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Editorial

Contents

Hope you enjoyed reading what we enjoyed writing & editing for your consumption in the earlier issues.

Antibiotic susceptibility testing (AST) is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection *in vivo*. Testing for antibiotic sensitivity is often done by the Kirby-Bauer method. Small wafers containing antibiotics are placed onto a plate upon which bacteria are growing. If the bacteria are sensitive to the antibiotic, a clear ring, or zone of inhibition, is seen around the wafer indicating poor growth. Other methods to test antimicrobial susceptibility include the Stokes method, E-test (also based on antibiotic diffusion).

The quality of dental unit water is of great importance since patients and dental staffs are regularly exposed to water from aerosols generated during work, and Dental Unit Water Line (DUWL) contamination has become a concern. Prior to the introduction of high-speed hand pieces, dentists did not use water as a coolant or as an irrigant. Along with the high-speed hand pieces came the dental unit water system that provided water for irrigation as well as a coolant during the cutting of teeth. Modernization of cutting instruments introduced a new problem of 'dental unit water system contamination' and 'release of bio-aerosols' in the dental clinic. Most modern dental unit water systems are made up of a complex maze of waterlines, control blocks, valves, barbs and connectors that are of various sizes and composed of different metals, plastics and rubbers.

Our In Profile section this time covers **The Astronaut Kalpana Chawla**. Born in Karnal & was the first Indian American astronaut and first Indian woman in space. She first flew on Space Shuttle Columbia in 1997 as a mission specialist and primary robotic arm operator.

Pelagibacter ubique and its relatives may be the most abundant organisms in the ocean, and quite possibly the most abundant bacteria in the entire world. It can make up about 25% of all microbial plankton cells, and in the summer they may account for approximately half the cells present in temperate ocean surface water. It is rod or crescent shaped and one of the smallest self-replicating cells known, with a length of 0.37-0.89 µm and a diameter of only 0.12-0.20 µm. 30% of the cell's volume is taken up by its genome. It is gram negative. It recycles dissolved organic carbon. It undergoes regular seasonal cycles in abundance - in summer reaching ~50% of the cells in the temperate ocean surface waters. Thus it plays a major role in the Earth's carbon cycle.

Tea is one of the most widely consumed beverages in the world, second only to water, and its medicinal properties have been widely explored. The tea plant, *Camellia sinensis*, is a member of the Theaceae family, and black, oolong, and green tea are produced from its leaves. Unlike black and oolong tea, green tea production does not involve oxidation of young tea leaves. Green tea is produced from steaming fresh leaves at high temperatures, thereby inactivating the oxidizing enzymes and leaving the polyphenol content intact.

Appropriate cleaning, disinfection and sterilization of patient care equipment are important in limiting the transmission of organisms related to reusable patient care equipment. The reprocessing method required for a specific item will depend on the item's intended use, the risk of infection to the patient, and the amount of soiling.

Also enjoy the jokes in our Relax Mood section & give a try to the interesting Brain Teasers.

Our JHS team would like to thank all our readers for their continuous support. Also awaiting for your valuable Feedback & suggestions.

Antimicrobial Susceptibility Testing

Antibiotics : Mechanism of Action

Antibiotics are low molecular weight substances that interfere with specific activities in certain types of organisms. These effects could be cidal (killing) and/or static (inhibitory). If an antibiotic has a widespread effect on Gram-positive and Gram-negative bacteria it is said to be a **broad spectrum antibiotic**. **A narrow spectrum antibiotic** will only act on either Gram-positive or Gram-negative bacterial strains. Antibiotics are found throughout nature and are used by organisms like moulds and soil inhabitants to gain advantages over their competitors. The early antibiotics were isolated from these natural sources, however today many are genetically engineered to be even more effective than their natural counterparts. For an antibiotic to be useful to humans it must have the ability to destroy pathogens while being relatively non-toxic to the host organism. It should be chemically stable and be able to reach the part of the host organism in which the infection persists.

Antibiotics work in a variety of ways, most of which attack Bacterial cell components and their synthesis. Some broad-spectrum antibiotics use several of the following modes of cellular attack:

1. Cell Wall Synthesis Inhibitors
2. Cell Membrane Inhibitors
3. Protein Synthesis Inhibitors
4. Nucleic Acid Effectors
5. Competitive Inhibitors

1. Cell Wall Synthesis Inhibitors : These antibiotics are particularly selective because they typically target the formation of peptidoglycan cell walls which are only found in prokaryotic cells. Cell Wall Synthesis Inhibitors do not impact eukaryotic cells of humans due to the lack of peptidoglycan. These types of antibiotics are referred to as Beta lactam antibiotics. Some common Beta lactam antibiotics are Ampicillin, Cephalothin and Penicillin.

2. Cell Membrane Inhibitors : These are less common than other types of antibiotic inhibiting mechanisms. Cell membrane inhibitors attack integrity of the bacterial membranes. An example of these is Polymyxin, which binds to membrane phospholipids. Once antibiotic has disrupted the membrane, the cell loses its integrity and will die. However a problem arises when there is a similarity between phospholipids in bacterial and eukaryotic cell membranes. These drugs can therefore be very dangerous to the patient due to the lack of selectivity in target cells and thus have only topical application.

3. Protein Synthesis Inhibitors : These types of antibiotics have numerous ways of attacking protein synthesis in bacterial cells and will usually target activities occurring at the ribosome. These drugs affect the ribosome and donot bind to any other components of the protein synthesis process. Examples of these are Erythromycin, Streptomycin, Gentamicin and Tetracycline.

4. Nucleic Acid Effectors : Some antibiotics attack the DNA or RNA of a cell. These chemotherapeutic agents affect the synthesis of the DNA (in some case RNA). This serves to block the natural growth of the cell and will lead to death without replication.

One group is called Quinolones, and an example of this type is Nalidixic acid. Nalidixic acid binds itself to the enzyme topoisomerase, which uncoils supercoiled DNA before replication. The drug also inhibits the enzyme DNA gyrase, which returns the DNA to its supercoiled state after replication. This widely utilized nucleic acid effector possesses it's own caveats and has been known to affect animal cells in addition to bacterial DNA. A new class of drugs, called Rifamycins (e.g. Rifampicin), solves the specificity problem of the quinolones. These only attack the eubacterial RNA polymerase, which is essential to mRNA synthesis. It is inactive towards RNA polymerase found in eukaryotic cells.

5. Noncompetitive and Competitive Inhibitors : A competitive inhibitor blocks an enzyme from performing its normal function by mimicking the substrate and binding to the enzyme's active site. A noncompetitive inhibitor will bind to the enzyme at a different location (the allosteric site), which will change the structure of the enzyme affecting the rate at which it can perform a task.

The Sulfonamides are a class of competitive inhibitors. They inhibit Dihydropteroate Synthase (DHPS) an enzyme present exclusively in bacterial cells. DHPS enzyme is required in a series of reactions to synthesize folic acid. Inhibition of DHPS will results in many damaging effects including failure in biosynthesis of Purine and Thymylidate nucleotides and eventually inhibiting DNA synthesis.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing measure the ability of an antibiotic or other antimicrobial agent to inhibit bacterial growth *in vitro*. This ability may be estimated by either dilution method or diffusion method.

1. The dilution method

For quantitative estimates of antibiotic activity, dilutions of the antibiotic may be incorporated into broth or agar medium, which is then inoculated with the test organism. The lowest concentration that prevents growth after overnight incubation is known as the minimum inhibitory concentration (MIC) of the agent. The MIC value is then compared with known concentrations of the drug obtainable in the serum and in other body fluids to assess the likely clinical response.

2. The diffusion method

Paper discs impregnated with a defined quantity of antimicrobial agent are placed on agar medium uniformly seeded with the test organism. A concentration gradient of the antibiotic forms by diffusion from the disc and the growth of the test organism is inhibited at a distance from the disc that is related among other factors to the susceptibility of the organism.

The recommended method for intermediate and peripheral laboratories is the modified Kirby-Bauer method. **This method has been recommended by National Committee on Clinical Laboratory Services (NCCLS-USA) Subcommittee on Antimicrobial Susceptibility Testing.** This is the most thoroughly described disc diffusion method for which interpretive standards have been developed and which is supported by laboratory and clinical data.

Kirby-Bauer Disk Diffusion Method

To ascertain antibiotic efficacy, degree of antibiotic resistance or sensitivity to bacterium, is the objective of Kirby-Bauer Test.

To carry out Kirby-Bauer Test following components are used :

1. Mueller Hinton Agar - Culture Media

2. Antimicrobial Susceptibility Discs
3. Standard Strains for Quality Control
4. Turbidity Standard (McFarland standards)
5. Swabs

Mueller-Hinton Agar : Mueller-Hinton Agar is considered the best medium to use for routine susceptibility testing of nonfastidious bacteria for the following reasons:

- It shows acceptable batch-to-batch reproducibility for susceptibility testing
- It is low in sulfonamide, trimethoprim, and tetracycline inhibitors
- It supports satisfactory growth of most nonfastidious pathogens
- A large body of data and experience has been collected concerning susceptibility tests performed with this medium.

Please note that the use of media other than Mueller-Hinton Agar may result in erroneous results. Aerobic or facultative bacteria grow well on unsupplemented Mueller-Hinton Agar. Fastidious organisms require Mueller-Hinton Agar to be supplemented with additional nutrients.

1. Mueller-Hinton Agar (AM1071/AM5071) should be prepared from a dehydrated base or purchased as Mueller Hinton Agar ready prepared plates. Follow the manufacturer's recommendation for storage of prepared plates. Be sure to prepare the media according to the manufacturer's directions. The medium should be such that control zone sizes within the standard limits are produced. It is important not to overheat the medium.
2. If you prepare Mueller-Hinton Agar plates from dehydrated media, **the plates must be poured to a depth of 4 mm**. Plates that are too shallow will produce false susceptible results as the antimicrobial compound will diffuse further, creating larger zones of inhibition. Conversely, plates poured to a depth more than 4 mm will result in false resistant results.
3. While pouring media into plates medium temperature should be in between 45-50ºC.
4. Dry the plates at 35-37ºC in the incubator in an upright position for immediate use.
5. Any unused plates may be stored in a plastic bag, which should be sealed and placed in the refrigerator. Plates stored this way can be kept for 2 weeks.
6. pH of the Mueller-Hinton Agar should fall between 7.2 and 7.4 at room temperature after solidification and should be tested when the media is first prepared. If the pH is less than 7.2 certain drugs will appear to lose potency (aminoglycosides, quinolones, macrolides), while other agents may appear to have excessive activity (tetracycline). If the pH is more than 7.4, the opposite results may occur.
7. Excessive thymidine or thymine can reverse the inhibitory effects of sulfonamides and trimethoprim resulting in smaller and less distinct zones of inhibition, or no zones at all.
8. The incorrect concentration of divalent cations (calcium and magnesium) will affect the results of aminoglycoside and tetracycline tests against *Pseudomonas aeruginosa*. Excess cation concentration will result in reduced zone sizes and low concentration will increase zone sizes. Excess calcium will increase the zone size of *P. aeruginosa* against daptomycin. Excess zinc ions may reduce the zone size of carbapenems against *P. aeruginosa.*
9. Mueller-Hinton Agar should be tested with known strains of organism at least weekly in order to verify that the media and discs are working as expected.

Mueller-Hinton Agar Physical Parameters

PARAMETERS	SPECIFICATIONS
Dehydrated powder appearance	Yellow coloured, homogenous, free flowing powder
Final pH at 25ºC	7.3 $\pm$ 0.1
Gelling temperature	Gel is formed at 32ºC
Colour and clarity of prepared media	Light amber coloured, clear gel

Cultural Characteristics Observed with Mueller-Hinton Agar after incubation for 18 to 24 hours at 30 - 35ºC

ORGANISM	TYPE CULTURE	GROWTH
Escherichia coli	ATCC (8739)	Luxuriant
Streptococcus faecalis	ATCC (11420)	Luxuriant
Neisseria gonorrhoeae	ATCC (49226)	Luxuriant
Pseudomonas aeruginosa	ATCC (9027)	Luxuriant
Staphylococcus aureus	ATCC (6538)	Luxuriant
Escherichia coli	ATCC (25922)	Luxuriant
Staphylococcus aureus	ATCC (25923)	Luxuriant
Pseudomonas aeruginosa	ATCC (27853)	Luxuriant
Enterococcus faecalis	ATCC (29212)	Luxuriant

Antimicrobial Susceptibility Discs : Antimicrobial susceptibility discs with proper diameter and potency should be used. Discs should be made of an absorbent material, usually paper, which has no interfering effect either on bacterial growth or on the action of the antibiotic. It must be capable of absorbing moisture rapidly and the antibiotic should be evenly distributed in it. Sealed cartridges containing commercially prepared paper discs should be stored at either 8°C or frozen at -14°C in a non-self-defrosting freezer. A small working supply of discs can be kept in the refrigerator for one week. On removal from the refrigerator, the containers should be left at room temperature for about 1 hour to allow the temperature to equilibrate. **Once opened, store the cartridges in a storage container containing desiccant for not more than 1 week.**

Standard Strains for quality control : The quality control should use standard reference strains of bacteria that are tested in parallel with the clinical culture. They should preferably be run every week (Fig 11.1), or with every fifth batch of tests, and in addition, every time that a new batch of Mueller Hinton Agar or a new batch of discs is used.

Standard Strains :

Staphylococcus aureus (ATCC 25923)
Escherichia coli (ATCC 25922)
Pseudomonas aeruginosa (ATCC 27853)

Culture for day-to-day use should be grown on slants of Nutrient Agar or Tryptic Soya Agar and stored in the refrigerator. These should be subcultured onto fresh slants after every 2 weeks.

Turbidity Standard (McFarland standard) : McFarland standard may be prepared in-house as described below :

1. Add a 0.5-ml aliquot of a 0.048 mol/liter BaCl2 (1.175% wt/vol BaCl2 • 2H20) to 99.5 ml of 0.18 mol/liter H2SO4 (1% vol/vol) with constant stirring to maintain a suspension.
2. Verify the correct density of the turbidity standard by measuring absorbance using a spectrophotometer with a 1-cm light path and matched cuvette. The absorbance at 625nm should be 0.08 to 0.13 for the 0.5 McFarland standard.
3. Transfer the barium sulfate suspension in 4 to 6 ml aliquots

into screw-cap tubes of the same size as those used in standardizing the bacterial inoculums.
4. Tightly seal the tubes and store in the dark at room temperature.

Use of McFarland standard in the Kirby-Bauer procedure :

1. Prior to use, vigorously agitate the barium sulfate standard on a mechanical vortex mixer and inspect for a uniformly turbid appearance. Replace the standard if large particles appear. If using a standard composed of latex particles, mix by inverting gently, not on a vortex mixer.
2. While adding bacterial colonies to saline in the "preparation of the inoculum" step of the procedure, compare the resulting suspension to the McFarland standard. This is done by holding both the standard and the inoculum tube side by side and no more than 1 inch from the face of the Wickerham card (with adequate light present) and comparing the appearance of the lines through both suspensions. If the bacterial suspension appears lighter than the 0.5 McFarland standard, more organisms should be added to the tube from the culture plate. If the suspension appears denser than the 0.5 McFarland standard, additional saline should be added to the inoculum tube in order to dilute the suspension to the appropriate density. In some cases it may be easier to start over rather than to continue to dilute a bacterial suspension that is too dense for use.

Swabs : Sterilized cotton swab stick can used or cotton swabs sticks can be sterilized in tins, culture tubes, or on paper, either in the autoclave or by dry heat.

Procedure to Perform Kirby-Bauer Disc Diffusion Test

- To prepare the inoculum from a primary culture plate, touch with a loop the top of each of 3-5 colonies of similar appearance of the organism to be tested (Fig.1).

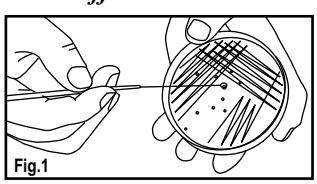


Fig.1

- When the inoculum has to be made from a pure culture, a loopful of the confluent growth is similarly suspended in saline (Fig. 2).

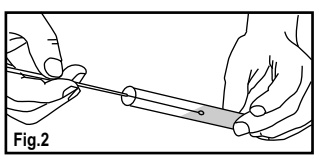


Fig.2

- Compare the tube with turbidity standard and adjust the density of test suspension to that of the standard by adding more bacteria or more sterile saline. Proper adjustment to the turbidity of the inoculum is essential to ensure that the resulting lawn of growth is confluent or almost confluent. Use this suspension within 15 minutes of preparation (Fig.3).

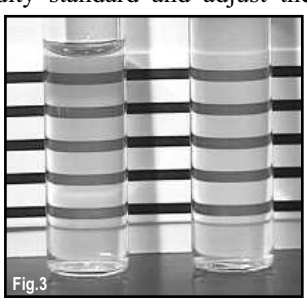


Fig.3

- Inoculate the plates by dipping a sterile swab into the inoculum. Remove excess inoculum by pressing and rotating the swabs firmly against the side of the tube above the level of the liquid (Fig. 4).

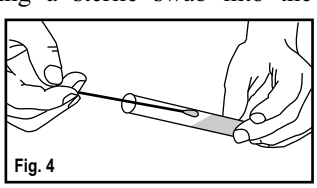


Fig. 4

- Streak the swab all over the surface of the medium three times, rotating the plate through an angle of 60º after each application. Finally, pass the swab round the edge of the agar surface. Leave the inoculum to dry for a few minutes at room temperature with the lid closed (Fig. 5).

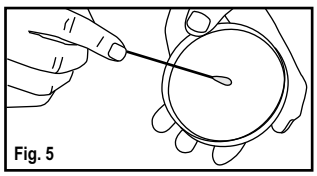


Fig. 5

- The antibiotic discs may be placed on the inoculated plates using a pair of sterile forceps or a sterile needle tip. Alternatively, an antibiotic disc dispenser can be used to apply the discs to the inoculated plate (Fig. 6).

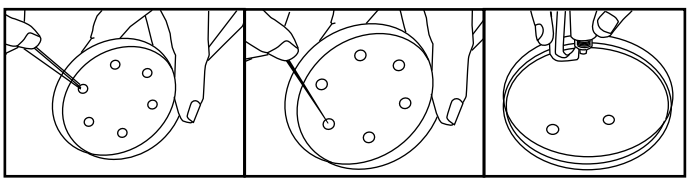


Fig. 6

- A maximum of 4-5 discs can be placed in a 90-100 mm diameter petridish.
- The plates should be placed in an incubator at 35-37ºC within 30 minutes of preparation.
- Incubate fastidious organism in an atmosphere of carbon dioxide.
- After overnight incubation, the diameter of each zone (including the diameter of the disc) should be measured and recorded in mm. The results should then be interpreted according to the critical diameters by comparing them with standard tables (Fig. 7).

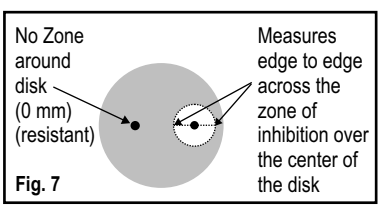


Fig. 7

- The measurements can be made with a ruler on the under surface of the plate without opening the lid (Fig. 8).

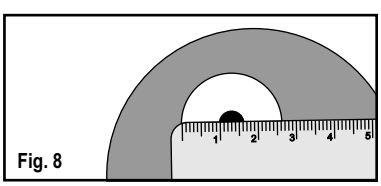


Fig. 8

- The endpoint of inhibition is judged by the naked eye at the edge where growth starts, but there are three exceptions,
  - With sulfonamides and co-trimoxazole, slight growth occurs within the inhibition zone; such growth should be ignored.
  - When β-lactamase producing staphylococci are tested against penicillin, zones of inhibition are produced with a heaped-up, clearly defined edge; these are readily recognizable when compared with the sensitive control and regardless of the size of the zone of inhibition, they should be reported as resistant.
  - Certain *Proteus* species may swarm into the area of inhibition around some antibiotics, but the zone of inhibition is usually clearly outlined and the thin layer of swarming growth should be ignored.

Water System Contamination Control in Dental

Introduction

Prior to the introduction of high-speed handpieces, dentists did not use water as a coolant or as an irrigant. Along with the high-speed handpieces came the dental unit water system that provided water for irrigation as well as a coolant during the cutting of teeth. Modernization of cutting instruments introduced a new problem of 'dental unit water system contamination' and 'release of bio-aerosols' in the dental clinic. Most modern dental unit water systems are made up of a complex maze of waterlines, control blocks, valves, barbs and connectors that are of various sizes and composed of different metals, plastics and rubbers.

The quality of dental unit water is of great importance since patients and dental staffs are regularly exposed to water from aerosols generated during work, and Dental Unit Water Line (DUWL) contamination has become a concern.

Traditionally, these water systems are directly rigged to the municipal water system and very few as of recent years are equipped with a bottle (self-contained water system) into which the treatment water or irrigants are added. The design of all dental unit water systems allows settling of contaminants from water and air. These contaminants can be inorganic materials such as salts from the hardness of the source water that coat the lines and cause corrosion of metals and allow settling of microbes. The other types of contaminants are organic in nature including extremely high counts of bacteria, fungi, viruses and even nematodes. These problems of contamination have been known by dentists and researchers for over 50 years. Only recently have agencies such as the American Dental Association (ADA), the Centers for Disease Control and Prevention (CDC) the dental industry seriously broached this topic in providing some methodologies and measures to modify the equipment, treat the contamination and monitor the level of contamination.

Factors associated with treatment water contamination

Some factors associated with water system contamination are—
1) Nutrient content of source water; 2) Extended period of stagnation per day; 3) High surface to volume ration; 4) Low flow-rate; 5) Laminar flow; 6) Microbial quality of source water; and lastly 7) Equipment design.

Influence by Adjacent Communities

One of the many possibilities is contamination or microbes transferred from patient's mouth to dental apparatuses, following back siphonage, then to dental water line. Also, microbes can be transferred from the hands of dental staff while treating patients

Bacteria who found in Dental water line

Achromobacter xylosoxidans, Acidovorax defluvii, Acidovorax spp., Acinectobacter spp., Actinomyces spp., Aeromonas hydrophila, Alcaligenes dentifricans, Bacillus spp., Bacteroides spp., Caulobacter spp., Flavobacterium spp., Fusobacterium spp., Klebsiella pneumoniae, Lactobacillus spp., Legionella pneumophila, Legionella spp., Methylophilus spp., Micrococcus spp., Moraxella spp., Mycobacterium avium, Nocardia spp., Pasteurella spp., Porphyromonas gingivalis, Proteus vulgaris, Pseudomonas aeruginosa, Burkholderia cepacia, Sphigomonas paucimobilus, Sphingomonas spp., Streptococcus spp., Staphylococcus aureus, and *Xanthomonas spp.*

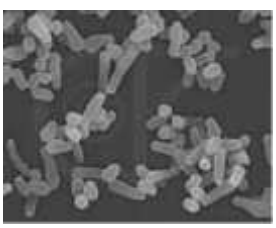
Among bacteria that are listed above, there are four most commonly discussed bacteria within biofilms of Dental Water Line. They are *Legionella pneumophila, Mycobacterium spp., Pseudomonas aeruginosa,* and *Staphylococcus spp.*

Microbe:

Legionella pneumophila

L. pneumophila is a gram-negative bacterium which spreads via the air-conditioning system. They are called chemo-organotrophs because they are capable of using certain amino acids as primary carbon and energy sources. Their cell walls are unique in a way that they contain a huge amount of branched fatty acids. Once they enter the cells, they reside and multiply in numbers within a membrane-bound compartment. A respiratory trace and the lungs are the main sites of infections. Legionnaire's disease is an atypical lung infection caused by *Legionella pneumophila.*

Mycobacterium spp.

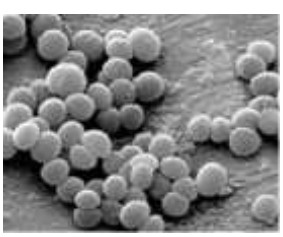


It is a bacterial genus which contains a vast number of species. The best known species are *M. leprae* and *M. Tuberculosis* which cause leprosy and tuberculosis respectively. In their cell wall, it consists of special fatty molecules called mycolic acids, and these complexes make the cell walls less permeable. They are aerobic, non-motile rods .

Pseudomonas aeruginosa

It is a heterogeneous genus comprising gram-negative, aerobic, rod-shaped bacteria. They exhibit motility since they have one or more polar flagella. They also contain fimbriae as an attachment to surfaces of other organisms. They grow fastest at the room temperature. *Pseudomonas aeruginosa* is the best known and the most virulent pathogenic pseudomonad. It is known as one of the most abundant life forms on the planet. Despite their widespread distribution, *P. Aeruginosa* infections are relatively rare.

Staphylococcus spp.



They are small cocci that exist in irregular clumps or grape-like clusters. There are three main species of *Staphylococcus* that share morphological and biochemical features. The pathogenic organisms produce many extracellular products known to play as a factor that leaves effects on humans.

Biofilm formation process

Biofilm formation takes place in a sequence of steps. At each step, the biofilm becomes more firmly attached and the microorganisms within it become more protected from the action of cleaners and sanitizers.

The first step is the formation of a conditioning layer. This consists of a loose collection of organic material such as protein, carbohydrates that combine with minerals in water. The conditioning step may begin within seconds of exposure to the surface.

Within 8 to 24 hours, bacteria in the growing biofilm become firmly attached to the surface and to each other by means of tendrils or filaments. They do this by exuding a polysaccharide material

that entraps cells and debris within a glue-like matrix. The biofilm environment is now a rich layer of nutrients that is capable of supporting rapid growth of the microorganisms within the biofilm.
As the biofilm becomes more established and grows in thickness and is capable of blocking cleaners and sanitizers from penetration. A mature biofilm can contain as many 100 billion bacterial cells per milliliter. Complex diffusion channels deliver nutrients, oxygen and other elements that cells need to grow and carry away metabolic waste products, debris, and cells. The thriving and well protected colony provides a continuous supply of cells that easily slough away and contaminate other surfaces.

Potential Risks

This biofilm is found to be teaming with microbes and chunks of which detach contaminating the dental treatment water. While municipal water normally may have less than 500 "planktonic" or free-floating microbes per milliliter, the water coming out of the dental handpiece and air/water syringe may have more than a million microbes per milliliter. The units of measurement for the microbial contamination in water is "colony 52forming units per milliliter" or cfu/mL. An example of usage is – 100 microbes in one milliliter of water is referred to as 100 cfu/mL. These environmental microbes survive even under very low nutrient conditions and may shrink to a tenth of their size, but still have the capacity to live on salts and other contaminants.
All environmental microbes are not pathogenic, but the sheer numbers that are found in the dental treatment water is of concern. Most of the microbes found in the dental water system biofilms are gram negative rods. When these gram negative rods die, they release a toxin called "bacterial endotoxins". These endotoxins in large amounts (as seen in un-cleaned water systems) have a potential in causing health problems in patients.
Among the potentially pathogenic microbes isolated in the dental unit water systems are-- Pseudomonas (potential for super infections), Mycobacterium (potential for causing lung infections), Legionella (potential for causing Legionnaire's Disease), and Moraxella (potential for causing bacterial endocarditis). Therefore, one has to consider the potential for being exposed to life-threatening infectious diseases from the dental treatment water of un-cleaned water systems. Investigators have also isolated amoebae, fungi and nematodes in the dental water systems.
Although there have not been studies linking association between the dental unit waterline contamination and mortality/morbidity among the dental patient population, it is pragmatic to keep the water system decontaminated and reduce the potential risks. It is difficult to conduct either retrospective or prospective epidemiological studies to find association between diseases and water source due to legal issue and logistical issues. Some studies have demonstrated significantly high titers of antibodies to Legionella among dental health care providers as opposed to a normal population and another study has shown that a large percentage of the dental water samples contaminated by at least one variety of Legionella.
During oral prophylaxis, use of a high speed handpiece or even the air/water syringe we generate aerosols that may be laden with microbes and endotoxins. These organic matter laden aerosols are referred to as "bioaerosols". These aerosols may reach up to 12 – 16 feet from the source during patient care and may stay suspended in the air for over 24 hours if there is "inadequate ventilation or air exchanges" and therefore, having a potential for exposing the employees to contaminants over time.
Microorganisms, blood and saliva from the oral cavity can enter the dental unit waterline system during patient treatment. Thus handpieces, ultrasonic scalers and air/water syringes should be operated for a minimum of 20 to 30 seconds after each patient to flush out retracted material. Even for devices fitted with anti retraction valves, flushing devices for a minimum of 20 to 30 seconds after each patient is appropriate**.**

Interaction between each other

Biofilm provides a suitable niche for conjugation to occur. Bacteria cells formed in biofilms will transfer genetic elements at a greater rate than those in the planktonic phase. This is primarily due to the close cell to cell contact and the minimal shear.
With this closely packed community, there are several possible ways that bacteria inhibit the forming of biofilms. *Pseudomonas aeruginosa* exhibit the cell-cell signaling, which plays a major role in attachment and detachment. At higher densities of biofilm colonies, the signal molecules, such as homoserine lacones in Gram negative bacteria and peptides in Gram positive bacteria, reach sufficient concentrations to activate genes involved in biofilm break-down. Similarly, *Streptococcus cristatus* is able to inhibit attachment of *Porphyromonas gingivalis* to a plaque biofilm. *Aeromonas hydrophila* is known to form biofilms in water systems. However, mutant forms of *A. hydrophila* were weakened in cell-cell communication, which resulted in their incapability to form biofilms.
As diverse microorganisms engage in forming the biofilm, the interactions may include incidents of antagonism and commensalism. In case of antagonism, there may be competition and predation due to bacteriovores, like free-living protozoa, which have been known to be 300 times more in number in Dental Unit Water than in city water, and bacteriophage that attacks certain bacteria in the biofilm.
There are also some commensalism cases in that several free-living amoebae, which have been found that while, encysted, they protect the inner microorganism from disinfectants and other biocides, and the microorganism may include legionella and other pathogenic microbes.
There have not been many studies done onto how a certain bacterium is interacting with other microbial cells specifically with the dental unit water line. *Pseudomonas fluorescens* and *Bacillus cereus* show significant differences in behavior and composition of planktonic and sessil dual species communities of them. Iron availability was used to measure differences in planktonic mixed growth of *P. fluorescens* and *B. cereus*. Under iron deficient condition, *P. fluorescens* prevented the vegetative growth of *B. cereus*. Dual biofilms were formed in a greater extent by *B. cereus*. *B.cereus*, being the primary surface colonizer, attached more effectively than *P. fluorescens*. Thus, the biofilms had a structure with a mid-layer composed of *P. fluorescens*, which was surrounded by two layers of *B. cereus*. The dominance of *B. cereus* in the outer layer of the dual biofilm is due to the constant supply of medium, and such flow may minimize the inhibitory factors formed by *P. fluorescens*. Also, the two specie dual biofilms had shown its weakened physical stability compared to that of the single species biofilms. In other words, there is some antagonism relationship, or some similar effect between *P. fluorescens* and *B. cereus* in biofilms.

Microbes change their environment through binding to surface/Biofilm formation

Hypothetically, the flow rate situated next to the substratum-liquid interface is insignificant. This area is coined with the term "hydrodynamic boundary layer" whose dimensions are inversely related to the water speed. Once a microbial cell has arrived at the hydrodynamic boundary layer, it can start the process of attachment.
The initial attachment of bacteria to a surface is often described by the DLVO Theory proposed by Derjaquin and Landau and Verwey

and Overbeek. Once a cell is inside the hydrodynamic boundary layer, it experiences an area of non-specific attraction about 10~20nm from the surface. This area is created due to the Van der Wall's forces of attraction and electrostatic repulsion. At this stage, various properties of bacterial cell affects adhesion: Hydrophobic non-flagella appendages such as fimbrae help in cell attachment by overcoming the electrostatic repulsion barrier that is located between the cell and the substratum; Lipopolysaccharides and exo-polysaccharides, protein, and protein-carbohydrate interactions also play important roles in the process of cell attachment at this stage.

Overall microbial attachment involves a dynamic complex process. This can be affected by different variables such as flow rate, surface roughness and hydrophobicity, and the presence and properties of conditioning films. DUWS contains several features that encourage increase in bacterial contamination. Since the tubing of DUWS is composed of polyurethane and polyvinylchloride, it can serve as nutrient sources for the bacteria. Moreover, DUWS is faced with long periods of stagnation that will encourage biofilm formation as well.

When the cell is attached to a surface, cells start to grow quickly and form microcolony. Then secondary colonies, which are colonies that lack the ability to initially attach to a surface by themselves to the primary colonisers.

A biofilm community can provide an ideal condition for the exchange of extrachromosomal DNA and plasmids. For example, plasmid recipient strains have been proven to demonstrate increased biofilm growth (penicillin binding proteins and IgA proteases).

Lastly, when the biofilm is at the climax number of community, individual cells and parts of the biofilm can be lost from the surface due to the loss of nutrient, quorum sensing or shear forces. This occurs when a newly divided daughter cell is shed from the outermost layer of the biofilm. Detachment from physical process can result from erision or shear, rapid loss and abrasion. Even though high shear forces may reduce the biofilm, the laminar flow rates in DUWS are insufficient to result in a great loss of the biofilm structure (DUWS has the maximum flow rate of 100mL/min)

Disinfection-by-products

When certain chemicals come in contact with biofilms, they react adversely and can lead to production of an array of toxic substances called disinfection-by-products (DBPs). When bleach comes in contact with biofilms a group of DBPs call trihalomethanes are produced that are considered to be carcinogens. Therefore, use of certain agents such as bleach to constantly or periodically decontaminate the water system may prove to be more deleterious than beneficial. Further, we have to consider corrosive effects of the chemical on the components of the water system as well as its safety on human health.

Some methods of control

Microbial control of dental treatment water/irrigant contamination is a two pronged issue. Firstly, the dental unit water system has to be cleaned and kept free of biofilms and other inorganic contaminants by initially and frequent periodic cleaning/ disinfecting the system with a decontaminating agent. Secondly, the water/ irrigant used for patient care must be microbe-free, and therefore, the water needs to be filtered, sterile, and distilled/boiled or a regulatory body approved low-grade antimicrobial added prior to use.

Periodic cleaning methods of the water system with a cleaning/ decontamination agent alone may remove some amount of biofilm or inorganic materials but will not prevent re-growth of biofilms. The water system can get re-colonized with biofilms very soon and therefore may show high CFUs/ml within days after periodic cleaning. Periodic cleaning products should not only disrupt and remove well established biofilms but also be effective on dissolving the inorganic salts coating the water system that may act as neutralizing agents against the low-grade disinfectants/ irrigants.

Selection of decontaminating agents and irrigants should entail asking manufacturers' information on— (1) Registration of the chemicals with respective regulatory agencies for safety issues (2) Biofilms and other deposit removal efficacy (3) Compatibility with metals, plastics and rubbers in the water system (4) Evidence of easy removal of periodic cleaning agent from the water system (5) Biocompatibility of periodic cleaning agent and irrigant with humans (6) Should not produce dangerous disinfection-by-products such as trihalomethanes (7) Compatibility of irrigant with composites bonding to enamel and dentin.

Approaches to improve dental unit output water quality.

Four methods are now widely advocated to reduce the level of bacterial contamination in dental unit water. The methods are:

(1) flushing waterlines for several minutes at the beginning of the day and after periods of disuse; (2) using an independent water reservoir system that is separate from the municipal water source (sterile water); (3) use of an independent water reservoir system combined with period or continuous application of chemical germicides; and (4) use of microfiltration to trap microbes before they reach the dental client.

Although flushing recommendations are found in a number of published infection control guidelines, there is little scientific support for this practice. Studies have shown that biofilms cannot be removed by flushing alone, and that biofilm bacteria can quickly recontaminate treatment water. Flushing between clients however may be beneficial to eliminating retracted client material.

The largest number of published studies have investigated the use of separate water reservoirs that isolate the dental water supply from the public water system. Since the unit itself is the main source of bacterial contamination, germicidal treatment is necessary to suppress or remove biofilms. Several studies have shown varying degrees of success using a number of chemicals and protocols.

Microbial filtration of ultrasonic and dental handpieces was first reported in 1978 by Dayoub, et al. More recent research has been published on point-of-use microfiltration in dentistry. This technology is widely used in medicine and industry and has the potential to reduce the levels of bacteria in treatment water. The use of filters is effective in reducing the CFU/ml to close to zero; however because they are placed at the end of the line, they have no effect on the formation of biofilms in waterlines.

Both separate water reservoir systems and microfiltration devices have been commercially available for a number of years. Most dental unit manufacturers now offer reservoir systems as optional equipment. Retrofittable systems are also available to add to existing dental units. These devices are of no value, however, without germicidal treatment, even when sterile water is used in the reservoirs. Users must follow routine maintenance protocols to control biofilm formation. These protocols, as well as safety and compatibility information, should be supplied by the manufacturer of the system.

An inline disposable microfiltration cartridge also is on the market. This device must be inserted as close to the water-using instrument as possible, and should be replaced at least daily on each line. Although this technology can dramatically reduce bacterial contamination in dental coolant and irrigating solutions, the biofilms that colonize the unit are unaffected. Filtration cartridges can be used in combination with water reservoirs to assure

improved water quality.

Other Technological approaches, including continuous chemical treatment devices and antimicrobial tubing, are currently under development. All devices and chemicals that claim to improve the quality of water used in dental treatment and/or control biofilms are subject to regulation as medical devices by respective regulatory agencies. To be assured of a device's safety and efficacy, users should select products that have been cleared.

Dental practitioners should consult the dental chair unit manual or contact the manufacturer for advice on products and procedures for waterline disinfection. In dental units supplied with a bottle reservoir, approved biocides can be added to the bottle, aspirated into the waterlines and left for an appropriate time to disinfect. Following disinfection, all of the waterlines should be thoroughly flushed to eliminate biocide. In dental chair units supplied with mains water, the dental practitioners should contact the manufacturer for advice on biocide delivery. Some brands of dental chair are supplied with an integrated waterline cleaning system.

Chemical treatment of biofilms

Over the past three decades, different cleaners, antiseptics, and disinfectants have been proposed or evaluated for their effectiveness in controlling microbial contamination. These solutions can be employed in two ways.

- Periodic or "shock" treatment with (hopefully) biocidal levels of chemicals.
- Continuous application of chemicals at levels (again, hopefully) which can control or eliminate biofilms, but are below threshold levels for toxicity in humans.

Other approaches being researched involve the use of cleaning agents that remove, rather than kill, biofilms; and the use of waterline materials which resist microbial adhesion.

Although the remarkable ability of biofilms to resist chemical attack has been repeatedly demonstrated, several chemicals have shown promise. The most extensively evaluated substances are chlorine compounds, including sodium hypochlorite (household bleach). Unfortunately, many of the compounds which have shown promise in controlling biofilms can also corrode or degrade dental unit materials. Moreover, these chemicals can react with dental unit materials, or with the biofilms, to produce disinfectant by-products which may have unanticipated effects on dental materials, clients, and healthcare workers.

Before initiating any chemical treatment regimen, the user should contact the dental equipment manufacturer to obtain treatment recommendations and safety precautions. All devices and chemicals which clam efficacy for the purpose of controlling biofilms or improving dental water quality are subject to FDA regulation as adjunctive medical devices. Select products cleared by FDA for marketing ensures users of their safety and efficacy.

The same chemical treatment regimens and technology used in the dental unit should be effective with the ultrasonic scalers and air polishing equipment, which also pose risk of bacterial contamination via biofilm in tubing. The user should contact the manufacturer for information on compatibility with the equipment and any chemical disinfectant or antimicrobial used. Microfiltration also has shown promise in clinical evaluations with these devices. Some of the ultrasonic scalers now on the market permit the use of sterile irrigants and have autoclavable or disposable waterlines and handpieces.

Non-chemical Treatment

DUWS engineering and redesigning: DUWS manufacturing is critical to achieve the improvements of water quality. Designing and evaluating DUWS is required to solve the problem of biofouling in the pipes. The length of the stagnant section can be reduced by redesigning the units and keep the water flow continuous. Also, the materials used could be re-evaluated to provide tubing that has less susceptibility to biofouling.

Flushing: Flushing the water line for several minutes before the first patient and for 20-30s between patients is recommended so that the number of bacteria in the water line can be decreased. However, this reduction is only temporary because the microorganisms will multiply back in a very short time. Therefore, an alternative strategy could be a flushing the dental unit at the end of the day and dry it overnight to reduce development of the biofilm.

Filtration: In-line 0.2 µm membrane filters is used for the filtration of the dental unit water when they need to reduce the need for chemical treatment. The length of the DUWS does not matter too much, but the system beyond the filter will be susceptible to microbial contamination.

Ultraviolet Light (UV): As the water flows out over the UV lamp, the bacterial numbers will be reduced. It is mainly supplemented by filtration and/or alternating chemical treatment.

Physical Cleaning: This method uses sponges or balls by making them pass through the pipeline at high pressure in order to remove the biofilm and destroy biofouling. (This technique is not applicable to a current design of DUWS).

Monitoring the quality of treatment water

Dental treatment water or irrigants should have less than 500 cfu/mL of heterotrophic mesophilic microorganisms. Apart from the microbial quality, the water should not have high endotoxin content and should at least be as clean as drinking water. Traditionally, the microbes found in dental treatment water are slow-growing and need low nutrient agars for growth. The ideal agar (for providing the diagnostic or contamination) for heterotrophic counts (total counts) is R2A agar, which can grow these environmental microbes at room temperature over 1-4 weeks. Normally, after plating, the microbial colonies can be counted after one week (7 days). Prior to plating the water/irrigant samples, the latter has to be neutralized using an appropriate chemical that will neutralize the antimicrobial agent to provide a natural environment for microbial growth. Using R2A agar is not feasible in a dental office; therefore the water collected from the water system should be sent refrigerated/on ice over night to a water quality monitoring laboratory to plate the samples. For in-office use, there are very few microbial quality monitoring kits. Some kits provide heterotrophic counts of microbes in the water in about 3 days and some counts in about 7 days of incubation. Both the kits need no incubator for the growth of the microbial colonies as the incubation temperature is room temperature. Although both in office samplers are easy to use, they are not very accurate in predicting the level of contamination, however, they are very easy to use and not expensive, and do not need incubation. As of today, there are many devices available in the market for cleaning and maintaining the microbiological quality of dental unit water systems. In conclusion, dental unit water systems that have not been cleaned regularly are highly contaminated and release high amounts of microbes and endotoxins into patients' oral cavities. The water system should be cleaned periodically to dislodge the biofilm and should use an irrigant free of microbial contamination. Low grade antimicrobials may also be used as irrigants. Materials selected for cleaning/decontaminating the water system must be biocompatible and not harm the patient, employee or the equipment and should not affect composite bonding to enamel or dentin. Water quality should be periodically monitored and contamination levels kept below 500 cfu/mL (similar to CDC's Guidelines). Water samples may be screened for contamination using in-office kits or be sent to water quality testing laboratories.

Kalpana Chawla

Astronaut
Born: July 1, 1961, Karnal
Died: February 1, 2003, Texas, United States
Space missions: STS-87, STS-107
Spouse: Jean-Pierre Harrison (m. 1988)
Education: University of Colorado at Boulder (1988), More
Parents: Sanjyothi Chawla, Banarasi Lal Chawla

Introduction:
Kalpana Chawla was born in Karnal, India. She was the first Indian American astronaut and first Indian woman in space. She first flew on Space Shuttle Columbia in 1997 as a mission specialist and primary robotic arm operator.

Education:
Chawla completed her earlier schooling at Tagore Baal Niketan Sr. Sec. School, Karnal. She completed Bachelor of Engineering degree in Aeronautical Engineering at Punjab Engineering College at Chandigarh in 1982. She moved to the United States in 1982 and obtained a M.S. degree in aerospace engineering from the University of Texas at Arlington in 1984. Chawla went on to earn a second M.S. degree in 1986 and a PhD in aerospace engineering in 1988 from the University of Colorado at Boulder. Later that year she began working at the NASA Ames Research Center as vice president of Overset Methods, Inc. where she did CFD research on Vertical/Short Takeoff and Landing concepts. Chawla held a Certificated Flight Instructor rating for airplanes, gliders and Commercial Pilot licenses for single and multi-engine airplanes, seaplanes and gliders.

NASA Career:
Chawla joined the NASA Astronaut Corps in March 1995 and was selected for her first flight in 1996. She had traveled 10.67 million km, as many as 252 times around the Earth.
Her first space mission began on November 19, 1997 as part of the six-astronaut crew that flew the Space Shuttle Columbia flight STS-87. Chawla was the first Indian-born woman and the second Indian person to fly in space, following cosmonaut Rakesh Sharma who flew in 1984 in a spacecraft. On her first mission, Chawla traveled over 10.4 million miles in 252 orbits of the earth, logging more than 372 hours in space. During STS-87, she was responsible for deploying the Spartan Satellite which malfunctioned, necessitating a spacewalk by Winston Scott and Takao Doi to capture the satellite. A five-month NASA investigation fully exonerated Chawla by identifying errors in software interfaces and the defined procedures of flight crew and ground control.
After the completion of STS-87 post-flight activities, Chawla was assigned to technical positions in the astronaut office to work on the space station, her performance in which was recognized with a special award from her peers.
In 2000 she was selected for her second flight as part of the crew of STS-107. This mission was repeatedly delayed due to scheduling conflicts and technical problems such as the July 2002 discovery of cracks in the shuttle engine flow liners. On January 16, 2003, Chawla finally returned to space aboard *Columbia* on the ill-fated STS-107 mission. Chawla's responsibilities included the microgravity experiments, for which the crew conducted nearly 80 experiments studying earth and space science, advanced technology development, and astronaut health and safety.

Death:
Chawla died in the Space Shuttle Columbia disaster which occurred on February 1, 2003, when the Space Shuttle disintegrated over Texas during re-entry into the Earth's atmosphere, with the loss of all seven crew members, shortly before it was scheduled to conclude its 28th mission, STS-107.

Awards:
Posthumously awarded:
- Congressional Space Medal of Honor
- NASA Space Flight Medal
- NASA Distinguished Service Medal

Memorials:
- On November 18, 2012, in Karnal city, Haryana, India, Chawla's birthplace and first home town, the foundation stone of the Kalpana Chawla Government Medical College was laid by the Haryana Government in her memory.
- The Kalpana Chawla ISU Scholarship fund was founded by alumni of the International Space University (ISU) in 2010 to support Indian student participation in international space education programs.
- The Kalpana Chawla Memorial Scholarship program was instituted by the Indian Students Association (ISA) at the University of Texas at El Paso (UTEP) in 2005 for meritorious graduate students.
- In Karnal city, India, at least 30,000 school children and citizens joined hands to make a 36.4-km-long human chain to support the demand for a Kalpana Chawla medical college in the Karnal city, which was announced by then Health Minister of India Dr. C. P. Thakur and later promised by Prime Minister of India Dr. Manmohan Singh.
- Asteroid 51826 Kalpanachawla, one of seven named after the Columbia's crew.
- On February 5, 2003, India's Prime Minister announced that the meteorological series of satellites, MetSat, was to be renamed as "Kalpana". The first satellite of the series, "MetSat-1", launched by India on September 12, 2002, is now known as "Kalpana-1". "Kalpana-2" was expected to be launched by 2007.
- 74th Street in Jackson Heights, Queens, New York City has been renamed 74th Street Kalpana Chawla Way in her honor.
- The University of Texas at Arlington (where Chawla obtained a Master of Science degree in Aerospace Engineering in 1984) opened a dormitory named in her honor, Kalpana Chawla Hall, in 2004.
- The Kalpana Chawla Award was instituted by the government of Karnataka in 2004 for young women scientists.
- NASA has dedicated a supercomputer to Chawla.
- Novelist Peter David named a shuttlecraft, the Chawla, after the astronaut in his 2007 Star Trek novel, Star Trek: The Next Generation: Before Dishonor.
- The University of Texas at Arlington dedicated the Kalpana Chawla Memorial on May 3, 2010, in Nedderman Hall, one of the primary buildings in the College of Engineering.
- The Government of Haryana established the Kalpana Chawla Planetarium in Jyotisar, Kurukshetra.
- The Indian Institute of Technology, Kharagpur, named the Kalpana Chawla Space Technology Cell in her honor.
- A military housing development at Naval Air Station Patuxent River, Maryland, has been named Columbia Colony, and there is a street named Chawla Way.

Enjoy the humour

Three men were sitting in a bar talking about how whipped they had their wives.

The first two kept bragging about how they could get their wives to do anything.

They looked at the third man and he said, "I have my wife so whipped that the other day I had her crawling towards me on her hands and knees."

Both of the other men were very impressed and asked him how he had managed that.

The man replied, "Well, I was laying under the bed and she crawled over and said, 'Come out and fight like a man!'"

The boss was complaining in a staff meeting the other day that he wasn't getting any respect.

The next day, he brought a small sign that Read: "I'm the Boss !"

He then taped it to his office door.

Later that day when he returned from lunch, he found that someone had taped a note to the sign that said: "Your wife called, she wants her sign back!"

1. A doctor gave a microbiologist a juice glass, a dinner plate, water, a match, and a lemon wedge. The doctor poured enough water onto the plate to cover it.
   The doctor told the microbiologist, "If you can get the water on the plate into this glass without touching or moving this plate, I will give you $100. You can only use the match and lemon to do this."
   A few minutes later, the microbiologist walked away with $100 in her pocket. How did the microbiologist get the water into the glass?

2. Inside each set of the following words, there is a pair of smaller words. By putting "&" between them, you'll make a familiar phrase. For example, "Thighbone/Swallowtail" conceals "High & Low."
   1. Skyrocketing/Trolleyman
   2. Thermometer/Apoplexy
   3. Delaware/Bordering
   4. Surprised/Trashiness
   5. Throughout/Stumblebum

3. What do the following words have in common?

Assess	Potato
Banana	Revive
Dresser	Uneven
Grammar	Voodoo

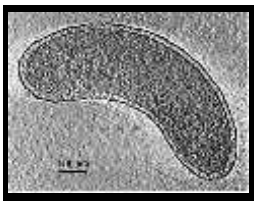
Solutions

1. First, the microbiologist stuck the match into the lemon wedge, so that it would stand straight. Then she lit the match, and put it in the middle of the plate with the lemon. Then, she placed the glass upside-down over the match. As the flame used up the oxygen in the glass, it created a small vacuum, which sucked in the water through the space between the glass and the plate. Thus, the microbiologist got the water into the glass without touching or moving the plate.

2. 1. Rock & Roll
   2. Mom & Pop
   3. Law & Order
   4. Rise & Shine
   5. Rough & Tumble

3. If you take the first letter and move it to the end and read the word backwards, you will have the same word.

Pelagibacter Ubique



Scientific classification: (Candidatus)
Kingdom: Bacteria
Phylum: Proteobacteria
Class: Alphaproteobacteria
Order: Rickettsiales
Family: "Pelagibacteraceae"
Genus: ***Pelagibacter***
Species: ***P. ubique***
Binomial name: ***Candidatus Pelagibacter ubique***

Description and significance:
Pelagibacter ubique, strain HTCC1062, is significantly known to be one of smallest and simplest, self-replicating, and free living cell. It is part of the SAR11 clade, which are small, heterotrophic alphaproteobacteria, equaling to ~25% of all microbial plankton cells. During the summer time, it can increase to ~50% of the cells in the temperate ocean. *P. ubique* has a coastal, ocean surface ecotype , living in a low nutrient habitat. As the first cultivated member of SAR11 clade (the most successful clade of organisms), *P. ubique* is completely sequenced and has the smallest genome (1,308,759 bp) for any free living organism, as well as the smallest number of predicted open reading frames (ORF). Differing from bacteria and archaea, which have small genomes as well, *P. ubique* has complete biosynthetic pathways for all 20 amino acids and all but a few cofactors. Also, it has no pseudogenes, introns, transposons, extrachromosomal elements, or inteins. In other words, *P. ubique* has no duplicate gene copies, no viral genes, and no junk DNA. The genome is about the size of intracellular parasites *R. conorii* and *W. pipientis*, however, its metabolic features are very simple. With a shorter DNA length to be copied for each generation, then the less work is to be done. Although there are numerous *P. ubique* microbes present in the ocean, mutation has not been a problem, mostly due to the fact that it is self-sufficient. Ongoing research is occurring to explain this phenomenon.
With a small genome size of 1,308,759 base pairs, whose DNA is completely sequenced, the “simplicity” of the *P. ubique* is more understood. It is a simple microbe, and with a refined understanding, we can identify what is needed for self cellular replication.
P. ubique was first isolated in 2002 from the Sargasso Sea, from which SAR11 obtained its name, and the Oregon coastal surface waters, by a team from Oregon State University. The first cultivated strains of *P. ubique* were by high throughput dilution-to-extinction culturing (HTC), using a natural seawater media for dilution. The cultures were regularly reproduced in autoclaved seawater, where they reached their typical cell densities as in their natural populations, which is about 10^6 cells/ml. In collaboration with Diversa Corporation, liquid chromatography and tandem mass spectrometry was used to find proteorhodopsin genes were present in the culture, from which the genes were abundant in the Oregon coast. The proteorhodopsin genes are light dependent proton pumps, supplying energy for microbial metabolism.

Genome structure:
The genome of *P. ubique* has 1,308,759 bp, constituting 30% of the cell volume, and is completely sequenced. It encodes the smallest number of predicted open reading frames and has complete biosynthetic pathways for all 20 amino acids and all but a few cofactors. With a small genome size, almost all the basic functions are encoded, which is attributed to the absence of nonfunctional or repeating DNA. There has been no evidence of DNA originating from any gene transfers. Although the number of *P. ubique* is vast in the ocean and there are many opportunities for random mutations, the microbe has been able to streamline its genes - simplify and organize its genes to be self-sufficient.
In addition, *P. ubique* has a circular shaped chromosome with 1354 protein genes, and 35 RNA genes. According to the Comprehensive Microbial Resource website, from the total size of the DNA molecules, 26.68% (388480 bp) is the amount of G+C bases and 95.46% (1249346 bp) is the amount of primary annotation coding bases. *P. ubique* has 1354 protein coding genes, with 85.89% (1163 genes) which are assigned a role category, 2.51% (34 genes) which are not assigned a role category, 2.88% (39 genes) which are conserved hypothetical genes, and 8.71% (118 genes) which are hypothetical genes.
The pattern of genome reduction observed is adherent with the hypothesis of genome streamlining, from a large population which originates in a low nutrient environment. This simply suggests that *P. ubique* is one of the most abundant organisms in the ocean because of its simple life form. Proteorhodopsins, which was active in the ocean, have been found to be first expressed in the SAR11 strain HTCC1062 – *P. ubique*, when cultured in both autoclaved seawater and in its natural environment. Proteorhodopsins are light dependent proton pumps which give energy for microbial metabolism. Some structural features of *P. ubique* proteorhodopsin genes had an effect on its absorbance. At position 105, if a leucine residue is present, then green light is absorbed, which is common of the shallow water proteorhodopsins. However, from the proteorhodopsin genes taken from the Sargasso Sea, glutamine residues were located at position 105, therefore, absorbing blue light. Through research, the gene is still expressed when cells are grown in both light and dark environments, showing not difference in growth rates or cell yields.

Cell structure and metabolism
P. ubique is rod or crescent shaped, with a length of 0.37-0.89 μm and an average cell diameter of 0.12-0.20 μm . The size range is due to natural variation associated with cell division. Also, the cells were measured after fixation in glutaraldehyde, which may have decreased the size. An average cell size after cell division is about 0.4 × 0.2μm^2, with the cell volume of 0.01 μm^3. It is stated many times that *P. ubique* is one of the most abundant and smallest organisms in the ocean. This is true because the combined weight of the SAR11 clade outweighs the total weight of all the fish present in the sea.
P. ubique is a self-sufficient organism. It is a small microbe, consisting of only 1,354 genes, which encodes for the bare essentials for life, compared to the 30,000 human genes. It gains energy from consuming dead organic carbon in its natural habitat, which is a low nutrient environment. From this process, the microbe releases nutrients needed by the ocean algae to convert carbon dioxide into oxygen, which is a photosynthetic process. Because nitrogen, which is a difficult element to attain, *P. ubique* favors base pairing, which requires less nitrogen.

Ecology

P. ubique is an efficient microbe. It is a heterotrophic alphaproteobacteria, and therefore is dependent on organic matter. It consumes the dead organic matter which is dissolved in the ocean water. Because the ocean is a vast area, there is no drawback of overabundance or under abundance; therefore, there is no need for a special metabolic cycle. Furthermore, adding nutrients to the ocean would not affect the microbe's feeding tendencies. By feeding off the dissolved organic carbon, *P. ubique* produced nutrients needed by the algae to grow, from which the algae is needed to convert carbon dioxide to oxygen. It is known that ocean algae are credited for most of photosynthetic oxygen. In addition, *P. ubique* uses less nitrogen, which is sometimes a difficult nutrient for organisms to attain. This is possible because *P. ubique* uses base pairs, which require less nitrogen.

Pathology

P. ubique has a completely coded genome, which does not consist of any junk DNA, duplicate entries, or viral genes. It is one of the simplest microbes found in the ocean, which simply eats to survive. As stated in the Ecology section, it feeds off dead organic matter and even uses base pairing to use less nitrogen. Therefore, currently, there is no research stating this organism causes or contributes to any disease.

Application to Biotechnology

P. ubique produces nutrients which are essential for the growth of ocean algae, by feeding off of dead organic carbon. In turn, the ocean algae convert carbon dioxide to oxygen by photosynthesis. Oxygen is necessary for most life forms on Earth to exist, and since algae constitutes more than half of the oxygen produced, *P. ubique* plays a crucial part in the production of oxygen.

Current Research

Giovannoni Lab

Dr. Stephen Giovannoni, from Oregon State University, is head of a lab which cultured SAR11 that was renamed *P. ubique*. On-going experiements are trying to understand how SAR 11 contributes to geochemical cycles and the basic architecture of cells and genomes. Currently, they are understanding the role of the proterohodopsin gene as part of the metabolism of *P. ubique*.

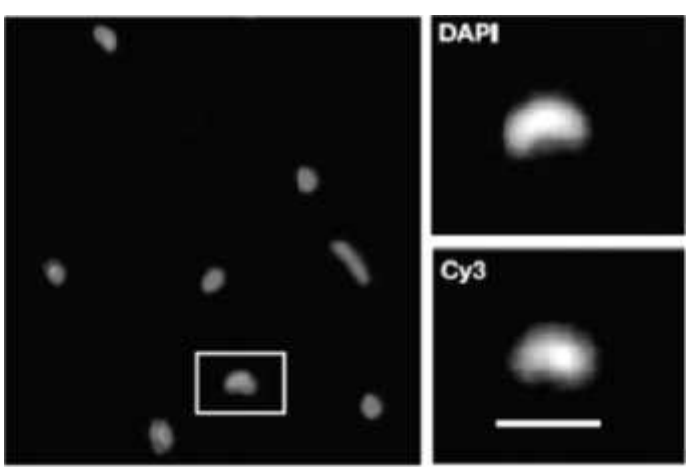


***Figure 2**: SAR11 fluorescence in situ hybridization image composite. Dual image overlay of DNA-containing cells stained with DAPI (blue) and the Cy3 probe (red). Cells emitting a signal for both DAPI and the Cy3 probe are both blue and red, and cells that did not hybridize to the set of SAR11 probes are blue. The identical fields of view in the DAPI and Cy3 stained images show the characteristic size and curved rod morphology of a magnified SAR11 cell.*

Artificial "Pelagibacter ubique"

Craig Venter, a bio-entrepreneur, is attempting to create an artificial version of *P. ubique* with about 300 genes. His purpose is to discover how few genes are needed for a living organism to survive.

Metabolic reconstruction

There is current research to predict the organic carbon sources used by *P. ubique* by metabolic reconstruction. Also, the proteome state of the microbe is studied, to be used as a means of finding information of the biological state of the system.

Improvement of techniques

In the Spring of 2007, Ulrich Sting, Harry James Tripp and Stephen Giovannoni, of Oregon State University, published a journal article in The ISME Journal, entitled Improvements of high-throughput culturing yielded novel SAR 11 strains and other abundant marine bacteria from the Oregon coast and the Bermuda Atlantic Time Series study site. In their research, they improved the high-throughput dilution-to-extinction culturing (HTC), leading to the isolation of 17 new SAR11 strains from the Oregon coast and the Sargasso Sea. Sterilized natural sea water was used as a medium. With phylogenetic analysis of the internal transcribed spacer (ITS) region, the new strains found in the Oregon coast were divided into three subclusters of SAR11, while the strains from the Sargasso Sea represented one ITS cluster. Using a PCR assay, proteorhodopsin (PR) was present in all the strains. By analyzing the PR amino acid sequences of the isolates, those from the Oregon coast were tuned to either blue or green light and those from the Sargasso Sea were tuned to only blue light. However, the phylogenies of the strains, according to the ITS and PR, did not correlate, therefore indicating some kind of lateral gene transfer. The new information about the SAR11 clade could possibly lead to more information of *P. ubique* and its minimal macromolecular machinery needed for self-replication.

Recombination

In 2007, a group of biologists contributed to an article in Environmental Microbiology, entitled High intraspecific recombination rate in a native population of *Candidatus Pelagibacter ubique* (SAR 11). The groups studied the recombination, which is an important progression in microbial evolution. Having low rates of recombination is enough to have lateral gene transfers, which will not disturb the clonal pattern of inheritance for other genes. However, a high rate of recombination can obscure clonal patterns, which can lead to sexually interbreeding eukaryotic populations. In the research, recombination between eight loci from nine strains of *Candidatus P. ubique* (SAR11), which was isolated from a 2L niskin sample of seawater. Using a test called the Shimodaira-Hasegawa test, it was found that there were phylogenetic differences in seven of the genes, showing that "frequent recombination obscures phylogenetic signals from the linear inheritance of genes in this population." At this point, the methods of genetic transfer is unknown. However, tests have shown that the recombination rates are higher than the point mutation rates, which is the source of genetic diversity within the SAR11 clade.

Antimicrobial properties of green tea (*Camellia sinensis*)

Description

Tea is one of the most widely consumed beverages in the world, second only to water, and its medicinal properties have been widely explored. The tea plant, *Camellia sinensis*, is a member of the Theaceae family, and black, oolong, and green tea are produced from its leaves. It is an evergreen shrub or tree and can grow to heights of 30 feet, but is usually pruned to 2-5 feet for cultivation. The leaves are dark green, alternate and oval,with serrated edges, and the blossoms are white, fragrant, and appear in clusters or singly.

Active Constituents

Unlike black and oolong tea, green tea production does not involve oxidation of young tea leaves. Green tea is produced from steaming fresh leaves at high temperatures, thereby inactivating the oxidizing enzymes and leaving the polyphenol content intact. The polyphenols found in tea are more commonly known as flavanols or catechins, and comprise 30-40 percent of the extractable solids of dried green tea leaves. The main catechins in green tea are epicatechin, epicatechin-3-gallate, epigallocatechin, and epigallocatechin-3-gallate (EGCG), with the latter being the highest in concentration. Green tea polyphenols have demonstrated significant antioxidant, anticarcinogenic, anti-inflammatory, thermogenic, probiotic, and antimicrobial properties in numerous human, animal, and *in vitro* studies.

Mechanisms of Action

The anticarcinogenic properties of green tea polyphenols, mainly EGCG, are likely a result of inhibition of tumor initiation and promotion, induction of apoptosis, and inhibition of cell replication rates, thus retarding the growth and development of neoplasms. Green tea polyphenols' antioxidant potential is directly related to the combination of aromatic rings and hydroxyl groups that make up their structure, and is a result of binding and neutralization of free radicals by the hydroxyl groups. In addition, green tea polyphenols stimulate the activity of hepatic detoxification enzymes, thereby promoting detoxification of xenobiotic compounds, and are also capable of chelating metal ions, such as iron, that can generate radical oxygen species.

Green tea polyphenols inhibit the production of arachidonic acid metabolites such as pro-inflammatory prostaglandins and leukotrienes, resulting in a decreased inflammatory response. Human and animal studies have demonstrated EGCG's ability to block inflammatory responses to ultraviolet A and B radiation, as well as significantly inhibiting neutrophil migration that occurs during the inflammatory process. Research on green tea's thermogenic properties indicates a synergistic interaction between its caffeine content and catechin polyphenols that can result in prolonged stimulation of thermogenesis.

Studies have also shown green tea extracts are capable of reducing fat digestion by inhibiting the activity of certain digestive enzymes. Although the exact mechanism is unknown, green tea catechins have been shown to significantly raise levels of Lactobacilli and Bifidobacteria while decreasing levels of numerous potential pathogens. Studies have also demonstrated green tea's antibacterial properties against a variety of gram-positive and gram-negative species.

Clinical Indications

Cancer Prevention/Inhibition

Several studies have demonstrated green tea polyphenols preventative and inhibitory effects against tumor formation and growth. While the studies are not conclusive, green tea polyphenols, particularly EGCG, may be effective in preventing cancer of the prostate, breast, esophagus, stomach, pancreas, and colon. There is also some evidence that green tea polyphenols may be chemopreventive or inhibitory toward lung, skin, and liver cancer, bladder and ovarian tumors, leukemia, and oral leukoplakia.

Antioxidant Applications

Many chronic disease states and inflammatory conditions are a result of oxidative stress and subsequent generation of free radicals. Some of these include heart disease (resulting from LDL oxidation), renal disease and failure, several types of cancer, skin exposure damage caused by ultraviolet (A and B) rays, as well as diseases associated with aging. Green tea polyphenols are potent free radical scavengers due to the hydroxyl groups in their chemical structure. The hydroxyl groups form complexes with free radicals and neutralize them, preventing the progression of the disease process.

Obesity/Weight Control

Recent studies on green tea's thermogenic properties have demonstrated a synergistic interaction between caffeine and catechin polyphenols that appears to prolong sympathetic stimulation of thermogenesis. A human study of green tea extract containing 90 mg EGCG taken three times daily concluded that men taking the extract burned 266 more calories per day than did those in the placebo group and that green tea extract's thermogenic effects may play a role in controlling obesity. Green tea polyphenols have also been shown to markedly inhibit digestive lipases *in vitro*, resulting in decreased lipolysis of triglycerides, which may translate to reduced fat digestion in humans.

Intestinal Dysbiosis and Infection

A small study in Japan demonstrated a special green tea catechin preparation (30.5% EGCG) was able to positively affect intestinal dysbiosis in nursing home patients by raising levels of Lactobacilli and Bifidobacteria while lowering levels of Enterobacteriaceae, Bacteroidaceae, and eubacteria. Levels of pathogenic bacterial metabolites were also decreased. An *in vitro* study also demonstrated green tea possesses antimicrobial activity against a variety of gram-positive and gram-negative pathogenic bacteria that cause cystitis, pyelonephritis, diarrhea, dental caries, pneumonia, and skin infections.

Other Applications

Sickle cell anemia is characterized by a population of "dense cells" that may trigger vasoocclusion and the painful sickle cell "crisis." One study demonstrated that 0.13 mg/mL green tea extract was capable of inhibiting dense-cell formation by 50 percent. Another potential therapeutic application of green tea is the treatment of psoriasis. The combination therapy of psoralens and ultraviolet A radiation is highly effective but has unfortunately been shown to substantially increase the risk for developing squamous cell carcinoma and melanoma. An *in vitro* study using human and mouse skin demonstrated that pre- and post treatment with green tea extract inhibited DNA damage induced by the psoralen/ ultraviolet A radiation exposure.

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Disinfection of some important medical/surgical equipment

Endoscopic instruments and their accessories

Fiber-optic endoscopic instruments can be divided into rigid endoscopes (laparoscopic instruments) and flexible endoscopes. There are significant processing and usage differences between rigid and flexible endoscopes.

However, both rigid and flexible endoscopes (and their accessories) require meticulous cleaning prior to undergoing the appropriate sterilization, or high level disinfection, process.

Rigid endoscopes are categorized as 'critical' items (refer to 'Spaulding's classification'), and must be sterilized between uses.

Flexible endoscopes are categorized as either 'critical' or 'semi-critical' in relation to the nature of their use. Those classified as 'critical' must be sterilized. In the case of 'semi-critical' flexible endoscopes, sterilization is preferred but not mandatory. Where sterilization of 'semi-critical' flexible endoscopes is not possible, high level chemical disinfection is required.

Both rigid and flexible endoscopes can be sterilized by low temperature processes, although some methods may be unsuitable for some flexible endoscopes. Many rigid endoscopes can also withstand steam sterilization. Endoscopes with no attached lenses, fiber-optic light carriers or cables, and suitable accessories, may be sterilized by steam (refer to 'steam sterilization' and 'low temperature sterilization processes'). In all instances, the instrument manufacturer's instructions as to the preferred method of sterilization should be followed.

A wide range of accessories is available for both invasive and non-invasive endoscopes, including forceps, laparoscopic scissors, diathermy, snares, sphincterotomy knives, and lasers. Accessories used in conjunction with rigid and flexible endoscopes should be treated as 'critical' items and sterilized.

Flexible Endoscope

Rigid endoscopes

Invasive ('critical') endoscopes are mainly laparoscopes, and rigid instruments with no operating channel. Arthroscopes and laparoscopes which are inserted into sterile body cavities shall be sterile. Rigid endoscopes are classified as 'critical' (refer 'Spaulding's classification') and must be sterilized. Although high level disinfection has been used in the past, it is now considered inadequate.

Sterilization of rigid endoscopes

Sterilization of invasive devices attains a higher standard of infection control than high level disinfection can achieve, is more thoroughly controlled, and cycle times are comparable with immersion in disinfectants. Low temperature sterilization methods (ethylene oxide, peracetic acid or hydrogen peroxide plasma sterilization processes) or steam sterilization may be used for rigid endoscopes. Low temperature methods are preferable as they may reduce instrument damage caused by repeated exposure to steam (even when the instrument is steam compatible). Refer to 'steam sterilization' and 'low temperature sterilization processes'. Laparoscopes and accessory instrumentation shall be dismantled, cleaned (specially designed ultrasonic irrigators are available to assist cleaning), dried thoroughly and then reassembled prior to sterilization.

Flexible endoscopes

Flexible endoscopes can be classified according to Spaulding's classification as 'critical' (e.g. invasive) instruments that penetrate the skin or are inserted into a sterile cavity, or as 'semi-critical' (e.g. non-invasive) items that are in contact with intact mucous membranes. However, even in cases where items are not classified as 'critical', sterilization is preferable to high level disinfection.

Sterilization of flexible endoscopes

Steam sterilization is unsuitable for flexible endoscopes because they are unable to tolerate temperatures greater than 60°C. Low temperature sterilization methods are required when sterilization of flexible endoscopes is required (i.e.: flexible endoscopes categorized as 'critical') and, where sterilization is desired (i.e.: flexible endoscopes categorized as 'semi-critical') but not mandatory. Low temperature systems such as ethylene oxide, and peracetic acid sterilization systems may be used to sterilize flexible endoscopes. However, the hydrogen peroxide plasma sterilization process is not commonly used for reprocessing flexible endoscopes due to the following technical problems:

- Very long narrow lumens and those closed at one end are unsuitable for sterilization using the hydrogen peroxide plasma process
- Process compatible packaging must be used
- Biological indicators are required for routine monitoring with lengthy incubation periods
- Entire cycle takes 75 minutes, making hydrogen peroxide plasma impractical for routine processing of most gastroenterological endoscopes

High level disinfection of flexible endoscopes

Non-invasive or 'semi-critical' items may either be sterilized or high level disinfected, however sterilization is the preferred method whenever possible. In the case of bronchoscopes that are high-level disinfected, care must be taken to observe the contact time required to inactivate Mycobacterium tuberculosis.

High level disinfection is a suitable process for non-invasive ('semi-critical') endoscopes such as gastroscopes, duodenoscopes, sigmoidoscopes, proctoscopes, colonoscopes, bronchoscopes, and laryngoscopes.

Reprocessing of anaesthetic and respiratory equipment

Most anaesthetic machines and breathing systems are contaminated to a minor degree with microorganisms. Equipment that is in direct contact with the patient (breathing circuits and masks) becomes more heavily contaminated.

All anaesthetic equipment that comes into contact with a patient's body fluids (including saliva) must be changed, cleaned and thermally disinfected before use on another patient.

This includes equipment that has come into indirect contact with the anaesthetist's hands, which may be contaminated with blood,

saliva or other body fluid. Additionally, unused items introduced into the anaesthetic work-field should be regarded as dirty and reprocessed. Attachment 3 outlines recommended processing information for anaesthetic and respiratory equipment.

To protect staff from aerosols generated during manual cleaning processes, preference should be given to the use of washer/disinfectors for the washing, disinfecting, rinsing and drying of respiratory apparatus. Most units automatically process through pre-wash, disinfection, rinse and drying cycles.

Endotracheal tube

Anaesthetic equipment: cleaning methods

Thorough cleaning of all instruments and equipment is an essential prerequisite in disinfection and sterilization processes:

- All systems must be disassembled completely to allow unrestricted contact of all parts with the cleaning and disinfection process
- measuring instruments and pressure gauges must be processed separately according to the recommendations of the manufacturer
- lumens of non-disposable endotracheal tubes, airways, facemasks, laryngeal masks, anaesthetic breathing circuits and cobbs connectors are to be placed over the appropriate nozzle/water jet on the washer/disinfector to ensure proper

Cleaning and rinsing

- The manufacturer's instructions for cleaning and reprocessing of anaesthetic equipment should be followed
- Mechanical washer/disinfectors must not be overloaded

The cleaning of external surfaces of anaesthetic machines and associated equipment should occur on a regular basis; the use of detergent and water is not sufficient. The internal components do not require routine cleaning and disinfection.

Anaesthetic equipment: disinfection requirements

Anaesthetic respiratory equipment is classified as 'semi-critical' (refer to 'Spaulding's classification') and requires thermal disinfection for reprocessing. Sterilization of anaesthetic and ventilator equipment is generally unnecessary.

Water temperatures for thermal disinfection

Rinse water temperature shall be between 80°C and 86°C (>80°C). Refer to 'Water temperature for thermal disinfection'.

Monitoring of washer/disinfectors

The requirement for routine microbiological monitoring of washer/disinfectors is unwarranted, as there are no current standards to determine if the washer/disinfector is microbiologically safe. Washer/disinfectors and instruments should be visually inspected and cycle parameters monitored to determine if the machine is functioning correctly:

- perform visual inspection and documentation of time at temperature.

Instruments and equipment should be free from disinfectant and rinse additive residues

- presence of chemical residue (if the washing machine is functioning as designed with temperature, detergent, wash and rinse pressure all at the correct levels, there will be virtually no chemical residue left on instruments)

Standardized test devices are available for testing the effectiveness of wash processes.

These tests are based on a visual indication of soil removal effectiveness.

Perform regular thermocouple testing of disinfection temperatures:

- rinsing 40°C to 50°C;
- washing 50°C to 60°C;
- disinfecting 80°C to 95°C, for up to 10 minutes

Drying

Drying reduces the risk of contamination during inspection and assembly of instruments:

- drying cabinets should be used for drying anaesthetic equipment. Drying cabinet operating temperatures shall be within the range 65°C to 75°C
- On completion of the cycle, the items shall be removed and placed in the anaesthetic apparatus drying machine (if drying cycle not installed). Tubing and other items with lumens shall be placed over appropriate connectors to ensure hot air dries all surfaces. Drying facilities may be available within the washer/disinfector

Single use items

- use single use sachets of lubricant for insertion into the patient's airway
- avoid using multi-dose vials. Such vials should always be accessed with a clean needle and syringe and dedicated for single patient use only

Soaking or 'cold sterilization'

Soaking or 'cold sterilization' (immersion of the different items in solutions containing disinfectants eg. Aldehydes) has a relatively high failure rate due to dosing errors, insufficient contact time (air trapping) as well as disadvantages of toxicity, skin irritation and allergy, and environmental concerns.

So, soaking/ cold sterilization should be done with regulatory body approved biocides with recommended contact time.

Management of patients with confirmed or suspected pulmonary tuberculosis

Ideally, elective operative procedures on patients who have pulmonary tuberculosis should be delayed until the patient is no longer infectious. However, if operative procedures must be performed, they should be done, if possible in operating rooms that have anterooms and staff must observe airborne precautions (refer to 'additional precautions').

For operating rooms with anterooms, the doors to the operating room should be closed, and traffic into and out of the room should be minimal to reduce the frequency with which the door opens and closes.

- A bacterial filter should be placed as close as possible to the patient to help reduce the risk of contamination of the anaesthetic equipment (ventilator and CO_2 absorbers) and prevent the discharge of tubercle bacilli into the ambient air
- Preference may be given to a disposable anaesthetic breathing circuit with appropriate filters.

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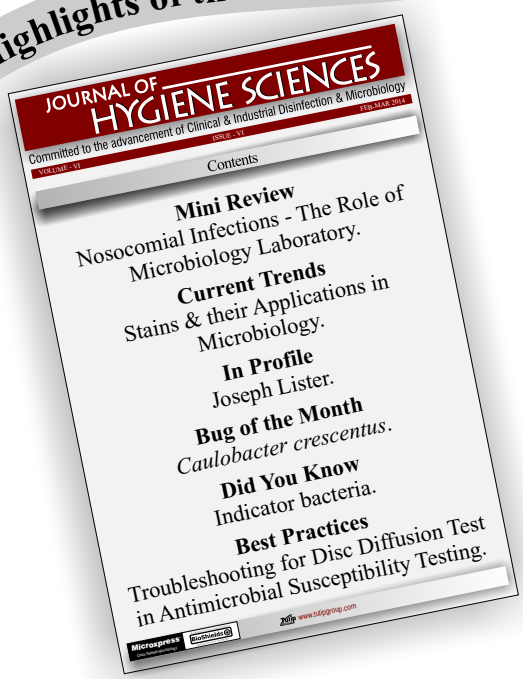
Highlights of the coming issue



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