

Committed to the advancement of Clinical & Industrial Disinfection & Microbiology VOLUME - IX ISSUE - V DEC 2016 - JAN 2017

Editorial

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Well to Jump start with this issue we have the 'Mini Review' section giving us a brief on **Single Cell Protein (SCP)**. Single-cell protein (SCP) refers to edible unicellular microorganisms. The biomass or protein extract from pure or mixed cultures of algae, yeasts, fungi or bacteria may be used as an ingredient or a substitute for protein-rich foods, and is suitable for human consumption or as animal feeds. The increasing world deficiency of protein is becoming a main problem of humankind. Since the early fifties, intense efforts have been made to explore new, alternate and unconventional protein. For this reason, in 1996, new sources mainly yeast, fungi, bacteria and algae named Single Cell Protein (SCP) as coined to describe the protein production from biomass, originating from different microbial sources.

Our Current Trends section highlights about Disinfection Validation Process. Fumigation is the process to disinfect the sterile manufacturing and microbiology testing area. Generally fumigation is not required when AHU runs continuously but when the microbial load increases in the controlled area it is controlled and minimized by fumigation of the area.

In Profile Scientist – Ignaz Philipp Semmelweis, Semmelweis was one of the most prominent medical figures of his time. His discovery concerning the etiology and prevention of puerperal fever was a brilliant example of fact-finding, meaningful statistical analysis, and keen inductive reasoning. The highly successful prophylactic hand washings made him a pioneer in antisepsis during the pre bacteriological era in spite of deliberate opposition and uninformed resistance.

Bug of the month - *Borrelia recurrentis* is the causative agent of louse borne relapsing fever (LBRF) and is closely related to the bacterium *B. duttonii*, the source of tick borne relapsing fever. A recent genomic study of the two strains of bacteria found that *B. recurrentis* is actually a subset of *B. duttonii* in which the genes of the latter underwent a decaying process that gave rise to *B. recurrentis*.

Did You Know? Triclosan is a nonionic, colorless substance that was developed in the 1960s. It has been incorporated into soaps for use by HCWs and the public and into other consumer products. Concentrations of 0.2%--2% have antimicrobial activity. Triclosan enters bacterial cells and affects the cytoplasmic membrane and synthesis of RNA, fatty acids, and proteins.

Best Practices - Like our skin, our scalp requires regular cleansing to remove excess oil and grime accumulated from exposure to atmospheric dust and dirt. Dead skin cells are removed at the same time to make way for cell renewal. Regular shampooing is key to maintaining good scalp health. An ideal shampoo is one that cleanses thoroughly but does not cause scalp dryness, does not contain conditioning agents that would clog pores, provides hydration, removes dry dead skin layers and oily skin layers and preferably does not contain allergens.

All work & no play makes Jack a dull boy! We don't forget that ever. Each issue comes with its own bouquet of jokes, so enjoy.....

Our JHS team is thankful to all our readers for their ever increasing appreciation that has served as a reward & motivation for us. Feedback & suggestions are always welcomed.

JOURNAL OF HYGIENE SCIENCES

Mini Review

Single Cell Protein

INTRODUCTION

The increasing world deficiency of protein is becoming a main problem of humankind. Since the early fifties, intense efforts have been made to explore new, alternate and unconventional protein. For this reason, in 1996, new sources mainly yeast, fungi, bacteria and algae named Single Cell Protein (SCP) as coined to describe the protein production from biomass, originating from different microbial sources. Microbial biomass has been considered an alternative to conventional sources of food or feed. Large-scale processes for SCP production show interesting features, including:

- The wide variety of methodologies, raw materials and microorganisms that can be used for this purpose
- High efficiency in substrate conversion
- High productivity, derived from the fast growth rate of microorganisms
- Independence of seasonal factors

Yeast was the first microorganism whose importance as animal feed supplement was recognized almost a century ago. During World War I, Germany replaced half of imported protein sources by yeast. Pruteen was the first commercial single cell protein used as animal feed additive. From a nutritional viewpoint, Nucleic Acids (NA) content in SCP is one of the main factors hindering its utilization as food. Excessive intakes of NA lead to **uric acid** precipitation, causing health disorders, such as gout or kidney stone formation.

SINGLE CELL PROTEIN DEFINITION

A variety of microorganisms and substrate are used to produce single cell proteins. Yeast is suitable for single cell protein production because of its superior nutritional quality. The supplementation cereals with single cell proteins, especially yeast, make them as good as animal proteins. The necessary factor considered for use of SCP is the demonstration of the absence of toxic and carcinogenic compounds originated from the substrates, biosynthesized by the microorganisms or formed during processing. High nucleic acid content and low cell wall digestibility are two of the most important factors limiting nutritional and toxicological value of yeast for animal or human consumption. As constituents of nucleic acid, purine compounds in human diet mostly metabolized to yield uric acid whose high concentration may lead to gout or renal stones. However, nucleic acid is not a toxic component and it causes only physiological effects at higher levels like any other essential dietary ingredients taken in larger amounts. Algae grown in ponds can produce 20 tons (dry weight) of protein, per acre, per year. Bacteria are usually high in protein (50 to 80%) and have a rapid growth rate. The principal disadvantages are as follows:

- Bacterial cells have small size and low density, which makes harvesting from the fermented medium difficult and costly
- Bacterial cells have high nucleic acid content relative to yeast and fungi. To decrease the nucleic acid level additional processing step has to be introduced and this increases the cost
- The general public thinking is that all bacteria are harmful and produce disease. An extensive education program is required to remove this misconception and to make the public accept bacterial protein

Yeasts have advantages such as their larger size (easier to harvest), lower nucleic add content, high lysine content and ability to grow at acidic pH. However, the most important advantage is familiarity and acceptability because of the long history of its use

in traditional fermentations. Disadvantages include lower growth rates, lower protein content (45 to 65%) and lower methionine content than in bacteria. Filamentous fungi have advantages in ease of harvesting, but have their limitations in lower growth rates, lower protein content and acceptability. Algae have disadvantages of having cellulosic cell walls which are not digested by human beings. Secondly, they also concentrate heavy metals. In the case of algae it has to be stressed that, due to technical and economical reasons, it is not the general intention to isolate and utilize the sole protein, but to propagate the whole algal biomass. So, the term SCP is not quite correct, because the microalgal material is definitely more than just protein. To date, worldwide various sophisticated technologies are employed for mass production and processing of photoautotrophic microalgae. The annual world production of all microalgae species is estimated to about 10,000 tons year. The algal biomass as sun dried or in compressed form as pastilles is the predominant product in microalgal biotechnology. More than 75% of the annual microalgal biomass production is used for the manufacture of powders, tablets, capsules, or pastilles. This biomass is harvested from natural waters or artificial ponds or photo bioreactors (PBR) and subsequently separated from the growth media followed by drying. The two major species cultivated for this purpose are the unicellular green alga, Chlorella and more recently, filamentous blue-green alga (Cyanobacterium), Spirulina. The production of SCP from various microbes, particularly from fungi and bacteria has received considerable attention, in contrast, only a few studies have dealt with the feasibility of using SCP from microalgae. Algal proteins are of high quality and comparable to conventional vegetable proteins. However, due to high production costs as well as technical difficulties, cultivation of algae as protein is still in evaluation. The celluloid cell wall, which represents about 10% of the algal dry matter, poses a serious problem in digesting/ utilizing the algal biomass, since it is not digestible for humans and other non-ruminants. Hence, effective treatments are necessary to disrupt the cell wall to make the protein and other constituents accessible for digestive enzymes. Several authors have studied the effect of different post-harvesting treatments on the digestibility of various algal species. Different species of algae, fungi, yeasts and bacteria are used as single cell protein and produced at commercial scale (Table 1). These organisms are grown on different carbon sources. Although, microalgae with some other supplements have been used as an essential food for the larval stages of fish and shellfish. Yeast cells have been considered as a substitute because of their small particle size, high protein content as SCP and relatively low production costs. However, poor digestibility may be an important constraint in the use of this SCP as a food source in seed production of aquacultural organisms, since yeast has a complex and thick cell envelope. The external mannoprotein layer of the yeast cell wall is probably the major barrier to digestion. Several methods have been developed to improve the digestibility of SCP products: mechanical disruption, autolysis and enzymatic treatment. For maintenance of a stock collection of microorganisms preservation by drying or freeze-drying for long periods of time was reported to be good for yeast or algae. It has been agreed that the criteria used to evaluate SCP production are growth yield, total protein (39-73%) and nucleic acid contents (1-11%). Marine yeasts are better candidates for marifeed production according to their easy cultivation in the fermenter, high cell density and high content of

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essential **amino acids**. The substrates which have been used for SCP production by yeasts so far include sorghum hydrolysate, sulfate waste liquor, pawn-shell wastes, dairy wastes, methanol, molasses, starch and plant origin liquid waste. Several fungi like

Fusarium oxyporum var., *lini* and *Chetomium cellulolyticum*, algae like *Chlorella* and *Spirulina*, yeast like *Candida lipolytica* and *Saccharomyces sereviciae* and phototrophic bacteria like *Rodospirillum* sp., had been explored for SCP.

Table 1: Microorganism	and substrates used for	or single cell	protein production

Microorganism	Substrate	
Bacteria		
Aeromonas hydrophylla	Lactose	
Acromobacter delvacvate	n-Alkanes	
Acinetobacter calcoacenticus	Ethanol	
Bacillus megaterium	Non-protein nitrogenous compounds	
Bacillus subtilis, Cellulomonas sp., Flavobacterium sp., Thermomonospora fusca	Cellulose, Hemicellulose	
Lactobacillus sp.	Glucose, Amylose, Maltose	
Methylomonas methylotrophus, M. clara	Methanol	
Pseudomonas fluorescens	Uric acid and other non-protein	
	nitrogenous compounds	
Rhodopseudomonas capsulata	Glucose	
Fungi		
Aspergillus fumigatus	Maltose, Glucose	
Aspergillus niger, A. oryzae, Cephalosporium eichhorniae, Chaetomium cellulolyticum	Cellulose, Hemicellulose	
Penecillium cyclopium	Glucose, Lactose, Galactose	
Rhizopus chinensis	Glucose, Maltose	
Scytalidium aciduphlium, Thricoderma viridae, Thricoderma alba	Cellulose, pentose	
Yeast		
Amoco torula	Ethanol	
Candida tropicalis	Maltose, Glucose	
Candida utilis	Glucose	
Candida novellas	n-alkanes	
Candida intermedia	Lactose	
Saccharomyces cereviciae	Lactose, pentose, maltose	
Algae		
Chlorella pyrenoidosa, Chlorella sorokiana, Chondrus crispus, Scenedesmus sp.,	Carbone dioxide through	
Spirulina sp., Porphyrium sp.	photosinthesis	

SCPPRODUCTION

The worldwide, large-scale development of SCP processes has contributed greatly to the advancement of present day biotechnology. Research and development of SCP processes has involved work in the fields of microbiology, biochemistry, genetics, chemical and process engineering, food technology, agriculture, animal nutrition, ecology, toxicology, medicine and veterinary science and economics. In developing SCP processes new technical solutions for other related technologies in waste water treatment, production of alcohol, enzyme technology and nutritional science also improves. The future of SCP will be heavily dependent on reducing production costs and improving quality by fermentation, downstream processing and improvement in the producer organisms as a result of conventional applied genetics together with recombinant DNA technologies.

Single cell proteins have application in animal nutrition as: fattening calves, poultry, pigs and fish breading in the foodstuffs area as: aroma carriers, vitamin carrier, emulsifying aids and to improve the nutritive value of baked products, in soups, in readyto-serve meals, in diet recipes and in the technical field as: paper processing, leather processing and as foam stabilizers. The production of single cell protein takes place in a fermentation process. This is done by selected strains of microorganisms which are multiplied on suitable raw materials in technical cultivation process directed to the growth of the culture and the cell mass followed by separation processes. Process development begins with microbial screening, in which suitable production strains are obtained from samples of soil, water, air or from swabs of inorganic or biological materials and are subsequently optimized by selection, mutation, or other genetic methods. Then the technical conditions of cultivation for the optimized strains are done and all metabolic pathways and cell structures will be determined.

The classical raw materials are substances containing mono and disaccharides, since almost all microorgansims can digest glucose, other hexose and pentose sugars and disaccharides. These materials also are utilized in other branches of industry with a high price level, which puts the economic aspect of the production of microbial biomass in doubt. The choice of substrates that are normally abundant has determined the design and strategy of SCP processes. The most widespread and commonly used substrates for SCP production have been those where the carbon and energy source is derived from.

SCP derived from high energy sources: Materials with high commercial value as energy sources or derivatives like gas oil, methane, methanol and n-alkanes are of interest in SCP production. The microbes involved are mostly bacteria and yeast and several processes are now in operation. The wisdom of using such high-energy potential compounds for food production has been questioned by many scientists. British Petroleum uses two yeasts, Candida lipolytica and C. tropicalis and C12-C20 alkanes

as substrate which is of the wax fraction of gas oils for treating. Some crude oils contain up to 15% in wax. The product produced was called TOPRINA. For 12 years TOPRINA was tested for toxicity and carcinogenecity and was marketed as a replacement for fish meal in high protein feeds and as a replacement for skimmed milk powder in milk replacers. After a while the main opposition came from Japan. In 1972 a specialised committee decided that SCP was only for animal feeding but later, Japan was the first country to ban petrochemical protein. In 1977 Italy stopped the SCP production from alkanes due to the increase in oil prices. The price of soya was more competitive. Now there is no factory which produces any petrochemical protein. Methane as a SCP source has been extensively researched. The species which has been extensively studied is Methylomonas methanica and nitrates or ammonium salts can serve as N-source. The technology used for this purpose faced too many technical difficulties to optimize the production. In contrast, methanol offers great economic SCP interest. A large-scale fermentation plant for producing the methanol-utilizing bacterium Methylophilus methtlotrophus was constructed by ICI (Imperial Chemical Industries Company), UK. The ICI SCP protein was used exclusively for animal feeding. Methanol as a carbon source for SCP has many inherent advantages over n-paraffins, methane gas and even carbohydrates composition is independent of seasonal fluctuations. There are no possible sources of toxicity in methanol and it dissolves easily in the aqueous phase in all concentrations. The ICI Pruteen (72% protein) plant (by Pseudomonas methylotrophus) was the only process of its kind in the Western world but could not operate economically at present methanol prices and cannot compete with soya and fish meal so has ceased production. Methanol represents approximately 50% of the costs of the product. In the USA the cost of SCP derived from methanol is two-to five-folds the cost of fishmeal. In the Middle East the low cost of methanol and higher costs of fishmeal coupled with a need to produce more animal products could make SCP an attractive proposition. Ethanol is a particular suitable source if the SCP is intended for human consumption. The process comes from the Amoco Company in the US utilizing food grade yeast: Torula. The product is sold by the name "Torutein" even in Canada and Sweden. The yeast is about 52% protein. Torutein is being marketed as a flavor enhancer of high nutritional value and a replacement for meat, milk and egg protein. However, it is not very successful in the United States since soya which is plentiful and cheap can serve as an alternative or substitute to meat and egg diets.

SCP from wastes: The amount of agricultural and some industrial wastes used for SCP production can be locally very high and may contribute to a significant level of pollution in water courses. Thus, the utilization of such materials in SCP processes serves two functions as reduction in pollution and creation of edible protein. Cellulose from agriculture and forestry sources constitutes the most abundant renewable resource in the planet as potential substrates for SCP production. Cellulose has emerged as an attractive substrate for SCP production but in nature it is usually found with lignin, hemicellulose, starch, etc., in a complex form. Therefore, if cellulose is to be used as substrate it must be pretreated chemically (acid hydrolysis) or enzymatically (cellulases) to remove cellulose as fermentable sugars. For the utilization of lignocellulose, a pre-treatment is usually necessary. Many pre-treatment methods have been reported which vary from alkali or acid treatment, steam explosion or even x-ray radiation. To the present time the only economical utilization of lignocellulosic wastes is in mushroom production. Besides our well known cultivated mushroom Agaricusbisporus there are

many important ones which contain lignocellulolytic enzymes and are cultivated for food mainly in Asia and Africa. Some are of great economic significance and are cultivated on an industrial scale. Examples of important ones include Volvariella sp., *Lentinusedodes* and *Pleurotus* sp. In the manufacture of industrial cellulose for paper and tissue production the cost of these steps has prevented the generalized production of SCP from cellulose. Wood can be also cooked in a medium containing calcium sulfite with excess free sulfur dioxide. Lignin is thus converted to lignosulfonates and hemicellulose is hydrolysed to monosaccharides and may be further broken down to furfurols. The amount of free sugars in the spent liquor is variable with the type of procedure chosen, as various cellulose fibers may be obtained with different degrees of degradation. Spent sulfite liquor has been used as a substrate for fermentations since 1909 in Sweden and later in many other parts of the world. The first organism to be used was Saccharomyces cerevisiae, although this organism is unable to metabolise pentoses which are found in considerable amounts in this waste product. Later, other organisms better suited for the assimilation of all the sugar monomers were chosen, namely Candida tropicalis and Candida utilis. Yeast produced from sulfite liquor has been used for feeding at war periods, but lost favour in peace time. However, experiences of baker's yeast produced from sulfite liquor exist in Finland by Peliko process. The protein content of the fungus Paecilomyces variotii exceeds 55% (w/w) and has been officially approved as a food in Finland. In 1983, the projected biomass production of the process was estimated to be around 7000 tons per year. Nowadays, extracellular cellulases are commercially used in cellulose separating process. Cellulase is a complex of three enzymes (endocellulase, cellobiohydrolase, cellobiase). A number of efficient cellulase producers have been reported but Trichoderma viride continued to be well known high cellulaseproducing organism. Chaetomium cellulolyticum is another cellulolytic fungus which grows faster and forms 80% more biomass-protein than Trichoderma. This means that C. cellulolyticum is suitable for SCP production while T. viride is a hyper producer of extracellular cellulases. The amino acid composition of C. cellulolyticumis generally better than that of T. viride and similar to alfalfa and soya meal protein. A cheaper, more amenable SCP substrate of carbohydrate origin is starch. This very abundant carbohydrate may be obtained from rice, maize and cereals. In tropical countries, cassava has been proposed as a good source of starch for SCP processes. The Symba process developed in Sweden utilized starchy wastes combining two yeasts in sequential mixed culture: the amylase producing Endomycopsis fibuligira and the fast growing Candida utilis. The process consists of three phases: The incoming starch waste is fed through heat exchangers and sterilized. The medium is then fed to a first bioreactor where the starch hydrolyzing yeast grows and hydrolyses starch. The hydrolyzed solution is then fed to a second reactor where culture conditions favor the proliferation of C. utilis. Whey traditionally originates from the curding process in cheese production, but can now be obtained after ultra filtration procedures for the production of spreading cheeses, where the protein fraction corresponding to lactalbumins and lactoglobulins is incorporated to the casein fraction and all the proteins are in native form with the principal component as lactose (4-6%) (w/v). Although other nutrients can be find in significant amounts. Whey has been presented as an extremely suitable substrate for the production of SCP. In 1956 The French dairy company Fromageries Bel pioneered a project to produce yeast from whey, using lactose assimilating Kluyveromyces marxianus (formerly K. fragilis).

To Be Continued in the next issue...



Disinfectant Validation (Part 2)

Fumigation is the process to disinfect the sterile manufacturing and microbiology testing area. Generally fumigation is not required when AHU runs continuously but when the microbial load increases in the controlled area it is controlled and minimized by fumigation of the area.

Validation of Fumigation in Cleanroom Area Fumigation in cleanroom areas is done by fogging with hydrogen peroxide and it is validated with the bacterial spore strips. Validation of fumigation is also required to verify the effectiveness of the fumigation. There are some indicators for the validation of fumigation efficiency in cleanroom areas. It is validated by the strip of *Geobacillus stearothermophillus* ATCC 7953. These are perforated stainless steel coupons having spores.



Bacterial spore strips

Spore coupons biological indicators are placed at the different locations in the cleanroom especially at the critical locations as the corners and behind the equipments. After fogging in room with Hydrogen Peroxide the strips are collected and incubated in the MC20 growth medium provided with the biological indicators at 60 ± 2 °C for 24 hours.

Validation Criteria: The color of MC20 growth medium should not be changed to yellow after incubation at 60 ± 2 °C. Change in color of growth medium indicates the presence of the living microbes in the strips and shows the unsuccessful fumigation of the area. While no change in color of growth medium shows the effective fumigation of the area.

Focus is set to the proper and simplified validation of the process using standardized biological indicators with defined concentrations of the test organism *Geobacillus stearothermophilus*.

Recent applications with VHP have included cleanrooms, research environments and whole facility decontamination and these are all validated in a similar way to include: a) Area review: size, shape, contents and temperature. Based on this review the area is prepared for fumigation. Depending on the room size, fans may be distributed to ensure adequate circulation; alternatively, the HVAC system can also be used for efficient VHP delivery and decontamination. b) Distribution studies: the distribution of VHP can be verified in the room using electrochemical or spectrophotometric sensors, as well as chemical indicators. c) Validation studies: microbiological and chemical indicators may be used to verify antimicrobial efficacy in the area. Geobacillusstearothermophilus spores have been verified as the most resistant organism to peroxide gas and are recommended as biological indicators for efficient fumigation. Alternatively, other microorganisms may be used depending on the specific concerns of the facility.

Of particular note, recent large scale studies have used VHP for the

remediation of building contaminated with Bacillus anthracis spores and are discussed in more detail below. Pathogen contamination of a facility is a significant concern. Examples include contamination in hospitals (e.g., methicillin-resistant Staphylococcus aureus, vancomycin-resistant Enterococcus and spore-forming Clostridium difficile), laboratories (Class 2-4 bacteria and viruses) and general area contamination (e.g., fungal spores and *Legionella* contamination in air handling systems). These concerns were recently highlighted with the widely reported bioterrorism episodes in the. In these cases, a series of letters containing high concentrations of Bacillus anthracis spores led to the contamination of buildings up to 45,000m3 volume. B. anthracis is the causative agent of anthrax and, as a spore-forming microorganism, can persist in the environment. VHP was successfully used for the remediation of small and large areas. Previous applications with VHP decontamination systems were limited by their capacity, with one VHP1000 system being capable of decontaminating an area up to 170m3. Larger high capacity systems were therefore developed and used for the remediation of contaminated buildings and their air handling systems, under approval of the US Environmental Protection Agency. All areas were reviewed prior to remediation. Excess paper or other cellulosic-based materials were removed from the area prior to fumigation; this was primarily due to the absorptive nature of this material which can extend the total fumigation time. Air fans were positioned around the area to ensure adequate air movement; in the case of HVAC systems, the VHP system was directly linked to the air handling unit and flowed through the ductwork for the required fumigation time. Biological (>106 G. stearothermophilus or B. subtilis spores, the latter being widely used as a stimulant for B. anthracis) and VHP chemical indicators were distributed around the area to test for distribution during fumigation. A typical area fumigation cycle included reducing the relative humidity to below 40%, introducing VHP until the concentration had stabilised (this was set at >0.1mg/L and depended on the room volume and contents) and then maintaining the concentration at that level for the desired exposure time. At the lowest concentration of 0.1mg/L the time for a 12-log reduction of spores would be three hours (12 x 15 secs = 180 mins). Due to the potentially high contamination rate in some of the areas, extended exposure times (3-12 hours) ensured overkill. Following exposure time, the area was then aerated to reduce the concentration of VHP to less than 1ppm. For all areas, inspection of the chemical indicators confirmed the presence of VHP and analysis of the biological indicators did not indicate the presence of the growth of the indicator organism. Subsequent environmental sampling did not detect the presence of B. anthracis spores, allowing for the safe re-entry of the buildings. Further, no damage to the building or contents (including inspection of electrical equipment) was observed.

Biological Indicators Preparation:

Spores of *Bacillus atrophaeus* (ATCC9372) and *Geobacillus stearothermophilus* (ATCC7953) were used to evaluate the biodecontamination efficiency of VHP fumigation for the HEPA filter unit in this study. Spores preparation and coupons inoculation were carried out in accordance with slightly modified standard method. Cotton cloth coupons (2×107 cfu spores of *Bacillus atrophaeus*) were prepared for quantitative evaluation of bio-decontamination efficiency. Cotton cloth coupons (3×106 cfu spores of *Bacillus atrophaeus*) and the commercially available common filter paper coupons (3×106 cfu spores of *Bacillus atrophaeus*) or

Geobacillus stearothermophilus) were prepared for qualitative evaluation of bio-decontamination efficiency.

Spore Extract on from Coupons All quantitatively tested coupons were transferred into 5ml eluant (0.05% Tween 80, and 0.01% catalase) in a 30 mL glass sterile tube. The coupons were submerged in eluant for 30 min and then tubes were knocked violently to dislodge the survival spores from coupons. Serial dilutions of extract at 1:10 were performed as needed and 1.0 mL aliquots of undiluted extract or dilutions were added in triplicate to plates. 15 to 20 mL volume of liquefied nutrient agar (45-50 °C) was added to each plate and was mixed with the extract solution by gentle rotational swirling. The plates were incubated at 37 °C for 24 h. All qualitative coupons were put into 8ml nutrient broth (for Bacillus atrophaeus) or glucose peptone water medium (for Geobacillus stearothermophilus) directly and incubated for 7 days at 37°C (for Bacillus atrophaeus) or at 56°C (for Geobacillus stearothermophilus). The turbidity and the color change of the culture medium were the indications of bacterial growth. Data Collection and Statistical Analysis Plates were enumerated and the number of CFUs per coupon was determined by multiplying the average number of colonies per plate by the dilution factor and the coefficient of eluant volume. The sporicidal efficiency was expressed in terms of a Log reduction that was computed by subtracting the log survival spores CFUs of fumigated coupons from the log viable spores CFUs of the positive control coupons. The mean $(\pm SD)$ log reduction was calculated from three independent experiments. The (coupons inoculated with spores, not decontaminated by VHP fumigation) were left at room temperature when the test coupons were decontaminated.

Working with Chosen Bacterial Strains and Culture

This work involved preparation of seeded steel discs, using hightitre microbial challenges, which then required their placement, recovery, and cultivation. In view of the amount of samplehandling required and the need to deploy these discs within a chamber environment, HSL followed principles recommended by HSE in The Control of Substances Hazardous to Health (COSHH) Regulations (2002) to control and so minimize any contamination risk to those performing experimental work. There is a primary duty under COSHH to prevent exposure of laboratory staff to biological agents during planned research (HSE-ACDP, 2005), by either avoiding their use or substituting with a safer alternative. For some types of laboratory work, such as diagnostic work, this may not be possible. However, it can be achieved for other types of work, such as planned experimental testing. With this in mind, the following cultures and methods were used for fumigation testing:

Clostridium difficile. NCTC 11209, a widely used reference strain, was used as a surrogate for epidemic C. difficile. Cultures of C. difficile were grown anaerobically in cooked meat broth (Oxoid Ltd., Cambridge, England) using a shaking incubator at 37°C for 48 hours. Oxoid-cooked meat broth is designed to promote anaerobic growth conditions in a sealed flask without the need for additional anaerobic controls. However, as an additional precaution, all culture flasks were placed in anaerobic jars containing anaerobic gas packs (Anaerogen, Oxoid Ltd.). Liquid culture was agitated to ensure a uniform suspension before decanting 50 ml of the suspension. This was centrifuged at 3,000 rpm for 10 minutes, and the pellet was then resuspended in 5 ml freshcooked meat broth to concentrate the bacteria. The combination of using meat-broth culture and drying the seeded residues onto discs effectively induced sporulation. Spore concentrations were confirmed by staining the dried suspensions with malachite green. This process showed between 85% and 90% spore formation for each independent experiment (data not shown). Fifty microliters of C. difficile stock typically contained approximately 106 to 107 cells. This volume was seeded onto

sterile stainless steel discs in quadruplet (triplicate replicas for exposure to fumigant, plus a single comparative positive process control for each room location). Seeded discs were dried for 1.5 hours prior to fumigation and then positioned in predetermined chamber locations prior to the start of the test fumigation.

Mycobacterium fortuitum. NCTC 10394, a fastgrowing, nontuberculous Mycobacterium species, was used as a safe surrogate of Mycobacterium tuberculosis. M. fortuitum has the added advantage of growing as quantifiable colonies on standard agar plates, unlike other members of this genus, which require growth on slopes. M. fortuitum was grown aerobically in Middlebrook 7H9 broth containing 10% Middlebrook ADC enrichment broth and 1% Tween and was maintained on Middlebrook 7H10 agar plates (Becton Dickinson, Franklin Lakes, NJ) at 37°C for 4 days. To prepare a sample for fumigation experiments, 10 ml was removed from a 4-day-old broth culture of M. fortuitum and pipetted into a universal tube containing two glass beads. The sample was vortexed for 1 minute to disperse any bacteria and minimize cell clumping, and the resulting suspension was used to seed steel discs in 50-µl aliquots, as for C. difficile. The 50-µl aliquots of the M. fortuitum stock typically contained approximately 106 to 107 cells. Commercially prepared spore discs of Geobacillus stearothermophilus 7953, at a concentration of approximately 106 spores/disc (ATI Atlas, West Sussex, UK), were used as an additional control and point of comparison. Recovered G. stearothermophilus was grown on Tryptone Soya Agar (TSA-Oxoid, Cambridge, England) at 55°C overnight. This bacterium has been used extensively for the evaluation of disinfection and sterilization methods and was included here as a recognized standard within the industry. The steel disc preparation method was used for this organism, rather than the cellulose strip process, because the steel discs are designed for fumigation assessment.

Bacterial Recovery from Steel Discs

Following exposure to the fumigant, each disc was placed in a tube containing two 5-mm sterile glass beads and 10 ml of PBS; the mixture was vigorously agitated for 1 minute by vortexing. The tubes were left at room temperature for 30 minutes and then vortexed again to ensure efficient rehydration and removal of the dried bacterial sample from the disc. The simulated spills were recovered from the wells and each transferred into a sterile tube. In some cases, particularly following overnight exposure or venting, the liquid in the spill wells had evaporated partially or completely. In these cases, the sample was reconstituted to its original 750-µl volume using PBS before transfer to a sterile tube. The samples were serially diluted, 10-fold, and 100- µl aliquots of each dilution were plated onto duplicate agar plates; mycobacteria were recovered on Middlebrook 7H10 agar plates (Becton Dickinson), clostridia on Columbia blood agar, and Geobacillus on TSA. Following appropriate incubation, the plates were counted and the number of bacterial colonies recovered from each tile or spill well was calculated. Exposed samples were compared with unexposed control samples to determine the percentage kill and the log10 reduction.

http://www.pharmaguideline.com/2016/05/validation-of-fumigation-in-cleanroom.html

https://www.ncbi.nlm.nih.gov/pubmed/22767884

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http://cleanhospital.com/pdfs/absa fumigation comparisons.pdf

In Profile

HYGIENE SCIENCES

Ignaz Philipp Semmelweis



Semmelweis was one of the most prominent medical figures of his time. His discovery concerning the etiology and prevention of puerperal fever was a brilliant example of fact-finding, meaningful statistical analysis, and keen inductive reasoning. The highly successful prophylactic hand washings made him a pioneer in antisepsis during the pre bacteriological era in spite of deliberate opposition and uninformed resistance.

Semmelweis was born in Tabán, an old commercial sector of Buda. The fifth child of a prosperous shopkeeper of German origin, he received his elementary education at the Catholic Gymnasium of Buda and then completed his schooling at the University of Pest between 1835 and 1837.

In the fall of 1837, Semmelweis traveled to Vienna, ostensibly to enroll in its law school. His father wanted him to become a military advocate in the service of the Austrian bureaucracy. Soon after his arrival, however, he was attracted to medicine; and seemingly without parental opposition he matriculated in the medical school.

After completing his first year of studies at Vienna, Semmelweis returned to Pest and continued at the local university during the academic years 1839–1841. The backward conditions in the school, however, caused his return to Vienna in 1841 for further studies at the Second Vienna Medical School, which became one of the leading world centers for almost a century with its amalgamation of laboratory and bedside medicine. During the last two years of study, Semmelweis came in close contact with three of the most promising figures of the new school: Karl von Rokitansky, Josef Skoda, and Ferdinand von Hebra.

After voluntarily attending seminars led by these teachers, semmelweis completed his botanically oriented dissertation early in 1844. He remained in Vienna after graduation, repeating a twomonth course in practical midwifery and receiving a master's degree in the subject. He also completed some surgical training and spent almost fifteen months (October 1844–February 1846) with Skoda learning diagnostic and statistical methods. Finally Semmelweis applied for the position of assistant in the First Obstetrical Clinic of the university's teaching institution, the Vienna General Hospital.

In July of 1846 Semmelweis became the titular house officer of the First Clinic, which was then under the direction of Johann

Klein. Among his numerous duties were the instruction of medical students, assistance at surgical procedures, and the regular performance of all clinical examinations. One of the most pressing problems facing him was the high maternal and neonatal mortality due to puerperal fever, 13.10 percent. Curiously, however, the Second Obstetrical Clinic in the same hospital exhibited a much lower mortality rate, 2.03 percent. The only difference between them lay in their function. The First was the teaching service for medical students, while the Second had been selected in 1839 for the instruction of midwives. Although everyone was baffled by the contrasting mortality figures, no clear explanation for the differences was forthcoming. The disease was considered to be an inevitable aspect of contemporary hospital-based obstetrics, a product of unknown agents operating in conjunction with elusive atmospheric conditions.

After a temporary demotion to allow the reinstatement of his predecessor, who soon left Vienna for a professorship at Tübingen, Semmelweis resumed his post in March 1847. During his short vacation in Venice, the tragic death of his friend Jakob Kolletschka, professor of forensic medicine, occurred after his finger was accidentally punctured with a knife during a postmortem examination. Interestingly, Kolletschka's own autopsy revealed a pathological situation akin to that of the women who were dying from puerperal fever.

Prepared through his intensive pathological training with Rokitansky, who had placed all cadavers from the gynecology ward at his disposal for dissection, Semmelweis made a crucial association. He promptly connected the idea of cadaveric contamination with puerperal fever, and made a detailed study of the mortality statistics of both obstetrical clinics. He concluded that he and the students carried the infecting particles on their hands from the autopsy room to the patients they examined during labor. This startling hypothesis led Semmelweis to devise a novel system of prophylaxis in May 1847. Realizing that the cadaveric smell emanating from the hands of the dissectors reflected the presence of the incriminated poisonous matter, he instituted the use of a solution of chlorinated lime for washing hands between autopsy work and examination of patients. Despite early protests, especially from the medical students and hospital staff, Semmelweis was able to enforce the new procedure vigorously; and in barely one month the mortality from puerperal fever declined in his clinic from 12.24 percent to 2.38 percent. A subsequent temporary resurgence of the dreaded ailment was traced to contamination with putrid material from a patient suffering from uterine cancer and another with a knee infection.

In spite of the dramatic practical results of his washings, Semmelweis refused to communicate his method officially to the learned circles of Vienna, nor was he eager to explain it on paper. Hence, Hebra finally wrote two articles in his behalf, explaining the etiology of puerperal fever and strongly recommending use of chlorinated lime as a preventive. Although foreign physicians and the leading members of the Viennese school were impressed by Semmelweis' apparent discovery, the papers failed to generate widespread support.

During 1848 Semmelweis gradually widened his prophylaxis to include all instruments coming in contact with patients in labor. His statistically documented success in virtually eliminating puerperal fever from the hospital ward led to efforts by Skoda to create an official commission to investigate the results. The proposal was ultimately rejected by the Ministry of Education, however, a casualty of the political struggle between the defeated liberals of the 1848 movement and the newly empowered conservatives in both the university and the government bureaucracy.

Angered by favorable reports concerning the new methods that indirectly represented an indictment of his own beliefs and actions, Klein refused to reappoint Semmelweis in March 1849. Undaunted, he applied for an unpaid instructorship in midwifery. In the meantime he began to carry out animal experiments to prove his clinical conclusions with the aid of the physiologist Ernst Brücke and a grant from the Vienna Academy of Sciences.

Semmelweis was at last persuaded to present his findings personally to the local medical community. On 15 May 1850 he delivered a lecture to the Association of Physicians in Vienna, meeting under the presidency of Rokitansky. The following October he received the long-awaited appointment as a *Privatdozent* in midwifery, but the routine governmental decree stipulated that he could only teach obstetrics on a mannequin. Faced with financial difficulties in supporting his family, and perhaps discouraged, Semmelweis abruptly left the Austrian capital, returning to Pest without notifying even his closest friends. Such a hasty decision jeopardized forever his chances to overcome the Viennese skeptics gradually with the dedicated help of Rokitansky, Skoda, Hebra, and other colleagues.

In Hungary, Semmelweis found a backward and depressed political and scientific atmosphere following the crushing defeat of the liberals in the revolution of 1848. Despite the unfavorable circumstances, he managed to receive an honorary appointment and took charge of the maternity ward of Pest's St. Rochus Hospital in May 1851, remaining there until 1857. He soon was able to implement his new prophylaxis against puerperal fever, with great success, while building an extensive private practice.

Following the death of the incumbent, Semmelweis was appointed by the Austrian Ministry of Education to the chair of theoretical and practical midwifery at the University of Pest in July 1855, although he had been only the second choice of the local medical faculty. He subsequently devoted his efforts to improving the appalling conditions of the university's lying-in hospital, a difficult task in the face of severe economic restrictions. In 1855 Semmelweis instituted his chlorine hand washings in the clinic, and he gradually achieved good results despite initial carelessness by the hospital staff. His lectures, delivered in Hungarian by decree of the Austrian authorities, attracted large student audiences. Semmelweis also became active in university affairs, serving on committees dealing with medical education, clinical services, and library organization.

In 1861 Semmelweis finally published his momentous discovery in book form. The work was written in German and discussed, at length, the historical circumstances surrounding his discovery of the cause and prevention of puerperal fever. A number of unfavorable foreign reviews of the book prompted Semmelweis to lash out against his critics in a series of open letters written in 1861–1862, which did little to advance his ideas. After 1863 Semmelweis' increasing bitterness and frustration at the lack of acceptance of his method finally broke his hitherto indomitable spirit. He became alternately apathetic and pathologically enraged about his mission as a savior of mothers. In July 1865 Semmelweis suffered what appeared to be a form of mental illness; and after a journey to Vienna imposed by friends and relatives, he was committed to an asylum, the Niederösterreichische Heil- und pflegeanstalt. He died there only two weeks later, the victim of a generalized sepsis ironically similar to that of puerperal fever, which had ensued from a surgically infected finger.

Semmelweis' achievement must be considered against the medical milieu of his time. The ontological concept of disease insisted ion specific disease entities that could be distinctly correlated both clinically and pathologically. Puerperal fever, however, exhibited multiple and varying anatomical localizations and a baffling symptomatology closely related to the evolution of generalized sepsis. The apparent connection between this fever and erysipelas further clouded the issue. Moreover, the idea of a specific contagion causing the disease was not borne out by the clinical experience.

In the face of such theoretical uncertainties and the profusion of causes attributable to the disease, Semmelweis displayed a brilliant methodology borrowed from his teachers at the Second Vienna Medical School. He partially solved the puzzle through extensive and meticulous dissections of those who had succumbed to the disease, eventually recognizing the crucial similarities of all septic states. The methodical exclusion of possible etiological factors–one variable at a time–followed Skoda's diagnostic procedure, while the employment of statistical data was transferred from therapeutic analysis to the elucidation of the decisive factor responsible for the disease. In finally arriving at his discovery, Semmelweis successfully seized upon his built-in control group of women at the Second Clinic, a fortunate situation unparalleled elsewhere.

The subsequent lack of recognition for Semmelweis' prophylaxis can be attributed to several factors. An initial lack of proper publicity among Viennese and foreign visiting physicians led to misunderstandings and an incomplete assessment of the intended procedure. Further, political feuds led to an identification of Semmelweis with the liberal and reform-oriented faction of the Viennese medical faculty, a group temporarily thwarted in their objectives by the crushing defeat of 1848. Finally, Semmelweis' abrupt departure from the arena robbed him of the possibility of eventually persuading his Viennese colleagues of the soundness of the chlorine washings. Operating from a politically suppressed and scientifically backward country with a second-rate university, Semmelweis was effectively hampered in the promulgation of his ideas. His later, rather violent and passionate polemics added little further credence to a somewhat cumbersome method that was difficult to implement among hospital staff members content with the status quo. Most important, however, was the lack of a good explanation for Semmelweis' empirically derived procedure, a development made possible only through the ensuing work of Pasteur.

Relaxed Mood

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Wife: You delivered an excellent speech.

Husband: Thanks, dear, but the audience was full of fools and idiots.

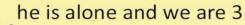
Wife: Hmm... so, that explains why you addressed them as

brothers and sisters.



What's winning attitude? 3 ants saw an elephant coming.

Ant1: We will kill him Ant2: We will break his legs. Ant3: Forgive him guys,







Wife : I am not talking to you. Husband : Okay Wife : Don't you want to know the reason. Husband : No, I respect & trust your decision



Sali ne DIL tod diya aur sali ne block mar diya ab computer hi tod deta hoon..!!!

Kamine rahne de meri wali online aane wali hai..!!!

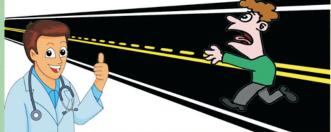
Height of English Misunderstanding

In a Train , a lady sleeping at Santa's son's place and refused to get Up.





Santa went to TC and complained:-This Lady is not giving "Birth" to my "Child", (a) (a) (a) (b)



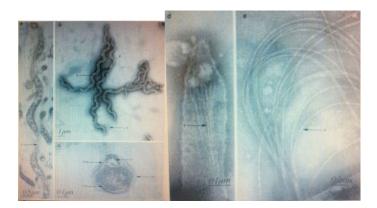
The doctor told a Patient that if he ran Eight kilometres a day for 200 days, he would lose 34 kg. After 200 days, the patient called the doctor to report he had lost weight, but he had a problem. Doctor: 'What is the Problem?'

Patient: 'I am 1600 kms. from home.'

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Bug of the Month

Borrelia recurrentis



Classification Domain (Bacteria); Phylum (Spirochaetes); Class (Spirochaetes); Order (Spirochaetales); Family (Borreliaceae); Genus (*Borrelia*)

Electron micrographs of B. recurrentis Al. F, flagella; SL, surface layer; OM, outer membrane; CM, cytoplasmic membrane. (a) Spirochetal morphology of cells. (b) Negative staining (phosphotungstic acid) of spirochetal cells, illustrating the tapering ends. (c) Transverse section of cell, showing the periplasmic flagella. (d) Negatively stained preparation (ammonium molybdate), showing terminally associated flagella. (e) Negatively stained preparation (ammonium molybdate), showing flagella associated with the central portion of a cell. [2]

Description and significance

Borrelia recurrentis is the causative agent of louse borne relapsing fever (LBRF) and is closely related to the bacterium *B. duttonii*, the source of tick borne relapsing fever. A recent genomic study of the two strains of bacteria found that *B. recurrentis* is actually a subset of *B. duttonii* in which the genes of the latter underwent a decaying process that gave rise to *B. recurrentis*. This process could be due to the inactivation of genes encoding for DNA repair mechanisms (recA and mutS), causing an accumulation of errors in the genome

B. recurrentis is the cause of systemic inflammatory disease, characterized by one to five fever relapses, distinctive hemorrhagic syndrome, a high rate of spontaneous abortion in pregnant women, and a 2-4% mortality rate despite modern antibiotics. B.recurrentis is unlike its counter part (B. duttonii), which produces more relapses and a lower mortality rate. B. recurrentis is a slender, pathogenic spirochete whose habitat is usually associated with humans and is vector borne via lice. LBRF was once a world wide epidemic, but over the last century has since been eradicated due to better personal hygiene and way of living. Now the disease is prevalent in areas that have major lice problems such as the Andean foothills, the highlands of eastern Africa, southern Sudan, and Rwanda. The lice become infected by feeding on humans that are infected by the spirochetes. When the lice are transferred to another human, the infection spreads by the contact of the hemolymph (the fluid found in the circulatory system of arthropods) with abraded skin.

This contact can occur by scratching, which then opens the skin and crushes the body of the lice, exposing the hemolymph. New evidence suggests that another means of infection can occur through contact with infected feces.

Genome structure

According to JGI Genome encyclopedia, the fully sequenced bacteria *Borrelia recurrentis* has a genome with 1025 genes and 1,242,163 base pairs which contain 8 linear fragments ranging from 6,131 bp to 930,981 bp. This is quite atypical of prokaryotes since they are normally seen having a single, circular chromosome. *B. recurrentis* contains a linear chromosome with a size of approximately 1 Mb (27.5 GC%) along with both linear and circular plasmids. All strains contain one large plasmid anywhere from 183 to 194 kb as well a small one (11 kb). Strains have 5 main differences in the pattern of the other plasmids, which range in size from 25 kb to 62 kb.

Cell and colony structure

Electron microscopy has revealed B. recurrentis as a spirochetal cell with pointed ends containing 8-10 periplasmic flagella, an average wavelength of 1.8 pm and an amplitude of 0.8 pm. Because of the flagella, this is a motile bacterium which leads to greater spreading in the bloodstream of the host. Contrary to most pathogenic bacteria, B. recurrentis is gram negative.

Metabolism

This microaerophilic microbe is also mesophilic (it prefers moderate temperatures of 68-113° F), where the human host provides perfect conditions for the bacteria to thrive. It is also an auxotroph for most amino acids, meaning that it is unable to produce them itself and absorbs them from the environment instead.

Ecology

B. recurrentis is known for its inhabitance in human hosts and has only successfully been able to infect primates.

Pathology

These spirochaetes normally cause a blood infection but they can also infect the nervous system along with other tissues. There are no known virulence factors, but there are a few novel ways that this sneaky pathogen evades the host immune system. The body has its way of recognizing and enhancing the recognition of foreign invaders called opsonization, which targets them for destruction through a cascade of reactions known as the complement system. Recent studies show that B. recurrentis expresses a multifunctional surface lipoprotein, termed HcpA, that exploits the host's proteins and offers resistance to complement attack and opsonization while increasing the potential to invade the host's tissues. Since HcpA outlines the high virulence potential of B. recurrentis, it makes a good target for therapeutic treatment of LBRF, however, none have been created yet. It was also found that this spirochete binds to the PLG(human plasminogen/Plasmin) receptor on endothelium cells and exploits their increased proteolytic capacity to breach tight junctions of endothelium, cross basement membranes, and to initiate patho-physiological processes in the affected organs. Another novel approach is its ability to undergo antigenic

variation, meaning that once the innate immune system is able to identify and start fighting off the first antigenic type, another antigenic type appears. This impairs the host immunes system from being able to clear the infection and explains why there are multiple recurrences of fever. To treat LBRF, tetracyclines and penicillins are commonly used and are usually quite effective; however they may cause a severe Jarisch-Herxheimer reaction, which can be fatal. This reaction causes fever, chills, rigor, hypotension, headache, tachycardia, hyperventilation, vasodilation with flushing, myalgia (muscle pain), and exacerbation of skin lesions because the death of the bacteria causes the release of harmful endotoxins faster than the body can get rid of them.

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Did You Know

Triclosan

Triclosan (chemical name: 2,4,4' --trichloro-2'-hydroxydiphenyl ether) is a nonionic, colorless substance that was developed in the 1960s. It has been incorporated into soaps for use by HCWs and the public and into other consumer products. Concentrations of 0.2%--2% have antimicrobial activity. Triclosan enters bacterial cells and affects the cytoplasmic membrane and synthesis of RNA, fatty acids, and proteins. Recent studies indicate this agent's antibacterial activity is attributable to binding to the active site of enoyl-acyl carrier protein reductase.

Triclosan has a broad range of antimicrobial activity, but it is often bacteriostatic. Minimum inhibitory concentrations (MICs) range from 0.1 to 10 ug/mL, whereas minimum bactericidal concentrations are 25--500 ug/mL. Triclosan's activity against gram-positive organisms (including MRSA) is greater than against gram-negative bacilli, particularly P. aeruginosa. The agent possesses reasonable activity against mycobacterial and *Candida* spp., but it has limited activity against filamentous fungi. Triclosan (0.1%) reduces bacterial counts on hands by 2.8 log₁₀ after a 1-minute hygienic handwash. In several studies, log reductions have been lower after triclosan is used than when chlorhexidine, iodophors, or alcohol-based products are applied. In 1994, FDA TFM tentatively classified triclosan $\leq 1.0\%$ as a Category IIISE active agent (i.e., insufficient data exist to classify this agent as safe and effective for use as an antiseptic handwash). Further evaluation of this agent by the FDA is underway. Like chlorhexidine, triclosan has persistent activity on the skin. Its activity in hand-care products is affected by pH, the presence of surfactants, emollients, or humectants and by the ionic nature of the particular formulation. Triclosan's activity is not substantially affected by organic matter, but it can be inhibited by sequestration of the agent in micelle structures formed by surfactants present in certain formulations. The majority of formulations containing <2% triclosan are well-tolerated and seldom cause allergic reactions. Certain reports indicate that providing hospital personnel with a triclosan-containing preparation for hand antisepsis has led to decreased MRSA infections. Triclosan's lack of potent activity against gram-negative bacilli has resulted in occasional reports of contamination.

On September 2, 2016, the FDA issued a final rule on consumer antibacterial hand soaps that prohibits the continued use of

triclosan and 18 other antibacterial ingredients, as of September 6, 2017. The FDA concluded that soap manufacturers had not submitted adequate safety evidence for use in these products; the agency did not conclude that triclosan in soap is unsafe.

Independent scientific committees of the European Commission have evaluated triclosan several times and concluded that it is a safe ingredient in toothpaste. The Scientific Committee on Cosmetic Products and Non-food Products (SCCNFP) in 2002, the Scientific Committee on Consumer Products (SCCP) in 2002 and 2006, and the Scientific Committee on Consumer Safety (SCCS) in 2011 concluded that triclosan use at 0.3% in toothpaste, the level found in Colgate Total, is considered safe. Accordingly, an April 2014 amendment to the EU Cosmetic Regulation confirmed triclosan's use in toothpastes up to 0.3%. In addition, the two EU medicinal authorities (the UK's MHRA and the Irish HPRA) who have reviewed Colgate Total[®] with triclosan have both approved it as a safe and effective toothpaste.

Reviews by the U.S. Food and Drug Administration, the American Dental Association, government agencies, and other independent sources in Europe and around the world confirm triclosan's safe use in toothpaste and recognize that Colgate Total[®] toothpaste provides an important oral health benefit.

Colgate Total[®] is the only toothpaste on the market that has undergone the U.S. Food and Drug Administration's rigorous New Drug Application (NDA) review process for efficacy and safety, including periodic reviews of scientific literature and safety information. As recently as November 25, 2013, the FDA continues to affirm its support for the use of Colgate Total[®] toothpaste to fight gum problems Colgate Total[®] is the only toothpaste that is both approved by the US FDA and accepted by the American Dental Association as safe and effective in helping to prevent gum problems.

References:

https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5116a1.htm http://www.colgatetotal.com/health-benefits/triclosan-safety-intoothpaste

HYGIENE SCIENCES

Best Practices in Scalp Hygiene

To give us a clearer picture, let's look at our hair and scalp separately.

First, our scalp has a skin-like structure, the cell layers are subject to constant rejuvenation and renewal. New cells replaces old cells. It is normal for us to lose up to 100 strands of hair a day. Our hair is held at its root to the scalp by hair follicle. Sebaceous glands secrets oil or sebum. Oil and moisture strengthen the hair follicles.



Normal human skin is colonized with bacteria; different areas of the body have varied total aerobic bacterial counts (e.g., 1×10^6 colony forming units (CFUs)/cm² on the scalp.

Caring for a Bald Head

The bare skin on the head requires just as much attention as hair. A hairless head is exposed to the sun, air, pollutants, chemicals, chlorine and allergens and can therefore develop scalp problems. Acne, rashes and problems like ingrown hair can also create hygiene and health issues.

Treating the skin on the scalp in the same way as the skin on the body means that you reduce the likelihood of dealing with issues like sunburn, flaky skin and infections. Rashes can be treated by applying cortisone cream that can be bought over the counter. Regular washing and moisturising of the scalp will reduce problems caused by perspiration, dryness and clogged follicles that may encourage acne and pimple inflammation.

Caring for a Shaved Head

Many men choose to remove their hair as a fashion statement. This requires daily shaving of the scalp and this regular action can encourage scalp dryness if particular attention is not given to the care of the scalp before and after shaving. Shaving too often can dry out and irritate the skin on the scalp so adequate moisturisation is recommended to help overcome this problem. It is also important to shave when the pores are open so after a hot shower is the ideal time.

Caring for a head that is regularly shaved is a simple routine. After shaving the head, using the preferred shaving cream, the scalp should be washed with gentle shampoo. After patting dry moisturiser can be applied all over the scalp. For men with a more defined sense of style there are a variety of moisturising products available that provide a matt or shiny finish on the scalp.

Dealing with Dandruff

Dandruff is a disorder of the scalp that causes itching and flaking of the skin and can affect the confidence and self-esteem of the sufferer.

The largest organ of the human body, the skin, is designed to slough-off the dead cells as new ones are produced. In many people there seems to be an increase in the amount of dead skin shed during this process. Dandruff can affect anyone of any age, race or culture and can vary in severity. It can present as large amounts of very small particles, to extremely large flakes, in both dry and greasy hair, and tends to be worse in the winter months.

Causes of Dandruff

Most experts agree that dandruff is the excessive production of a type of **fungus called Pityrosporumovale** which lives normally on our skin all of the time. What cannot be agreed upon is the cause for this over production. Theories range from the use of hair products, hormonal imbalances, dietary influences or from stress.

Treatments for Dandruff

The most obvious and easiest treatment for dandruff would be to change the shampoo and conditioners used and to limit or discontinue the use of styling products. Select products that are specially designed for the treatment of dandruff and use the same product continually; changing the product will prevent the full benefit of the anti-dandruff lotions from functioning as these sometimes take up to three weeks to take effect.

In severe cases, experts may recommend the use of solutions that contain zinc pyritheone or selenium sulphide. Both of these ointments contain active ingredients aimed at combating dandruff and are not harmful to the skin.

If you are worried about dandruff, seek advice from a skin specialist who will be able to advise you on whether a specialised lotion is required.

If there are any additional symptoms such as soreness or crusting of the scalp, this may indicate a different disorder such as ringworm or psoriasis which both need treating in a separate manner.

Preventing Dandruff

Many experts suggest that by gently massaging the scalp, aiding in increasing the blood circulation, instead of scratching it will help to prevent the skin being disrupted.

Limiting the use of hair products will also help to reduce the likelihood of flakes as many people find that certain hairsprays or gels dry over time and can appear as dandruff when the effects are wearing out.

It is thought a diet with sufficient vitamin B6 will help to prevent the scalp from sloughing-off dead skin cells, if you think you may be lacking in this substance, a supplement can be taken.

Hygiene Concerns

There is a growing concern that barbering procedures could create opportunities for HIV transmission. The instruments used were razor blades (11.1%), manual clippers (8.9%) and electric clippers (80%). Clippers were sterilized in 10% and disinfected in 72.5%, while no decontamination was carried out in 17.5% of the sessions. Fifty two percent of the disinfections involved the use of kerosene, a disinfectant not recommended for HIVinactivation; 48.3% of the disinfectants were not in the original containers while 53.4% of the sessions involved the use of same brush for cleaning clipper and brushing hair. Hand-held flame and Ultraviolet light sterilizer were used in 50% of the sterilization process. Barbers in the high-class peripheral communities were more likely to practice appropriate equipment decontamination than those from lower-class inner-core communities. There was blade-

Best Practices

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to-skin contact in all and accidental cuts occurred in three of the sessions and none was properly managed.

Head lice infestation is an infection of the scalp and skin which causes blood loss, discomfort, and social and psychological distress with the possibility of secondary bacterial infections occurring at scratch sites.

Pediculosis capitis, also known as head lice infestation, is caused by *Pediculushumanus capitis* an ectoparasite of man found on the hair and scalp.

TINEA CAPITIS (TC), a dermatophyte infection of the scalp and hair, is a common infection in children. It has become an important public health problem in the United States. The most prevalent causative organism in most of the country is *Trichophyton tonsurans*, which presents in various ways, from minimally symptomatic dandruff like scaling to tender, highly inflamed, purulent nodules known as kerions. If untreated, TC may lead to scalp scarring and permanent hair loss. Alternatively, asymptomatic infection may persist undetected for years, with shedding of spores and spreading to susceptible contacts.



Scalp *Tinea capitis* (scalp ringworm). (Source: University of California, Dermatology Glossary)

Clean your scalp: Massaging your hair with warm oil will help to clean your scalp. Massaging will help remove dandruff or dirt from the scalp. Oil can act as a conditioner and will protect your hair from getting rough and dry. Remove excess oil: While applying oil is healthy for hair, keeping excess oil on hair without washing will affect the health of your hair. It will accelerate the accumulation of dirt on the scalp. Always remember to wash off the excess oil with a mild shampoo. Clean your hair accessories and tools: Washing your comb is very important to maintain hair hygiene. Do not share your comb. Clean your hair accessories like hair clips or hair bands.

- Wash clothes, hats, and bedding that has been recently used in very hot water for at least 20 minutes and then dry in a hot dryer.
- Objects that cannot be washed can be bagged in plastic and put away for 10 days, or dry cleaned.
- Wash all combs in very hot (130° F) water every day.
- Put stuffed toys in the dryer at a high setting.
- In freezing weather (32° F or below), put items outside for 72 hours to kill lice and nits. Small items can go in the freezer.
- Thoroughly vacuum car seats, the family couch, carpets, and floors.
- Disinfectant should only be used on equipment or surfaces that have first been cleaned with a detergent.
- Wipe over all parts of the clippers with a 70% alcohol preparation(small alcohol wipes in individual sachets are ideal for this)
- Allow to dry before reusing.

Washing the scalp and the hair regularly can help avoid issues such as folliculitis and seborrheic dermatitis.

Folliculitis

Folliculitis is a rash-like appearing superficial infection of the hair follicles with purulent material in the epidermis.

Folliculitis Symptoms

It manifests as multiple small, raised, sometimes pruritic, erythematous lesions that are less than 5 mm in diameter. Pustules (or small white heads) may be present at the centers of the lesions. Hair follicles are often seen in the center of the bumps. Folliculitis is frequently observed in areas of repeated shaving. It can occur almost anywhere on the skin, but it is most commonly found on the neck, thighs, buttocks, or armpits. Coalescence of several follicular lesions or extension of a lone follicular lesion into deeper portions of the dermis may give rise to abscess formations, typically with pain and tenderness, and possible purulent discharge from the area.

Causes of Folliculitis

- Usual causes include normal skin floral carriage of *Staphylococcus aureus* that gets into the superficial portion of the hair follicle through shaving, friction, etc.
- Pseudomonas is a causative pathogen in the setting of inadequately chlorinated hot tubs, whirlpools, and swimming pools
- Fungal folliculitis is sometimes seen in the setting of broadspectrum antibiotic administration, glucocorticoid therapy, and/or immunocompromised individuals

Folliculitis Prevention

To reduce likelihood of folliculitis, take these steps to protect yourself:

- Wash your hands frequently.
- Use antibacterial soap and a clean washcloth and towel every time you shower or bathe.
- Avoid sharing towels, wash cloths, razors or other personal care items.
- Change clothes and shower with antibacterial soap after participating in sports or exercising.
- Avoid tight-fitting clothes that trap sweat and bacteria. Wash clothes worn next to the skin in very hot water.
- Avoid overuse of skin oils or make up, which can trap bacteria in your pores.
- Avoid public hot tubs or spas and shower with antibacterial soap after using one.
- Shave in the direction of hair growth with shaving gel or cream. Frequently cleanse razor w/antiseptic cleansing agent and change razor blade after each shaving.
- May use antiseptic cleansing agent designed for bodily use to shaved areas.

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AlcoMopTM is a perfumed disinfectant cleaner for floor and hard surfaces. Smart action formula with two active ingredients viz. Benzalkonium Chloride, kills the bacteria and other microbes leaving the surface squeaky clean and Ethanol, a good cleanser for hard tiles leaves no residue making the surface look glossy. **AlcoMop**TM spreads a distinctive aroma throughout the room adding to its fresh appeal.



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