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Editorial

Contents

- Editorial 1
- Mini review 2
- Current Trends 5
- In Profile 8
- Relaxed Mood 9
- Bug of the Month 10
- Did you Know 12
- Best Practices 13
- In Focus 15

Mini review section – Humans around the world have been consuming fermented, pickled foods and beverages for years. Fermentation was an extremely important technique in preserving a wide range of foods, allowing them to be transported and stored. Fermented foods are food substrates that are invaded or overgrown by edible microorganisms whose enzymes, particularly amylases, proteases, lipases hydrolyze the polysaccharides, proteins and lipids to nontoxic products with flavors, aromas and textures pleasant and attractive to the human consumer.

Current Trends section – Chlorine has been used as a disinfectant for the treatment of drinking water for more than 100 years. It is by far the most commonly used means of disinfecting water, and its effectiveness as a microbicide has been widely assessed. While most conventional systems in developed countries treat water with chlorine gas, other common alternatives include calcium hypochlorite, sodium hypochlorite, lithium hypochlorite and chloroisocyanurates (sodium dichloroisocyanurate or trichloroisocyanuric acid). Until recently, the isocyanurates were used chiefly in the disinfection of water for swimming pools and industrial cooling towers. They are also a common microbial agent in cleaning and sanitizing applications, including baby bottles and contact lens.

In Profile Scientist – Asima Chatterjee was an Indian chemist noted for her work in the fields of organic chemistry and phytomedicine. Her most notable work includes research on vinca alkaloids, and the development of anti-epileptic and anti-malarial drugs. She also authored a considerable volume of work on medicinal plants of the Indian subcontinent.

Bug of the month – *Burkholderia pseudomallei* is a Gram-negative, bipolar, aerobic, motile rod-shaped bacterium. It is a soil-dwelling bacterium endemic in tropical and subtropical regions worldwide, particularly in Thailand and northern Australia. It infects humans and other animals and causes the disease melioidosis.

Did You Know? – Bacterial infections that don't respond to treatment are a leading cause of death around the world. In 2019, antimicrobial resistance caused an estimated 1.27 million deaths. More people died from untreatable bacterial infections that year than from HIV or malaria.

Best Practices – Methods aimed at prevention of infection in the operating room have varying levels of data to substantiate their practice, in some cases vetted by strong randomized, controlled trials showing clear benefit, whereas in others propagated through lore or common sense. Either way, awareness of the important implications of SSI for patient health and costs of care is paramount for any surgeon, and surveilling one's own practices in the operating room with respect to the existing literature is an important step in controlling infection and maximizing beneficial outcomes.

Tickle yourself enjoying the jokes in our **Relax Mood** section.

Our JHS team is thankful to all our readers for their ever increasing appreciation that has served as a reward & motivation for us. Looking forward for your continuous support.

FOOD AND FERMENTATION

Humans around the world have been consuming fermented, pickled foods and beverages for hundreds, and in some cases thousands, of years. Fermentation was an extremely important technique in preserving a wide range of foods, allowing them to be transported and stored. This helped to ensure the survival of diverse human cultures in times when cultivation of fresh foods was insufficient.

Fermented foods are food substrates that are invaded or overgrown by edible microorganisms whose enzymes, particularly amylases, proteases, lipases hydrolyze the polysaccharides, proteins and lipids to nontoxic products with flavors, aromas and textures pleasant and attractive to the human consumer. If the products of enzyme activities have unpleasant odors or undesirable, unattractive flavors or the products are toxic or disease producing, then these food are termed as spoiled food.

The French chemist Louis Pasteur founded zymology, when in 1856 he connected yeast to fermentation. Zymology is an applied science that studies the biochemical process of fermentation and its practical uses. Common topics include the selection of fermenting yeast and bacteria species and their use in brewing, wine making, fermenting milk, and the making of other fermented foods.

Microorganism in fermentation

Fermentation is generally defined as the production of foods, beverages, or other useful metabolites by aerobic or anaerobic microorganisms via enzymatic conversions of substrates and controlled microbial growth.

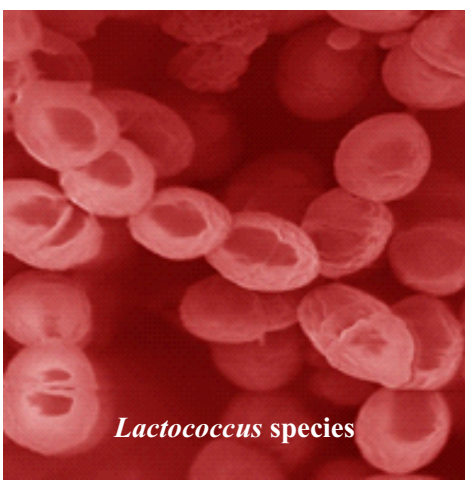
Fermented foods are foods and beverages that have undergone controlled microbial growth and fermentation which gives them their unique and desirable taste, aroma, texture and appearance. Fermentation operates via the natural activities of microorganism metabolism, which ensures the growth and reproduction of microorganisms it is one of the basic characteristics of natural microbial activities.

Bacteria

Bacteria such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, are known as lactic acid bacteria, or LAB. They use lactose, the sugar commonly found in milk, to complete their fermentation duties and produce lactic acid. Another type of bacteria, Acetobacter spp., can produce acetic acid from alcohol. Acetic acid more commonly as vinegar. These acids are sour which provide the characteristic tastes in items such as yogurt, cheese, vinegar, and sauerkraut.

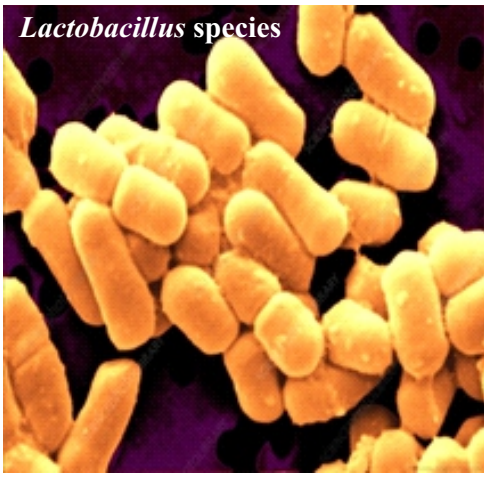
Lactic acid bacteria (LAB) are common in the dairy industry. Lactic acid bacteria produce lactic acid as the principal by-product of sugar fermentation. They are gram-positive and rod or cocci shaped. These bacteria are more tolerant of low pH than other bacteria associated with the dairy industry. They are commonly used in starter cultures and dairy fermentations. Lactic acid bacteria cause milk to be sour. Moreover, these bacteria are classified as homofermentative and heterofermentative bacteria based on their by-product in sugar fermentation.

Homofermentative Bacteria



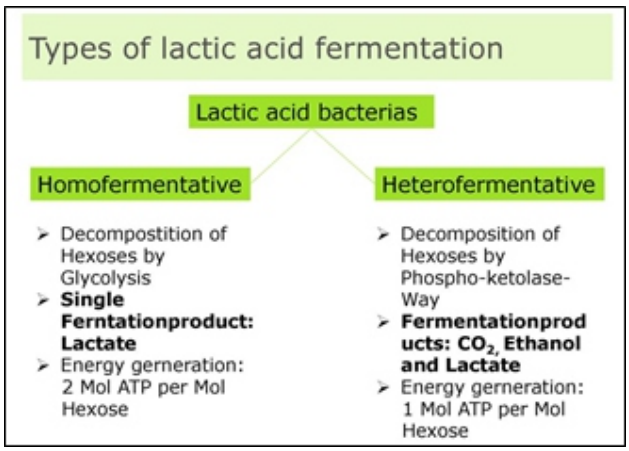
Homofermentative bacteria are a type of lactic acid bacteria that produce only lactic acid as a primary by-product in glucose fermentation. In biochemistry, homofermentative bacteria convert glucose molecules into two lactic acid molecules. They use this reaction to make two ATP molecules through substrate-level phosphorylation. Homofermentative bacteria include *Lactococcus* species, which is used in dairy starter cultures to rapidly-produce lactic acid in reduced pH conditions. In the dairy industry, *Lactococcus* species can be used in single-strain starter cultures or in mixed-strain cultures with other lactic acid bacteria *Lactococcus* species are commonly used in the manufacture of fermented dairy products such as cheeses. Anyhow, the homofermentative state of *Lactococcus* can be adjusted by altering the environmental conditions such as pH, glucose concentration and nutrient limitation. Thermophilic strains of *Lactobacillus helveticus* are also used in cheese production. The homofermentative bacteria used in the yogurt industry includes strains of *Lactobacillus delbruckii, Lactobacillus acidophilus* and *Streptococcus salivarius*. Furthermore, *Sterptococcus* spp., *Enterococcus, Pediococcus*, and *Aerococcus* are other homofermentative bacteria used in the milk industry, but they are rarely used as starter cultures.

Heterofermentative Bacteria



Heterofermentative bacteria are a type of lactic acid bacteria that produce ethanol/acetic acid and CO_2 in addition to lactic acid as by-products in glucose fermentation. In heterofermentative bacteria, other than the lactic acid as the principal end product, a significant amount of one or more metabolites (ethanol/acetic acid, CO_2) are also produced in the glucose fermentation. In biochemistry, heterofermentative bacteria produce one lactic acid and one ATP, together with CO_2 in glucose fermentation. But they also may produce several other end products such as ethanol, acetic acid, propionic acid, acetaldehyde, or diacetyl

Testing for heterofermentative bacteria involves the detection of CO_2 gas. Though they are uncommon in the milk and dairy industry, they are rarely used as starter cultures in the dairy industry. Sometimes, if they allow growth in significant numbers, heterofermentative bacteria can cause defects such as slits in hard cheeses and bloated packaging in other dairy products. Heterofermentative bacteria include *Leuconostoc* spp., *Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus reuteri, Lactobacillus plantarium, Lactobacillus casei*, and *Lactobacillus curvatus*.



Acetic acid bacteria (AAB) are mainly associated with the biotechnological process of vinegar and cellulose production. AAB are not studied to the same extent as many other food-grade and industrially important microorganisms AAB are predominantly known for their use in the production of vinegar, vitamin C, and cellulose

AAB are commonly found on plants, flowers, and fruits. These aerobic environments are rich in carbohydrates, sugar alcohols, and/or ethanol. This enables AAB to rapidly and incompletely oxidize these substrates into organic acids for energy production through a specific respiratory chain. Consequently, an acidification of the environment takes place, thereby preventing the growth of competitors, while the producing cells possess several mechanisms to tolerate the acidity. Also, they can utilize the accumulated organic acids later to further sustain their growth. AAB cells capable of cellulose production form biofilms that allow their retention on the culture surface, which is favourable for the survival of these strictly aerobic bacteria. All these physiological features explain their occurrence and underlines their functional role in the production of diverse fermented foods and beverages such as lambic beer, water kefir, kombucha, and cocoa.

Natural food fermentation processes with acetic acid bacteria. Examples of spontaneous food and beverage fermentation processes in which acetic acid bacteria participate. From left to right: the end of a cocoa bean heap fermentation; fermenting lambic beer in oak casks; water kefir fermentation in a closed jar with the water kefir grains visible as a sediment; and kombucha fermentation in a vessel with the tea fungus visible as a floating cellulose layer.

Fungi

Yeasts are predominant in several fermented foods prepared from ingredients of plant as well as animal origin. The diversity of foods in which, yeasts predominate ranges from alcoholic beverages such as wines (e.g., fruit, palm and rice wines), cereal based leavened products (e.g., sourdough and idli), milk products (e.g., cheese and dahi) and condiments such as soy sauce.

Yeast Fermentation Processes

Alcoholic Fermentations

The production of alcoholic beverages from fermentable carbon sources by yeast is the oldest and most economically important of all biotechnologies. Yeast plays a vital role in the production of all alcoholic beverages.

Wine fermentation: The process of fermentation in winemaking turns grape juice into an alcoholic beverage. During fermentation, yeasts transform sugars present in the juice into ethanol and carbon dioxide

Beer fermentation: Is traditionally made from four key ingredients: malted cereals (barley or other), water, hops, and yeast. Each of these ingredients contributes to the final taste and aroma of beer. During fermentation, yeast cells convert cereal-derived sugars into ethanol and CO_2. *S. cerevisiae* as the top-fermenting yeast to make ales while *S. pastorianus* is a bottom-fermenting yeast used in lager brewing processes.

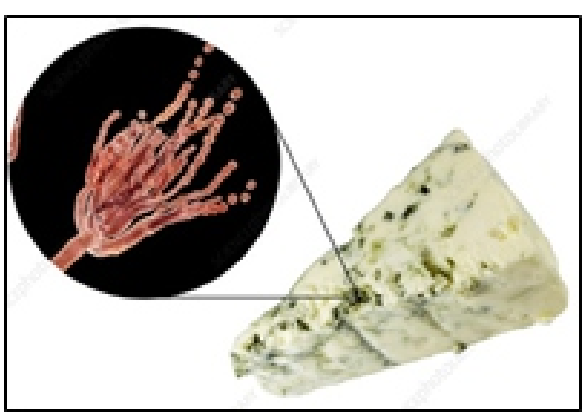
Cider Fermentation: Cider is another alcoholic beverage derived from the apple fruit industry, although traditional ciders are produced from spontaneous fermentation of juice carried out by autochthonous yeasts, selected *S. cerevisiae* strains are also commonly used to carry out alcoholic fermentation.

Non-Alcoholic Fermentations

Yeast can act in the fermentation of global non-alcoholic products like bread, chocolate or coffee, beverages such as kefir, sodas, lemonades, and vinegar or even biofuels and other chemicals.

Moulds

Moulds are aerobic microorganisms and therefore they cannot carry out fermentation. They produce extracellular enzymes and enzymes hydrolyze large organic compounds (such as polysaccharides, proteins, and fats) to smaller units (such as glucose, amino acids, and fatty acids).



Cheese making: Three main types of cheese rely on moulds for their characteristic properties: blue cheese, soft ripened cheese and rind-washed cheese.

Meat fermentation: A wide variety of moulds (i.e. *Penicillium chrysogenum* and *Penicillium nalgiovense*) are used to ripen surfaces of sausages.

Soy sauce Traditional soy sauce is made by mixing soybeans and other grains with a mould – either *Aspergillus oryzae* or *Aspergillus sojae* – and yeast.

Media used in brewery and fermentation

- Cooke Rose Bengal Agar Base
- Glucose Broth
- Lactobacillus MRS Agar
- MacConkey Agar Base
- MacConkey Agar with Crystal Violet, NaCl and 0.15% Bile Salts
- MacConkey Agar with Crystal Violet, NaCl and 0.15% Bile Salts
- MacConkey Broth Double Strength with Neutral Red
- MacConkey Broth Double Strength with Neutral Red IP
- MacConkey Broth Purple with BCP
- MacConkey Broth Purple with BCP (Medium 7) IP
- R-2A Agar
- R-2A Broth
- Rogosa S L Agar
- Rose Bengal Chloramphenicol Agar
- Soyabean Casein Digest Agar (Casein Soyabean Digest Agar) IP (Medium 2)
- Casein Digest Agar Plate (Triple Layer Pack, Gamma-Irradiated)
- Soyabean Casein Digest Agar (Tryptone Soya Agar)
- Soyabean Casein Digest Medium (Harmonized)

SODIUM DICHLOROISOCYANURATE (NaDCC)

Chlorine has been used as a disinfectant for the treatment of drinking water for more than 100 years. It is by far the most commonly used means of disinfecting water, and its effectiveness as a microbicide has been widely assessed (AWWA, 2000). While most conventional systems in developed countries treat water with chlorine gas (delivered as a liquid in pressurized systems), other common alternatives include calcium hypochlorite, sodium hypochlorite, lithium hypochlorite and chloroisocyanurates (sodium dichloroisocyanurate or trichloroisocyanuric acid). Until recently, the isocyanurates were used chiefly in the disinfection of water for swimming pools and industrial cooling towers. They are also a common microbial agent in cleaning and sanitizing applications, including baby bottles and contact lens (Dychdala, 2001).

All of these compounds disinfect water by releasing free available chlorine (FAC) in the form of hypochlorous acid (HOCl). For example,

$$NaOCl + H_2O \longrightarrow HOCl + NaOH$$

(Sodium hypochlorite dispersion in water)

$$NaCl_2(NCO)_3 + 2H_2O \longleftrightarrow 2HOCl + NaH_2(NCO)_3$$

(NaDCC disoolution in water)

FAC (chlorine in the +1 oxidation state) is an effective biocide against a wide range of bacteria, fungi, algae, and viruses (White, 1998). Regardless of the original source of the available chlorine, the active microbicidal agent is hypochlorous acid. This also means that the most common method used in the field to assess the safety of drinking water—measuring FAC using the DPD reagent—is equally applicable with respect to water treated with NaDCC.

While both NaOCl and NaDCC rely on HOCl as the active agent, there are important differences in the performance of the two compounds. Unlike NaOCl which releases all of its chlorine as FAC, NaDCC releases only approximately 50% of the chlorine as FAC, the balance remaining as "reservoir chlorine" (bound) in the form of chlorinated isocyanurates (Bloomfield and Miles, 1979). When the FAC is used up, the equilibrium is disturbed, immediately releasing further FAC from the "reservoir" until the total available is used up. Thus, as shown in Figs. 1 and 2, the stabilized chlorine in NaDCC acts as a reservoir of HOCl which is rapidly released when the free available chlorine is depleted (Kuechler 1997, 1999).

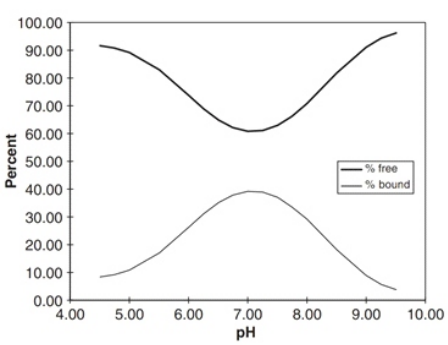


Fig. 1. Free and bound available chlorine in a solution of 1 mg/l NaDCC

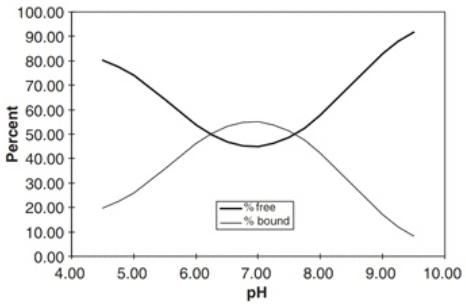


Fig. 2. Free and bound available chlorine in a solution of 3 mg/l NaDCC

This "reservoir" of FAC also enhances the biocidal protection over NaOCl when water is subject to high or variable organic loads (Bloomfield and Uso, 1985). Such conditions are common in some remote settings, forcing the use of more costly point-of-use water treatments (Crump et al., 2004).

NaDCC also presents certain advantages over NaOCl in those settings where the pH is high or variable. Hypochlorous acid is a weak acid, which tends to dissociate in water at increasing pH:

$$HOCl \longleftrightarrow H^+ + OCl^-$$

It is well known that chlorine loses its effectiveness to disinfect water at higher levels of pH, due to the dissociation of HOCl (Hurst, 2001). While 78% of chlorine exists in the active HOCl at neutral pH 7, at pH 8 the level drops to 26%. The capacity of NaDCC to continue to release significant amounts of HOCl allows it to operate over a wider pH range (Dychdala, 2001). Moreover, insofar as NaDCC tablets are acidic in solution, (the effervescent base contributes to their acidity), they tend to reduce the pH of water favouring the formation of undissociated HOCl; hypochlorites, being alkaline, tend to disadvantageously increase the pH and, therefore, the dissociation of HOCl (Macedo and Barra, 2002). This is another parameter that is difficult to control or adjust for in household treatment in the field.

Even in a tightly closed opaque bottle, NaOCl has a recommended life of only 6 months after opening. Decomposition produces undesirable by-products (chlorite or chlorate ions). Internal testing under industry standards has shown that tabulated and strippackaged NaDCC, on the other hand, has a shelf life of 5 years in temperate and tropical climates. The stability and retention of chlorine activity has been cited as an advantage of NaDCC not only over NaOCl but also over other donors of free chlorine (Macedo and Barra, 2002).

Finally, the different presentation of the chlorine sources makes effervescent (self-dissolving) NaDCC tablets considerably more convenient to use than NaOCl. Bleach, though less hazardous than elemental chlorine, is a corrosive liquid subject to spillage. For water treatment, users typically measure out the recommended dose using the bottle cap. NaDCC, on the other hand, is delivered as a solid tablet specifically sized to treat a given volume of water, typically 10 or 20 l in household applications. While liquid NaOCl (bleach) contains

approximately 5% available chlorine, anhydrous NaDCC contains about 62%, roughly the equivalent of calcium hypochlorite. A single 67 mg NaDCC tablet, for example, can treat 20 liter of clear water at a FAC dosage of 2 mg/l. (Two 67 mg NaDCC tablets are recommended for turbid waters, at a dosage of 4 mg/l FAC.) The potential for mis-dosing is minimized with the use of tablets, whereas the use of a bottle cap can lead to over or underdosing. Excess dosing would lead to an unpalatable level of residual chlorine and higher concentrations of potentially toxic chlorinated aromatic compounds (Crump et al., 2004). Investigators have found NaDCC to be advantageous to NaOCl in the production of trihalomethanes (Macedo, 1997).

Toxicity and Regulatory Issue

All chlorine products have some level of toxicity; this is what renders them such effective microbicides. When chlorinated water is ingested, however, the available chlorine is rapidly reduced by saliva and stomach fluid to harmless chloride ions salts (Kotiaho et al., 1992). This is true for all sources of chlorine, including both NaOCl and NaDCC. The unique characteristic of the isocyanurates is cyanuric acid, the carrier that allows the chlorine to be contained in a solid, stable and dry form. It is the potential toxicity of such cyanuric acid, therefore, that required review by regulatory agencies prior to the approval of NaDCC for the routine treatment of drinking water.

Cyanuric acid (H3C3N3O3), while confusingly similar in name, is not chemically related to cyanide. The toxicity of NaDCC and cyanuric acid have been extensively studied and documented in support of the registration of isocyanurates with the US EPA. These have been summarized (Hammond et al. 1986; US Environmental Protection Agency (US EPA), 1992). Studies performed on acute toxicity and irritancy were intended to assess the safety of handling the dry product. These studies found chlorinated isocyanurates no more than slightly toxic and not corrosive. Chronic and sub-chronic toxicity studies also found no toxicity. Developmental toxicity studies have also established that the compound is not fetotoxic, teratogenic (causing birth defects), mutagenic or carcinogenic. Chlorinated isocyanurates are not metabolized in the body and do not bioaccumulate.

Under the US Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), the manufacturer or distributor of disinfectants sold in the United States must be registered with the US EPA in a process required to demonstrate their safety and effectiveness. In July, 2001, OxyChem Corporation, the largest producer, secured such a registration for certain of its brands of isocyanurates for the routine treatment of drinking water. The US EPA approved label claims for NaDCC can be found at http://oaspub.epa.gov/pestlabl/ppls.home (registration number 935-41). NaDCC (up to 30mg/l) is also certified by NSF International under NSF/ANSI Standard 60 (Drinking Water Treatment Chemicals-Health Effects), which extends to the health impact of water treatment additives (http://www.nsf.org).

In 2002, the WHO requested a review of the use of NaDCC as a disinfectant for drinking water as part of the rolling revisions of its Guidelines for Drinking Water Quality. The review was conducted by the Joint Food and Agriculture Organization/WHO Expert Committee on Food Additives (JECFA) and, like the EPA review, required the submission of detailed toxicological data. In June, 2003, JECFA recommended that the tolerable daily intake (TDI) for anhydrous NaDCC from treated drinking water be set at 0–2.0 mg per kg of body weight per day (WHO, 2004). Using standard methods (WHO, 1993) guideline values (GVs) for NaDCC can be derived from the TDI. This translates into a GV for adults (60 kg, with a daily drinking water consumption of 2 l) of 60 mg/l NaDCC; a GV for children (10 kg, with a daily consumption of 1 l) of 20 mg/l NaDCC; and a GV for infants (5 kg, with a daily consumption of 0.75 l) of 13 mg/l. The dosage rate for Aquatabs, for example, is between 3.5 and 7 mg/l NaDCC (2–4 mg/l FAC), well within the JECFA value for daily intake (TDI).

Microbiocidal Effectiveness

As noted above, NaDCC is an alternative source of FAC (HOCl). Accordingly, the significant body of evidence on the antimicrobial action of chlorine is as relevant to NaDCC as it is to NaOCl and other sources of chlorine (White, 1998; Dychdala, 2001; CDC, 2005). While certain bacterial spores have shown greater resistance to NaDCC (Bloomfield and Arthur, 1992), thus at least suggesting the potential for differences in activity based on the chlorine donor, no differences have been reported in respect to waterborne pathogens. Susceptibility to hypochlorous acid has been established with respect to a wide variety of bacteria, including *Escherichia coli, Salmonella dysenteriae, Shigella sonnei, Campylobacter jejuni, Yersinia enterocolitica*; viruses, including hepatitis A, poliovirus (type 1), rotavirus, adenovirus and calicivirus; helminthes; and protozoa, including cysts of Entamoeba histolytica and Giardia lamblia (Dychdala, 2001).

Microbicidal activity is a function of chlorine concentration and contact time (White, 1998; Bloomfield, 1996). At doses of a few mg/l and contact time of about 30 min, free chlorine inactivates more than 4 logs of most waterborne pathogens. Cryptosporidium has demonstrated considerable resistance to chlorination (Korich et al., 1990; Venczel et al., 1997) and Mycobacterium has also been reported as resistant (Taylor et al., 2000; Le Dantec et al., 2002). It should also be noted that in some cases, certain viruses have also exhibited greater resistance to chlorine and chlorine compounds than common bacterial indicators of faecal contamination (Hurst, 2001). This may have implications for determining the required concentration and contact time required to kill or deactivate potential pathogens in the untreated water collected for use in emergency and development settings.

A number of studies have compared the biocidal effectiveness of NaDCC with NaOCl and other disinfectants against a variety of microbes. D'Auria et al. (1989) assessed the antimicrobial activity of NaDCC among 29 Gram-positive and 29 Gram-negative bacteria, as well as 66 fungi. They reported good activity and, significantly, no adverse influence by temperature and pH. Nascimento et al. (2003) found that at concentrations of 200 ppm, NaDCC yielded superior results compared to NaOCl and certain other agents used to sanitize fresh vegetables against aerobic mesophiles, molds and yeasts, total coliforms, *E. coli* and *Salmonella* sp. In another study at concentrations of 100 ppm, NaDCC was more effective than NaOCl against *Vibrio cholerae* (Eiroa and Porto, 1995). NaDCC has also been reported effective against encysted forms of *Acanthamoeba castellanii* (Khunkitti et al., 1996). Mazzola et al. (2003) compared the efficacy of NaDCC/sodium salt tablets with various chemical disinfectants, including a 10% solution of NaOCl on a variety of bacteria relevant to hospital settings. They recommended NaDCC over

NaOCl for certain hospital applications due to its biocidal effectiveness, its slow decomposition and liberation of HOCl, its capacity to maintain an appropriate level of available chlorine without affecting the pH of the water, its low level of toxicity and its lower corrosivity against metal, plastic and rubber.

While NaDCC was shown to be comparable or superior to NaOCl in these studies of non-water treatment applications, we found few studies that compared the microbiological performance of NaDCC with other agents in respect of the treatment of drinking water. In one study, Aquatabs™ tablets containing 3.5 mg of NaDCC in an effervescent base were compared to Drinkwell™ (25 mg/ml NaOCl), and Hydroclonazon® (12.2 mg chloramine) and a generic solution of 2% iodine in ethanol. Except for the Hydroclonazon®, the agents performed comparably in removing all coliforms and *E. coli* from low turbidity water (NTU < 1) and 1.8–2.8 logs of viable bacteria from raw river water (NTU > 10) (Schlosser et al., 2001). The unimpressive results on more turbid water demonstrate a general weakness of chemical disinfectants. Notably, however, the required contact time for the NaDCC and iodine was 30 min compared to 60 min for the hypochlorite and chloramine based agents. In a further study, NaDCC tablets were recommended over chloramine tablets for use by the military owing to superior microbiological performance under a variety of polluted water conditions and lack of toxicity (Baylac et al., 1996).

Owing to its widespread use by defense forces, water, and sanitation departments and ministries of health in developing countries, the microbiological effectiveness of NaDCC tablets has been assessed by governmental investigators in Brazil, El Salvador, France, Honduras, Portugal, South Africa, Tanzania, Vietnam, and Zimbabwe. However, only one study has assessed the microbiological performance of the disinfectant in the field in the context of a household-based water treatment intervention (Afroz Molla, 2005). In that study, which involved a pilot program in Dhaka, 84% of samples from households using NaDCC tablets to treat their water were free of fecal coliform (FC) and the maximum level was 23 FC/100 ml, compared to 1000–2400 FC/100 ml in pre-intervention source water.

BioShields offers NaDCC in the brand name of Puresafe with 2 g and 20 g sachets. We are the first in healthcare industries, who launched NaDCC in powder form.

Applications of Puresafe are:
1. Water purification
2. Surface Disinfection
3. Dairy / Vegetables / Fruit Washing / Food Processing
4. Hospital Biowaste
5. Swimming pool usage
6. Poultry farm usage, etc.

Puresafe Dilution Chart for Healthcare Settings

S. No.	Application Area	Req. Puresafe	Req. PPM	Req. %	Total Solution
1.	General Hospital Area / Non-Critical Area	20 g	1000 ppm	0.1%	5 Liter
2.	Critical Area	50 g	2500 ppm	0.25%	5 Liter
3.	Blood Spill / Body Fluid Spill	100 g	5000 ppm	0.5%	5 Liter
4.	Infected / Soiled Linen	10 g	160 ppm	0.016%	15 Liter

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BioShields Data.

Asima CHATTERJEE

Fields: Chemistry, Phytomedicine

Born: 1917 in Kolkata, Bengal (India)

Death: 2006 in Kolkata, West Bengal (India)

Main achievements: Research on vinca alkaloids, and the development of anti-epileptic and anti-malarial drugs.

Asima Chatterjee was an Indian chemist noted for her work in the fields of organic chemistry and phytomedicine. Her most notable work includes research on vinca alkaloids, and the development of anti-epileptic and anti-malarial drugs. She also authored a considerable volume of work on medicinal plants of the Indian subcontinent.

Asima Chatterjee was born on 23 September 1917 in Bengal. An excellent student, Chatterjee grew up in Calcutta, attending school and subsequently enrolling at the Scottish Church College, of the University of Calcutta, graduating with honours in chemistry in 1936.

Asima Chatterjee graduated in 1938 with a master's degree in organic chemistry from the University of Calcutta, completing a doctoral degree there in 1944. Her doctoral research focused on the chemistry of plant products and synthetic organic chemistry. Among her notable instructors at the time were Prafulla Chandra Roy and Prof S.N. Bose. Additionally she also had research experience from the University of Wisconsin, Madison and the Caltech. Chaterjee's research centered around natural products chemistry and resulted in anti-convulsive, anti-malarial and chemotherapy drugs.

She joined the Lady Brabourne College, of the University of Calcutta in 1940 as the founding head of the department of chemistry. In 1944, Chatterjee became the second woman to be conferred a Doctorate of Science by an Indian University. In 1954, Asima Chatterjee joined the University College of Science of the University of Calcutta, as reader in pure chemistry. In 1962, Chatterjee was appointed the prestigious Khaira professorship of Chemistry at the University of Calcutta, a position she held till 1982.

Awards and recognition: She was a Premchand Roychand Scholar of the University of Calcutta. She was the second woman after Janaki Ammal to be conferred Doctorate of Science by an Indian University, the University of Calcutta in 1944. From 1962 to 1982, she was the Khaira Professor of Chemistry, one of the most prestigious and coveted chairs of the University of Calcutta. In 1972, she was appointed as the Honorary Coordinator of the Special Assistance Programme to intensify teaching and research in natural product chemistry, sanctioned by the University Grants Commission (India). In 1960, she was elected a Fellow of the Indian National Science Academy, New Delhi. In 1961, she received the Shanti Swarup Bhatnagar Award in chemical science, in the process becoming the first female recipient of this award. In 1975, she was conferred the prestigious Padma Bhushan and became the first lady scientist to be elected as the General President of the Indian Science Congress Association. She was conferred the D Sc (Honoris causa) degree by a number of universities. She was nominated by the President of India as a Member of the Rajya Sabha from February 1982 to May 1990.

Jokes

"Women won't play football not coz they aren't gud at it..
But coz it's against their ego to b dressed up exactly like 10 other women infront of 10,000 people."

Saw It With My Eyes But Couldn't Understand It
Took It In My Hands, But Couldn't Understand It
Keep Thinking For A Long Time, But Again Couldn't Understand It
It was Not A Dream,
It was Is Not A Love,
It was Not Even Friendship,
Then I Realized: "It Was Question Paper"

Santa giving exam while standing at the door.
A man asked "Why are you standing at the door?"
Santa: "Idiot, I am giving entrance test."

Womens are like Fruits.
Every Woman has her own unique taste and colour…
But
The problem is the Men.
They seem to love Fruit salad..!!

Teacher: Why are you late?
santa: Because of the sign.
Teacher: What sign?
santa: The one that says,
"School Ahead, Go Slow."

New Teacher:
anybody who thinks he is stupid, stand up
pappu stoodup
Teacher: R U stupid?
Pappu: "nhi, Aap akeli khadi the mujhe acha nhi lag raha tha"
Foreigner: In India where does the,
Ice fall more?
Smart answer by Santa…..
Santa: Before 8 p.m. in Kashmir,
& after 8 p.m. in glass of whiskey…

Husband: Can you be the moon of my life?
Wife: Wow! Yes sweet heart…!
Husband: Great! then….
Stay 9,955,887.6kms away from me…!!

Wife: Tell me such a thing,
that I can become happy as well as sad…
Husband: You are my life,
& shame on such kind of life.

A police asked to a thief,
" Why you went to stole same rack 3 times in a store? "
The thief replied,
" Sir, I stole one dress for my wife,
& I came to change it twice.

Burkholderia pseudomallei

Domain: Bacteria
Phylum: Pseudomonadota
Class: Betaproteobacteria
Order: Burkholderiales
Family: Burkholderiaceae
Genus: *Burkholderia*
Species: *B. pseudomallei*

Burkholderia pseudomallei is a Gram-negative, bipolar, aerobic, motile rod-shaped bacterium. It is a soil-dwelling bacterium endemic in tropical and subtropical regions worldwide, particularly in Thailand and northern Australia. It infects humans and other animals and causes the disease melioidosis.

Burkholderia pseudomallei measures 2–5 μm in length and 0.4–0.8 μm in diameter and is capable of self-propulsion using flagella. The bacteria can grow in a number of artificial nutrient environments, especially betaine- and arginine-containing ones.

In vitro, optimal proliferation temperature is reported around 40°C in neutral or slightly acidic environments (pH 6.8–7.0). The majority of strains are capable of oxidation, not fermentation, of sugars without gas formation (most importantly, glucose and galactose; older cultures are reported to also metabolize maltose and starch). Bacteria produce both exo- and endotoxins. The role of the toxins identified in the process of melioidosis symptom development has not been fully elucidated.

Identification

Burkholderia pseudomallei is not fastidious and grows on a large variety of culture media (blood agar, MacConkey agar, EMB, etc.). Ashdown's medium (or *Burkholderia cepacia* medium) may be used for selective isolation. Cultures typically become positive in 24 to 48 hours (this rapid growth rate differentiates the organism from *B. mallei*, which typically takes a minimum of 72 hours to grow). Colonies are wrinkled, have a metallic appearance, and possess an earthy odour. On Gram staining, the organism is a Gram-negative rod with a characteristic "safety pin" appearance (bipolar staining). On sensitivity testing, the organism appears highly resistant (it is innately resistant to many antibiotics including colistin and gentamicin) and that again differentiates it from *B. mallei*, which is in contrast, exquisitely sensitive to many antibiotics. For environmental specimens only, differentiation from the nonpathogenic *B. thailandensis* using an arabinose test is necessary (*B. thailandensis* is never isolated from clinical specimens). The laboratory identification of *B. pseudomallei* has been described in the literature.

The classic textbook description of *B. pseudomallei* in clinical samples is of an intracellular, bipolar-staining, Gram-negative rod, but this is of little value in identifying the organism from clinical samples. Some suggest the Wayson stain is useful for this purpose, but this has been shown not to be the case.

Laboratory identification of *B. pseudomallei* can be difficult, especially in Western countries where it is rarely seen. The large, wrinkled colonies look like environmental contaminants, so are often discarded as being of no clinical significance. Colony morphology is very variable and a single strain may display multiple colony types, so inexperienced laboratory staff may mistakenly believe the growth is not pure. The organism grows more slowly than other bacteria that may be present in clinical specimens, and in specimens from nonsterile sites, is easily overgrown. Nonsterile specimens should, therefore, be cultured in selective media (e.g., Ashdown's or *B. cepacia* medium). For heavily contaminated samples, such as faeces, a modified version of Ashdown's that includes norfloxacin, amoxicillin, and polymyxin B has been proposed. In blood culture, the BacT/ALERT MB system (normally used for culturing mycobacteria) by bioMérieux has been shown to have superior yields compared to conventional blood culture media.

Even when the isolate is recognised to be significant, commonly used identification systems may misidentify the organism as *Chromobacterium violaceum* or other nonfermenting, Gram-negative bacilli such as *Burkholderia cepacia* or *Pseudomonas aeruginosa*. Again, because the disease is rarely seen in Western countries, identification of *B. pseudomallei* in cultures may not actually trigger alarms in physicians unfamiliar with the disease.

The pattern of resistance to antimicrobials is distinctive, and helps to differentiate the organism from *P. aeruginosa*. The majority of *B. pseudomallei* isolates are intrinsically resistant to all aminoglycosides (via an efflux pump mechanism), but sensitive to co-amoxiclav: this pattern of resistance almost never occurs in *P. aeruginosa* and is helpful in identification. Unfortunately, the majority of strains in Sarawak, Borneo, are susceptible to aminoglycosides and macrolides, which means the conventional recommendations for isolation and identification do not apply there.

Molecular methods (PCR) of diagnosis are possible, but not routinely available for clinical diagnosis. Fluorescence in situ hybridisation has also been described, but has not been clinically validated, and it is not commercially available. In Thailand, a latex agglutination assay is widely used, while a rapid immunofluorescence technique is also available in a small number of centres.

Disinfection

Burkholderia pseudomallei is susceptible to numerous disinfectants, including benzalkonium chloride, iodine, mercuric chloride, potassium permanganate, 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde, and to a lesser extent, phenolic preparations. *B. pseudomallei* is effectively killed by the commercial disinfectants, Perasafe and Virkon. The microorganism can also be destroyed by heating to above 74 °C for 10 min or by ultraviolet irradiation.

Medical importance

Burkholderia pseudomallei infection in humans is called melioidosis; its mortality is 20 to 50% even with treatment.

Antibiotic treatment and sensitivity testing

The antibiotic of choice is ceftazidime. While various antibiotics are active *in vitro* (e.g. chloramphenicol, doxycycline, co-trimoxazole), they have been proven to be inferior in vivo for the treatment of acute melioidosis. Disc diffusion tests are unreliable when looking for co-trimoxazole resistance in *B. pseudomallei* (they greatly overestimate resistance) and Etests or agar dilution tests should be used in preference. The actions of co-trimoxazole and doxycycline are antagonistic, which suggests these two drugs ought not to be used together.

The organism is intrinsically resistant to gentamicin and colistin, and this fact is helpful in the identification of the organism. Kanamycin is used to kill *B. pseudomallei* in the laboratory, but the concentrations used are much higher than those achievable in humans.

Pathogenicity mechanisms and virulence factors

Burkholderia pseudomallei is an opportunistic pathogen. An environmental organism, it has no requirement to pass through an animal host to replicate. From the point of view of the bacterium, human infection is a developmental "dead end".

Strains which cause disease in humans differ from those causing disease in other animals, by possessing certain genomic islands. It may have the ability to cause disease in humans because of DNA it has acquired from other microorganisms. Its mutation rate is also high, and the organism continues to evolve even after infecting a host.

Burkholderia pseudomallei is able to invade cells (it is an intracellular pathogen). It is able to polymerise actin, and to spread from cell to cell, causing cell fusion and the formation of multinucleated giant cells. It possesses a uniquely fusogenic type VI secretion system that is required for cell-cell spread and virulence in mammalian hosts. The bacterium also expresses a toxin called lethal factor 1. *B. pseudomallei* is one of the first Proteobacteria to be identified as containing an active type VI secretion system. It is also the only organism identified that contains up to six different type VI secretion systems.

B. pseudomallei is intrinsically resistant to many antimicrobial agents by virtue of its efflux pump mechanism. This mediates resistance to aminoglycosides (AmrAB-OprA), tetracyclines, fluoroquinolones, and macrolides (BpeAB-OprB).

Vaccine candidates

No vaccine is currently available, but a number of vaccine candidates have been suggested. Aspartate-β-semialdehyde dehydrogenase (*asd*) gene deletion mutants are auxotrophic for diaminopimelate (DAP) in rich media and auxotrophic for DAP, lysine, methionine and threonine in minimal media. The *asd* bacterium (bacterium with the *asd* gene removed) protects against inhalational melioidosis in mice.

Antimicrobial resistance is a leading cause of death globally

Bacterial infections that don't respond to treatment are a leading cause of death around the world.

In 2019, antimicrobial resistance caused an estimated 1.27 million deaths, researchers report January 19 in the *Lancet*. More people died from untreatable bacterial infections that year than from HIV or malaria.

Overall, bacterial antimicrobial resistance played a role in an estimated 4.95 million deaths globally, including the 1.27 million directly caused by resistant infections, the study found. The estimates are based on an analysis of hospital, surveillance and other sources of data covering 204 countries and territories by an international group of researchers called the Antimicrobial Resistance Collaborators.

Resistance to two classes of antibiotics, beta-lactams (which include penicillin) and fluoroquinolones, was behind more than 70 percent of resistance-caused deaths. Those drugs are the first-line options for many bacterial infections (*SN: 4/30/14*).

Among the bacteria responsible for fatal drug-resistant infections, the top three were *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus*, the researchers found. These pathogens can cause dangerous infections in health care settings in people with weakened immune systems.

Worldwide, 64 deaths per 100,000 people were associated with treatment-resistant bacterial infections and 16.4 deaths per 100,000 people were caused by such infections, the group found. Notably, western sub-Saharan Africa had the highest mortality rates: 114.8 deaths per 100,000 people were associated with bacterial antimicrobial resistance and 27.3 deaths per 100,000 people were due to resistance.

Overall, the mortality rate from bacterial antimicrobial resistance was higher in places with fewer health care resources. This illustrates that solutions need to consider regional differences, the research group says. Limits on antibiotic use to deter resistance is key in many places. But in western sub-Saharan Africa, for example, increasing access to antibiotics may lessen the mortality burden from resistance, since second-line antibiotics needed after first-line drugs fail aren't readily available.

Minimize Risk Of Surgical Site Infection (part 2)

Chlorhexidine: Chlorhexidine gluconate, a cationic bisbiguanide, has been widely recognized as an effective, safe antiseptic for nearly 40 years. Chlorhexidine formulations are used extensively for surgical and hygienic hand disinfection; other applications include preoperative showers (for whole-body disinfection), antisepsis in obstetrics and gynaecology, management of burns, wound antisepsis and prevention and treatment of oral disease (plaque control, pre- and postoperative mouthwash, oral hygiene). When chlorhexidine is used orally, its bitter taste must be masked; it can also stain the teeth. Intravenous catheters coated with chlorhexidine and silver sulfadiazine are used to prevent catheter-associated bloodstream infections.

Chlorhexidine is most commonly formulated as a 4% aqueous solution in a detergent base; however, alcoholic preparations have been shown in numerous studies to have better antimicrobial activity than detergent-based formulations. Bactericidal concentrations destroy the bacterial cell membrane, causing cellular constituents to leak out of the cell and the cell contents to coagulate. The bactericidal activity of chlorhexidine gluconate against vegetative Gram-positive and Gram-negative bacteria is rapid. In addition, it has a persistent antimicrobial action that prevents regrowth of microorganisms for up to 6 hours. This effect is desirable when a sustained reduction in microbial flora reduces the risk for infection, such as during surgical procedures. Chlorhexidine has little activity against bacterial and fungal spores except at high temperatures. Mycobacteria are inhibited but are not killed by aqueous solutions. Yeasts and dermatophytes are usually susceptible, although the fungicidal action varies with the species. Chlorhexidine is effective against lipophilic viruses, such as HIV, influenza virus and herpes simplex virus types 1 and 2, but viruses like poliovirus, coxsackievirus and rotavirus are not inactivated. Blood and other organic material do not affect the antimicrobial activity of chlorhexidine significantly, in contrast to their effects on povidone-iodine. Organic and inorganic anions such as soaps are, however, incompatible with chlorhexidine, and its activity is reduced at extremely acidic or alkaline pH and in the presence of anionic- and nonionic-based moisturizers and detergents.

Microorganisms can contaminate chlorhexidine solutions, and resistant isolates have been identified. For example, Stickler and Thomas found chlorhexidine-resistant *Proteus mirabilis* after extensive use of chlorhexidine over a long period to prepare patients for bladder catheterization. Resistance of vegetative bacteria to chlorhexidine was thought to be limited to certain Gram-negative bacilli such as *P. aeruginosa, Burkholderia (Pseudomonas) cepacia, P. mirabilis* and *S. marcescens*, but genes conferring resistance to various organic cations, including chlorhexidine, have been identified in *S. aureus* clinical isolates.

There are several other limitations to the use of chlorhexidine. When it is absorbed onto cotton and other fabrics, it usually resists removal by washing. Long-term experience with use of chlorhexidine has shown that the incidence of hypersensitivity and skin irritation is low, but severe allergic reactions including anaphylaxis have been reported. Although cytotoxicity has been observed in exposed fibroblasts, no deleterious effects on wound healing have been found in vivo. While there is no evidence that chlorhexidine gluconate is toxic if it is absorbed through the skin, ototoxicity is a concern when chlorhexidine is instilled into the middle ear during operations. High concentrations of chlorhexidine and preparations containing other compounds, such as alcohols and surfactants, may also damage the eyes, and its use on such tissues is not recommended.

Iodophors: Iodophors have essentially replaced aqueous iodine and tincture as antiseptics. These are chemical complexes of iodine bound to a carrier such as polyvinylpyrrolidone (povidone) or ethoxylated nonionic detergents (poloxamers), which gradually release small amounts of free microbicidal iodine. The most commonly used iodophor is povidone-iodine. Preparations generally contain 1–10% povidone–iodine, equivalent to 0.1–1.0% available iodine. The active component appears to be free molecular iodine. A paradoxical effect of dilution on the activity of povidone-iodine has been observed: as the dilution increases, bactericidal activity increases to a maximum and then falls. Commercial povidone–iodine solutions at dilutions of 1:2 to 1:100 kill *S. aureus* and *Mycobacterium chelonae* more rapidly than do stock solutions. *S. aureus* can survive a 2-minute exposure to full-strength povidone-iodine solution but cannot survive a 15-second exposure to a 1:100 dilution of the iodophor. Thus, iodophors must be used at the dilution stated by the manufacturer.

The exact mechanism by which iodine destroys microorganisms is not known. It may react with the microorganisms' amino acids and fatty acids, destroying cell structures and enzymes. Depending on the concentration of free iodine and other factors, iodophors exhibit a broad range of microbiocidal activity. Commercial preparations are bactericidal, mycobactericidal, fungicidal and virucidal but not sporicidal at the dilutions recommended for use. Prolonged contact is required to inactivate certain fungi and bacterial spores. Despite their bactericidal activity, povidone-iodine and poloxamer-iodine solutions can become contaminated with *B. (P.) cepacia* or *P. aeruginosa*, and contaminated solutions have caused outbreaks of pseudobacteraemia and peritonitis. *B. cepacia* was found to survive for up to 68 weeks in a povidone-iodine antiseptic solution. The most likely explanation for the survival of these microorganisms in iodophor solutions is that organic or inorganic material and biofilm provide mechanical protection.

Iodophors are widely used for antisepsis of skin, mucous membranes and wounds. A 2.5% ophthalmic solution of povidone-iodine is more effective and less toxic than silver nitrate or erythromycin ointment when used as prophylaxis against neonatal conjunctivitis (ophthalmia neonatorum). In some countries, povidone-iodine alcoholic solutions are used extensively for skin antisepsis before invasive procedures. Iodophors containing higher concentrations of free iodine can be used to disinfect medical equipment. However, iodophor

solutions designed for use on the skin should not be used to disinfect hard surfaces because the concentrations of antiseptic solutions are usually too low for this purpose.

The risk of side-effects, such as staining, tissue irritation and resorption, is lower with use of iodophors than with aqueous iodine. Iodophores do not corrode metal surfaces; a body surface treated with iodine or iodophor solutions may absorb free iodine, however. Consequently, increased serum iodine (and iodide) levels have been found in patients, especially when large areas were treated for a long period. For this reason, other disinfectants should be considered for patients with hyperthyroidism and other disorders of thyroid function. Because severe local and systemic allergic reactions have been observed, iodophors and iodine should not be used in patients with allergies to these preparations. Iodophores have little if any residual effect; however, they may have residual bactericidal activity on the skin surface for a limited time, because free iodine diffuses into deep regions and also back to the skin surface. The antimicrobial efficacy of iodophors is reduced in the presence of organic material such as blood.

Triclosan and chloroxylenol (para-chlorometaxylenol): Triclosan (Irgasan DP-300, Irgacare MP) has been used for more than 30 years in a wide array of skin-care products, including handwashes, surgical scrubs and consumer products. A review of its effectiveness and safety in health-care settings has been published. A concentration of 1% has good activity against Gram-positive bacteria, including antibiotic-resistant strains, but is less active against Gram-negative organisms, mycobacteria and fungi. Limited data suggest that triclosan has a relatively broad antiviral spectrum, with high-level activity against enveloped viruses such as HIV-1, influenza A virus and herpes simplex virus type 1. The nonenveloped viruses proved more difficult to inactivate.

Clinical strains of bacteria resistant to triclosan have been identified, but the clinical significance remains unknown. Triclosan is added to many soaps, lotions, deodorants, toothpastes, mouth rinses, commonly used household fabrics, plastics and medical devices. The mechanisms of triclosan resistance may be similar to those involved in antimicrobial resistance, and some of these mechanisms may account for the observed cross-resistance of laboratory isolates to antimicrobial agents. Consequently, concern has been raised that widespread use of triclosan formulations in non-health-care settings and products might select for biocide resistance and even cross-resistance to antibiotics. Environmental surveys have not, however, demonstrated an association between triclosan use and antibiotic resistance.

Triclosan solutions have a sustained residual effect against resident and transient microbial flora, which is minimally affected by organic matter. No toxic, allergenic, mutagenic or carcinogenic potential has been identified in any study. Triclosan formulations can help control outbreaks of methicillin-resistant *S. aureus* when used for hand hygiene and as a bathing cleanser for patients, although some methicillin-resistant *S. aureus* isolates have reduced triclosan susceptibility. Triclosan formulations are less effective than 2–4% chlorhexidine gluconate when used as surgical scrub solutions, but properly formulated triclosan solutions can be used for hygienic hand washing. para-Chlorometaxylenol (chloroxylenol, PCMX) is an antimicrobial agent used in hand-washing products, with properties similar to those of triclosan. It is available at concentrations of 0.5–3.75%. Nonionic surfactants can neutralize this compound.

Octenidine: Octenidine dihydrochloride is a novel bispyridine compound and an effective, safe antiseptic agent. The 0.1% commercial formulation compared favourably with other antiseptics with respect to antimicrobial activity and toxicological properties. It rapidly killed both Gram-positive and Gram-negative bacteria as well as fungi in vitro and in vivo. Octenidine is virucidal against HIV, hepatitis B virus and herpes simplex virus. Like chlorhexidine, it has a marked residual effect. No toxicological problems were found when the 0.1% formulation was applied according to the manufacturer's recommendations. The colourless solution is a useful antiseptic for mucous membranes of the female and male genital tracts and the oral cavity, but its unpleasant taste limits its use orally. In a recent observational study, the 0.1% formulation was highly effective and well tolerated in the care of central venous catheter insertion sites, and the results of this study are supported by those of a randomized controlled clinical trial. Octenidine is not registered for use in the United States.

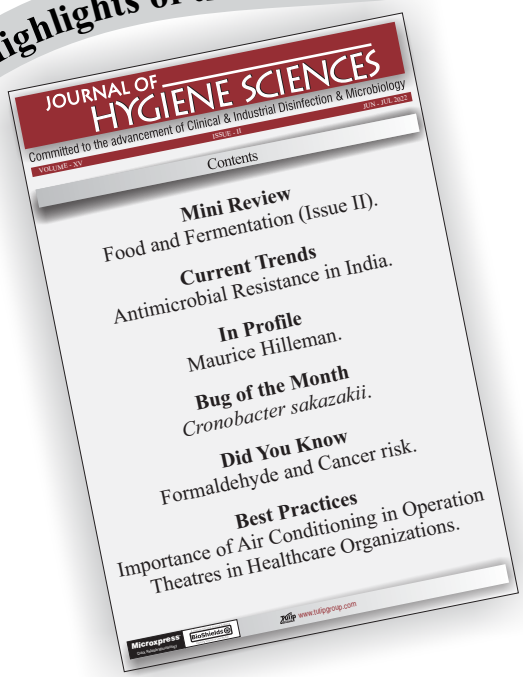
Table II – Antimicrobial agents recommended for surgical skin preparation

Solution	Comment
60-90% isopropanol	Not for use on mucous membranes
7.5-10% povidone-iodine	Can be used on mucous membranes
2-4% chlorhexidine	Not for use on eyes, ears, mucous membranes
Iodine, 3% preparation	Not for use on mucous membranes: can cause skin irritation if left for a long time
para-Chlorometaxylenol (PCMX)	Not for use on newborn babies; penetrates skin

References:

www.who.int/patientsafety/en/
www.who.int/patientsafety/safesurgery/en

Highlights of the coming issue



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