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Committed to the advancement of Clinical & Industrial Disinfection & Microbiology VOLUME - X ISSUE - IV OCT - NOV 2017

## Editorial

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**Mini Review Section** - The term, '*Antimicrobials*' include all agents that act against all types of microorganisms, such as, bacteria (antibacterial), viruses (antiviral), fungi (antifungal) and protozoa (antiprotozoal). In contrast,'*Antibiotic*' refers to those substances which are produced by microorganisms that act against another microorganism. An essential feature for an antimicrobial agent is the *selective toxicity*, which means that it selectively kills or inhibits the growth of the microbial targets while causing minimal or no harm to the host.

**Current Trends Section** – Povidone iodine is an iodinated polyvinyl polymer used as topical antiseptic in surgery and for skin and mucous membrane infections. This unique complex was discovered in 1955 at the Industrial Toxicology Laboratories in Philadelphia by H. A. Shelanski and M. V. Shelanski.

**In Profile** – **"Daniel Nathans**" was an American microbiologist who received the Nobel Prize in Physiology or Medicine in 1978 along with Hamilton Othanel Smith of the United States and Werner Arber of Switzerland. They were awarded the prize for the discovery of 'restriction enzymes' which can be used to break the molecules of DNA into small manageable portions so that the characteristics can be studied better. This discovery later became the basic tool for research in genetics.

**Bug of the Month -** *Azotobacter vinelandii* is an aerobic soil-dwelling organism with a wide variety of metabolic capabilities which include the ability to fix atmospheric nitrogen by converting it to ammonia. Two features of the biology of *Azotobacter* make it of particular interest to scientists studying the nitrogen fixation process.

**Did You Know?** – With the incidence of cancer cases rising globally, it has become imperative to find natural ways to reduce the adverse effects of the two major methods for cancer treatment, chemotherapy and radiation therapy. **Garlic oil could be used as complementary medicine for cancer patients receiving Chemotherapy / Radiotherapy, to ease the adverse effects of these treatments.** 

**Best Practices** - Prompt removal of spots and spills of blood and body substance followed by cleaning and disinfection of the area contaminated is a sound infection control practice and meets occupational health and safety requirements. OSHA promulgated a standard entitled "Occupational Exposure to Blood borne Pathogens" to eliminate or minimize occupational exposure to blood borne pathogens. One component of this requirement is that all equipment and environmental and working surfaces be cleaned and decontaminated with an appropriate disinfectant after contact with blood or other potentially infectious materials.

Tickle yourself with a light humour in our Relaxed Mood section.

Looking forward for your valuable Feedback & suggestions.

### Mini Review

## Common Antibacterial Agents Grouped by Mechanism of Activity

The term, '*Antimicrobials*' include all agents that act against all types of microorganisms, such as, bacteria (antibacterial), viruses (antiviral), fungi (antifungal) and protozoa (antiprotozoal). In contrast, '*Antibiotic*' refers to those substances which are produced by microorganisms that act against another microorganism. Thus, antibiotics do not include antimicrobial substances that are synthetic (sulfonamides and quinolones), or semisynthetic (methicillin and amoxicillin), or those which come from plants (quercetin and alkaloids) or animals (lysozyme).

More than 50 antibiotics that act as cell wall synthesis inhibitors are currently available, with individual spectra of activity that afford a wide range of clinical applications.

An essential feature for an antimicrobial agent is the *selective toxicity*, which means that it selectively kills or inhibits the growth of the microbial targets while causing minimal or no harm to the host. The major targets of the antimicrobial agents are as shown in the figure are, the cell wall of the microorganisms, the DNA and the ribosomes. Most of the antimicrobial agents currently in the clinical use are antibacterial because the prokaryotic cell provides a greater variety of unique targets for selective toxicity, in comparison to fungi, parasites and viruses.



## Figure: Major targets of Antimicrobial agents: (1) Cell wall, (2) DNA, (3) Ribosomes

Each class of antibacterial agents has a unique mode of action, that is, the way in which an antibiotic affects the microbes at the cellular level, and these are summarized in the figure below:



## Antibiotics that inhibit cell-wall synthesis (B-lactams and others)

Several different classes of antibiotics block the steps in the biosynthesis of peptidoglycan, making these cells more susceptible to osmotic lysis. Two types of antimicrobial drugs work by inhibiting or interfering with cell wall synthesis of the target bacteria. Antibiotics commonly target the bacterial cell wall formation of which peptidoglycan is an important component because animal cells do not have cell walls. The peptidoglycan layer is important for the cell wall structural integrity, being the outermost and primary component of the wall. Therefore, antibiotics that target cell wall biosynthesis are bactericidal in their action. Because human cells do not make peptidoglycan, this mode of action is an excellent example of *selective toxicity* (Ghooi, et al. 1995).



#### β-lactam antibiotics

A class of broad-spectrum antimicrobial drugs or the antibiotics that interfere with cell wall synthesis is the **\beta-lactam antibiotics** (beta-lactam antibiotics).  $\beta$ -lactam antibiotics include penicillins and cephalosporins, both of which possess a beta-lactam ring. These include penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems (Holten and Onusko, 2000).

β-lactam antibiotics are bacteriocidal and act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls. The final transpeptidation step in the synthesis of the peptidoglycan is facilitated by DD-transpeptidases which are penicillin binding proteins (PBPs). PBPs vary in their affinity for binding penicillin or other β-lactam antibiotics. These agents are very potent bactericidal agent that attaches to the PBPs on the bacterial cell membrane. These PBPs are enzymes (e.g., transpeptidase, carboxipetidase and endopeptidase) that are involved in the synthesis of the cell wall and the maintenance of the cell's structural integrity, especially in gram-positive organisms, being the outermost and primary component of the wall (Sykes and Matthew, 1976).

 $\beta$ -lactam antibiotics are analogues of D-alanyl-D-alanine— the terminal amino acid residues on the precursor NAM/NAGpeptide subunits of the nascent peptidoglycan layer. The structural similarity between  $\beta$ -lactam antibiotics and D-alanyl-D-alanine facilitates their binding to the active site of PBPs (Fisher et al., 2005). The  $\beta$ -lactam nucleus of the molecule irreversibly binds to acylates the Ser403 residue of the PBP active site. This irreversible inhibition of the PBPs prevents the final crosslinking (transpeptidation) of the nascent peptidoglycan layer, disrupting cell wall synthesis (Zapun et al., 2017).

### Mini Review

## HYGIENE SCIENCES



**Figure:** Diagram depicting formation of cross-links in the bacterial cell wall by a penicillin binding proteins (PBPs) and subsequent inhibition by penicillin.

- 1. Bacterial cell wall consists of strands of repeating Nacetylglucosamine (NAG) and N-acetylmuramic acid (NAM) subunits. NAM subunits have short peptide chains attached to them.
- 2. PBP binds the peptide side chains and forms the cross-link with the expulsion of one D-alanine from one peptide side chain.
- 3. PBP dissociates from the wall once the cross-link has been formed.
- 4. Penicillin is added to the system. It enters the active site of the PBP and reacts with the serine group that is important in its enzymatic activity.
- 5. The  $\beta$ -lactam ring of penicillin (denoted as "P") is irreversibly opened during the reaction with the PBPs. Penicillin remains covalently linked to the PBPs and permanently blocks the active site.

Under normal circumstances, peptidoglycan precursors signal a re-organization of the bacterial cell wall and, as a consequence, trigger the activation of autolytic cell wall hydrolases. Inhibition of cross-linkage by  $\beta$ -lactams causes a build-up of peptidoglycan precursors, which triggers the digestion of existing peptidoglycan by autolytic hydrolases without the production of new peptidoglycan. As a result, the bactericidal action of  $\beta$ -lactam antibiotics is further enhanced.

Bacteria often develop resistance to  $\beta$ -lactam antibiotics by synthesizing a  $\beta$ -lactamase, an enzyme that attacks the  $\beta$ -lactam ring. To overcome this resistance,  $\beta$ -lactam antibiotics are often given with  $\beta$ -lactamase inhibitors such as clavulanic acid (King et al., 2017).

#### Penicillins

Penicillins compounds are highly effective antibiotics with extremely low toxicity. The base compound is an organic acid with a ß-lactam ring obtained from culture of the mold, *Penicillium chrysogenum*.

Penicillins have been



shown to inhibit bacterial cell wall synthesis, and interact with penicillin binding proteins, leading to bacterial lysis. These two mechanisms, the former more than the latter are believed to be responsible for their therapeutic potential. It has further been demonstrated that only actively multiplying cells are susceptible to bactericidal effects of the antibiotic, which is in accordance

with the suggested mechanism of action. Bacterial growth takes place in terms of size and number, both r e q u i r i n g additional cell wall. An increase in bacterial size is due to an increase in the volume of cytosol and area of



the cell wall (Morin and Gorman, 2014).

#### **Pharmacokinetics of Penicillins**

Penicillins vary in their resistance to gastric acid and therefore vary in their oral bioavailability. Parenteral formulations of ampicillin, piperacillin and ticarcillin are available for injection. Penicillins are polar compounds and are not metabolized extensively. They are usually excreted unchanged in the urine via glomerular filtration and tubular secretion; the latter process is inhibited by probenecid. Nafcillin is excreted mainly in the bile and ampicillin undergoes enterohepatic cycling. The plasma half-lives of most penicillins vary from 30 min to 1 h. Procaine and benzathine forms of penicillin G are administered intramuscularly and have long plasma half-lives because the active drug is released very slowly into the bloodstream. Most penicillins cross the blood-brain barrier only when the meninges are inflamed (Yılmaz and Özcengiz, 2017).

Penicillins derivatives are achieved by adding or modifying the side chains to the structure of the basic ß-lactam ring.

#### 1. Penicillin G.

Penicillin G is a narrow spectrum activity antibiotic agent which includes gram-positive cocci such as *streptococci* (including *S pneumoniae*, *S. pyogenes*, *S. agalactiae*), non-penicillinase producing *staphylococci* and certain gram-negative cocci such as *Neisseriae* spp. Also it is effective against spirochetes and some anaerobic bacteria. However, on the other hand, Penicillin G is ineffective against the bacterial species that produce enzyme of penicillinase (or beta lactamase). Penicillin G is incompletely absorbed since it is inactivated by gastric acid. Thus, it is used mainly as parenteral drug for serious infections with penicillinsensitive organisms. The toxicity of Penicillin G is extremely low; hypersensitivity to penicillins is commonly present. 1% to 8% of the general populations are allergic to the penicillins.

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### Mini Review

hypersensitivity reactions range from immediate anaphylactic reactions to late manifestations such as a skin rash.

#### 2. Penicillinase-resistant Penicillins (Methicillin, Oxacillin, and Nafcillin)

These are used to treat infections caused by penicillinaseproducing staphylococci, including bacteremia, cellulitis, and ostemyelitis. Compared with Penicillin G, the Penicillin-resistant Penicillins are less effective against Penicillin G-sensitive organisms.

3. Aminopenicillins (Ampicillin and Amoxicillin).

Aminopenicillins, developed by introducing an alfa-amino group into the benzyl chain, had enhanced activity against some gramnegative bacteria such as Escherichia coli, Proteus mirabilis, Salmonella, Shigella and Haemophilus influenzae. Because Aminopenicillins are very sensitive to the Penicillinase (betalactamase), so finally, they have been combined with betalactamase inhibitors to treat infections caused by beta-lactamase producing bacteria.

4. Extended-spectrum Penicillins (Carbenicillin, Mezlocillin and Piperacillin)

These are wider spectrum of action than Penicillin G, with special activity against Pseudomonas aeruginosa and some strains of Proteus. They are not penicillinase resistant and are available parenterally to treat systemic infections.

#### **Cephalosporins**

The Cephalosporins are a large group of beta-lactam antibiotics derived from 7 amino-cephalosporanic acid, which was originally isolated for a Cephalosporium mold (now termed as Acremonium spp.). The antibiotics have the same mechanism of action as the penicillins but have a wider antibacterial spectrum, these



are resistant to many beta-lactamase and have improved pharmacokinetic properties (Morin and Gorman, 2014).



Cephalosporium mold (Acremonium spp.)

#### **Pharmacokinetics of Cephalosporins**

The mold Acremonium (previously called Cephalosporium) vielded three main compounds, historically called Cephalosporin N and C, and P, from which the first cephalosporins were derived. The therapeutic activity of antibiotics depends on several factors including absorption, elimination kinetics, distribution in the body, minimal inhibitory concentrations (MIC), stability against enzymes, and plasma-protein binding (Singhvi et al., 1978).

The pharmacokinetics of cephalosporins depends up on to which

class of generation they belong to. Some of the factors are interrelated, for example, the extent of protein binding of an antibiotic influences its elimination kinetics, distribution into tissues, MIC, and antibacterial activity. To evaluate the potential efficacy of an antibiotic, it is important to know the extent of its binding to plasma proteins especially since the protein-bound fraction of the antibiotic is devoid of antibacterial activity. Cephalosporins are a new class of broad-spectrum antibiotics that bind to plasma proteins in different degrees (Singhvi et al., 1978). Reported values for protein binding range from 6% for cephradine to 92% for cefazolin.

Resistance to cephalosporin antibiotics can involve either reduced affinity of existing PBP components or the acquisition of a supplementary  $\beta$ -lactam-insensitive PBP. Currently, some Citrobacter freundii, Enterobacter cloacae, Neisseria gonorrhoeae, and Escherichia coli strains are resistant to cephalosporins. Some Morganella morganii, Proteus vulgaris, Providencia rettgeri, Pseudomonas aeruginosa, and Serratia marcescens strains have also developed resistance to cephalosporins to varying degrees.

The antimicrobial agents are divided into "generations" based on their structure and spectrum of activity.

1. First-generation Cephalosporins (Cefazolin, Cephalothin)

They have good activity against gram-positive cocci, and also effective against some gram-negative bacteria including E.coli, the genera Klebsiella and Shigella.

2. Second-generation Cephalosporins (Cefoxitin, Cefuroxime) The spectrum of the 2<sup>nd</sup> Cephalosporins is expanded to include more gram-negative. They are may be less active than the 1<sup>st</sup> generation Cephalosporins against gram-positive cocci.

3. Third-generation Cephalosporins (Cefoperazone, Cefotaxime, Ceftriaxone)

Generally, are less active than the 1<sup>st</sup> generation Cephalosporins against gram-positive cocci, but they are more active than the 2<sup>nd</sup> generation Cephalosporins against gram-negative bacteria. These 3<sup>rd</sup> generation Cephalosporins are also effective against anaerobes.

4. Fourth-generation Cephalosporins (Cefepime, Cefpirome) They are new 4th generation parenteral cephalosporins with a spectrum of activity which makes them suitable for the treatment of infections caused by a wide variety of bacteria. Fourthgeneration Cephalosporins are considered to be 'a class of highly potent antibiotics that are among medicine's last defenses against several serious human infections' (Weiss, 2007).

They are structurally related to third-generation cephalosporins but possess an extra ammonium group, which allows them to rapidly penetrate through the outer membrane of gram-negative bacteria, enhancing their activity. They are also active against βlactamase producing Enterobacteriaceae which may inactivate third-generation cephalosporins. Some fourth-generation cephalosporins have excellent activity against gram-positive bacteria such as methicillin-susceptible staphylococci, penicillin-resistant pneumococci and viridans group streptococci. Cefepime is the only fourth generation cephalosporin available in the United States. Cefpirome is available overseas (www.drugs.com, 2017).

#### **Other Beta-Lactam Antibiotics**

Several beta-lactam antibiotics have slightly different biochemical structures from the penicillins and cephalosporins but have similar potent antibacterial activity.

**Imipenem** is a carbepenem with excellent *in-vitro* and *in-vivo* activity for aerobic and anaerobic gram-positive and gramnegative bacteria.

Aztreonam, a monobactam, is a narrow-spectrum antibiotic with activity specific for gram-negative bacilli.

....to be continued in next issue.

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## **Povidone Iodine and its Applications**

Povidone iodine is an iodinated polyvinyl polymer used as topical antiseptic in surgery and for skin and mucous membrane infections.

Povidone-iodine is a stable chemical complex of polyvinylpyrrolidone (povidone, PVP) and elemental iodine. It contains from 9.0% to 12.0% available iodine, calculated on a dry basis. This unique complex was discovered in 1955 at the Industrial Toxicology Laboratories in Philadelphia by H. A. Shelanski and M. V. Shelanski. They carried out tests in vitro to demonstrate anti-bacterial activity, and found that the complex was less toxic in mice than tincture of iodine. Human clinical trials showed the product to be superior to other iodine formulations. Povidone-iodine was immediately marketed, and has since become the universally preferred iodine antiseptic.



#### Compatibility

PVP-iodine systems should be mildly acidic since alkaline solutions, including ammonia, and reducing agents lower the available iodine which in turn, results in lower antiseptic activity pvp- iodine is compatible with steel, wool and plastic but reacts with silver.

#### **Biocidal Activity:**

In vitro results however should be considered only as preliminary measures of "cidal" efficacy. Factors such as skin penetration and reactivity can completely reverse any effectiveness rating determined by purely in vitro test methods. The invitro biocidal activity of PVP-iodine has been studied for years against bacteria, yeast and molds, Actinomycetes and rickettsia.

#### Activity of PVP iodine Vs organisms

Proteus Staphylococcus Pseudomonas Streptococcus Escherichia Salmonella Candida

Serralia Spores-baccillus; clostridium Trichomonas Enterobacter Klebsiella Clostridium Shigella Corynebacterium Diplococcus Mycobacterium Bacillus Sarcina Trichophyton Aspergillus Mima Herella Edwardsiella Citrobacter Providencia Acienetobacter Epidermophyton Microsporum Pencillium Nocardia Anti HIV

#### Indications:

Povidone-Iodine solutions in water or alcohol are better tolerated than iodine solutions with comparable efficacy.

#### **Pharmacology:**

Elemental iodine has a very broad antimicrobial spectrum: bacteria, viruses, bacteria endosperm, fungi, and protozoa's are destroyed through oxidative interaction and direct iodination of biological macromolecules. However, there have been reports of certain resistant germs. Povidone-iodine (synonym-PVP-iodine) is an iodophor, i.e. it is a labile complex of iodine with the polyvinylpyrrolidone, from which iodine is continually delivered. Only this free iodine has antimicrobial activity.

#### **Precautions:**

Spills should be cleaned as soon as possible wash affected area with dilute ammonia (or) dilute sodium thiosulfate solution and large amount of water.

#### **Application:**

#### **Treatment of wounds:**

PVP-Iodine Topical Solution (10% PVP Iodine containing 1.0% available iodine) is effective for ridding and preventing infections, including and preventing infections, including those with severe ulceration

#### Scrub and skin disinfection:

PVP-Iodine surgical scrub is a 7.5% PVP-Iodine solution (0.75% available iodine) containing various agents for wound and skin cleansing. It should be rinsed off immediately after use to minimize skin irritation and healing retardation. To reduce germs on skin and prevent infection in skin, the PVP-Iodine Topical solution containing 10% PVP-Iodine (1% available iodine) should be used. The PVP-Iodine film should remain on the skin so that it can act as a continued antimicrobial barrier. To measure the efficacy of surgical scrubs, samples of scrub juices were taken to establish immediate, cumulative and persistent effects. The immediate effect is the reduction of bacteria found immediately after scrubbing. A cumulative effect is seen when regular use of the scrub leads to increasing reductions of bacteria .The final measurements persistence of effect, is defined as a decline in the post wash bacterial count . Studies with PVP-Iodine scrubs showed an effective, extensive immediate effect, a definite cumulative effect, and a persistence of effect.

Preparation of the skin, pre-surgery: Numerous studies indicate the efficiency of PVP-iodine for presurgical skin preparation. There is even evidence that is effective against skin spores.

#### Skin Burns, Abrasions and Infections:

Topical PVP-Iodine Antiseptics, Aerosol Sprays, Ointments (5% PVP-Iodine, 0.5% available iodine) and creams (5% PVP-Iodine, 0.5% available iodine) have been used to prevent microbial contamination in burns, incisions and infected ulcers. Unlike iodine products, PVP- Iodine preparations may be bandaged. These products should not be used on deep wounds or serious burns without consulting a physician. Use should be discontinued if redness, irritation, swelling or pain persists or increases.

#### Scalp, Vaginal and Throat Infections:

Shampoo containing 7.5% PVP Iodine has been reported to yield a significantly larger reduction of the germ count in the scalp and hair than products without PVP Iodine. Douche and vaginal suppositories containing 10% PVP Iodine has been reported effective in the treatment of vaginal infections. A mouth wash /gargle product containing 0.5% PVP Iodine is effective in reducing odour causing bacteria.

For various surgical procedures:

PVP-Iodine products have been widely used in various surgical procedures and shown to significantly lower subsequent infection rates.

#### **Veterinary Medicine:**

PVP-Iodine products have been used topically in the treatment of various swelling, chronic inflammatory conditions, sprains, bruises, obstinate ulcers, and to disinfect the umbilical stump of foals and calves. Because of its low toxicity and highly effective antimicrobial activity, topical PVP-Iodine applications have particular advantages in treating skin infection of cats, dogs, or other animals that lick wounds. PVP-Iodine has also been found to be highly effective in treating bacterial and fungal fish eggs, thereby increasing the hatching yield. Additionally, scrub and antiseptic solutions containing PVP-Iodine have been reported as highly effective for use on dogs, cats and horses for various presurgical procedures.

#### Medical Equipment:

PVP-Iodine has been reported as an effective disinfectant for such diverse items as contact lenses, dialysis equipment, fiberscope, endoscopes, and a wide variety of dental and medical equipment

#### **Ophthalmic Applications**

5% Sterile Ophthalmic Prep Solution for the eye is indicated for prepping of the periocular region (lids, brow, and cheek) and irrigation of the ocular surface (cornea, conjunctiva, and palpebral fornices).

#### Advantages

- Stable complex
- No general odour
- No loss of iodine Rapid action even in presence of organic matter such as blood, pus, oil, grease, soap, etc.
- Film forming capacity
- Prolonged germicidal action
- Adheres to treated surface where applied colour delineates treated area.
- Water soluble so ease for formulation
- Non staining
- Low animal and phytotoxicity
- Non irritating to skin and mucous membranes
- Non sensitizing
- Non stinging
- It has reduced amount hazard
- Broad spectrum antiseptic
- Unparalleled for surface sterilization and in mixed infection

#### **Formulation: Application**

1. PVP iodine dermal solution 5%

Wounds and burns, pre-operative preparation of skin, mucous membrane, emergency microbial treatment of wounds, cuts and laceration skin

- 2. PVP iodine surgical scrub 7.5% disinfection in surgical procedure
- 3. PVP iodine hand cleaners liquid 7.5% Mild and gentle hand cleaner with iodine bactericidal activity
- 4. PVP iodine liquid soap 7.5% Antiseptic
- 5. PVP iodine shampoo 4% Antidandruff seborrhea scalp condition
- PVP iodine ointment 10%
  Boils, furnculosis, impetigo, sycosisbarbae otitis externa, paronychia, secondary infection of burns, wounds, fungi infection, taeniapediscorporis, cruris, versicolor, cutaneous candidacies.
- 7. PVP iodine mouse wound dressing with allantoin 10% Infected skin lesion, burns
- 8. PVP iodine cream 5% infections

### **Current Trends**

- 9. PVP iodine vaginal gel 10% Vaginal infection
- 10. PVP iodine vaginal tablets 200 mg Candidialtrychomonal non-specific vaginitis
- 11. PVP iodine vaginal douche 10% Candidialtrychomonal non-specific vaginitis
- 12. PVP iodine vaginal suppositories 10% candidiasis
- 13. PVP iodine gargle 1% acute oral mucosal infection
- PVP iodine dusting power 5% infected skin lesion, exudative wounds, post surgical dressing, episiotomy
- 15. PVP iodine aqueous spray with hydrocarbon propellant 5% Topical antiseptic
- PVP iodine dry power aerosol spray 5% Topical antiseptic
- 17. PVP iodine swab sticks 10% Topical antiseptic
- Lotion 10% Topical antiseptic
- 19. PVP iodine aqueous spray with allantoin 5% Topical antiseptic
- 20. VC-kit 10%

Candidialtrychomonal non-specific vaginitis

- 21. Liposomal PVP Iodine formulation containing 4.5 to 5% PVP Iodine
  - Viricidal efficacy as well as cytotoxicity effect
- 22. Liposomal Taurolidine & Povidone Iodine 0.01% Malignant pleural mesothelioma
- 23. Liposomal hydrogel with 3% PVP Antiinflammatory
- 24. Emulsions 4% PVP Iodine Topical Antiseptic
- 25. PVP Iodine gels as the lubricant Cathetarization of the urinary bladder
- 26. Povidone Iodine gel 7.5% Anti microbial
- 27. Solution of the Povidone Iodine diluted with the Ringers solution for reducing toxicity in the concentration of 0.23% Anti viral& Pathogenic H5N1 Avian influenza

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#### **Daniel Nathans**



Daniel Nathans was an American microbiologist who received the Nobel Prize in Physiology or Medicine in 1978 along with Hamilton Othanel Smith of the United States and Werner Arber of Switzerland. They were awarded the prize for the discovery of 'restriction enzymes' which can be used to break the molecules of DNA into small manageable portions so that the characteristics can be studied better. This discovery later became the basic tool for research in genetics. Smith had isolated the bacterium called 'Haemophilus influenzae' earlier which was used by Nathans in the investigations he carried out in relation to the structure of the DNA of the 'simian virus 40' or 'SV40'. This virus was the simplest one known for causing cancerous tumors. Nathans constructed a genetic map of the virus which helped in identifying the molecular structure of a cancer cell with the help of the 'restriction enzymes'. He also took part in developing prenatal procedures for testing genetic diseases such as 'sickle cell anemia' and 'cystic fibrosis'. He received many awards and honors for his work and was known as an outstanding mentor, teacher and researcher. He was also an able administrator and could execute his administrative work in a fair, thoughtful, deliberate and clearheaded manner.

#### Childhood & Early Life

- Daniel Nathans was born on October 30, 1928 in Wilmington, Delaware, USA. His father was Samuel Nathans and his mother was Sarah Levitan, both Jewish immigrants from Russia.
- □ He was the youngest of the eight children of Samuel and Sarah Nathans.
- □ He did his schooling from the public schools in Wilmington and had to do part-time jobs from the age of ten to support his family.
- □ After finishing school he attended the 'University of Delaware' to study philosophy, chemistry and literature.
- □ At his father's insistence he decided on a medical career after he received his B.Sc. degree in chemistry from the 'University of Delaware' in 1950.

- □ He joined the 'Washington University School of Medicine' in St Louis, Missouri. He planned of returning to Wilmington after becoming a doctor.
- □ Even though he loved the medical training very much, he was especially attracted towards laboratory research while working under the famous pharmacologist, Oliver Lowry during the summer of 1951.
- □ He earned his medical degree in 1954 and resolved to pursue a career in academic medicine where he could treat patients as well as carry out research work.

#### Career

- Daniel Nathans joined the 'Columbia Presbyterian Hospital' and worked as an intern for some time under the supervision of Robert Loeb.
- □ Before the start of his medical residency period, he joined the 'National Cancer Institute' under the 'National Institutes of Health' in Bethesda, Maryland as a clinical associate. While working there, he divided his time treating patients who were receiving experimental chemotherapy for cancer and on research related to 'plasma-cell tumors' found in mice which were similar to 'multiple myeloma' found in humans.
- □ Surprised at the lack of information on cancer biology, he started his research on the synthesis of proteins in 'myeloma tumors' and was soon able to publish his findings.
- □ He returned to the 'Columbia Presbyterian Hospital' in 1957 to complete a two year medical residency and to carry on with his ambition of following a career in academic medicine.
- □ He continuing with his research on the problem of protein synthesis whenever he had spare time after treating patients.
- □ In 1959 he decided to devote all his time to research and joined the 'Fritz Lipmann laboratory' at the 'Rockefeller Institute' in New York as a research associate.
- □ It was an interesting time for Nathans at the 'Rockefeller Institute', as great mysteries like the way in which 'genetic material DNA' directs the production of the required proteins and enzymes by the cells were being unraveled together by microbiologists, biochemists and geneticists.
- □ Nathans initially went on with his efforts in synthesizing protein from the extracts of cells affected by myeloma. He pursued the problem with cultures of the E.Coli bacteria when persuaded by Smith, a postdoctoral colleague.
- □ His three years at the 'Rockefeller Institute' convinced him that the 'science of medicine' was better than the 'practice of medicine' and he stared looking for a place where he could do more research as well as teach.
- He joined the 'John Hopkins University' in Baltimore as a professor of microbiology in 1972 when the post was offered to him by W. Barry Wood who had been one of his mentors during medical school and now held the chair at the 'John Hopkins University'.
- □ He continued with his research on protein synthesis and also on the blocking effect of antibiotics like 'puromycin' on the process.
- During the mid-1960s he was asked to give a few lectures on animal viruses when a couple of virologists left their posts. He became interested in viruses that cause tumors while preparing for his lectures.

### In Profile

## JOURNAL OF \_\_\_\_\_\_

- □ In 1969 he took a six-month sabbatical leave to be at the 'Weizmann Institute' in Israel to learn more about the techniques of cell-culture and about a small tumor virus known as the 'simian virus 40' or 'SV40'.
- □ While he was in Israel, his colleague Hamilton Smith at the JHU informed him about the bacteria called 'Haemophilus influenzae' that could cut the DNA of other species at particular places. He immediately recognized that such an enzyme could be used to make small uniform fragments of the DNA of a virus so that it could be mapped and its structure determined.
- □ He brought back some of the SV40 with him from Israel and started to apply the enzyme discovered by Smith on it and other 'restriction enzymes' on it. Soon they were able to map the 'SV40' virus and the 'restriction enzymes' was established as the tool for molecular biology and genetics.
- □ He continued his researches on SV40 through the 1980s and later shifted from tumor viruses to cellular genes which got activated when cells were given stimulation to grow and multiply.
- □ He served as the director of the 'Microbiology Department' of the JHU from 1972 to 1982.
- □ He served as the 'Senior Investigator' of the 'Howard Hughes Medical Institutes from 1981 to 1999.

□ He served as the interim president of JHU during from 1995 to 1996 after which he returned to his research work.

#### Major Works

□ Daniel Nathans published 138 books, articles and chapters during his entire career.

#### Awards & Achievements

- □ Daniel Nathans received the 'Selman Waksman Award' in Microbiology' in 1967.
- □ He was awarded the Nobel Prize in 1978 and the 'National Medal of Science' in 1993 along with six honorary doctorates.
- □ The 'McKusick-Nathans Institute of Genetic Medicine' was founded by JHU in 1999.

#### Personal Life & Legacy

- He was married to Joanne Gomberg, a lawyer, whom he had met in Bethesda. They had three sons from the marriage named Eli, Ben and Jeremy.
- □ Daniel Nathans died of leukemia on November 16, 1999 in Baltimore.



Teacher: "Kids, what does the chicken give you?" Student: "Meat!"

Teacher: "Very good! Now what does the pig give you?" Student: "Bacon!"

Teacher: "Great! And what does the fat cow give you?" Student: "Homework!"

Teacher: "If I gave you 2 cats and another 2 cats and another 2, how many would you have?"

Johnny: "Seven."

Teacher: "No, listen carefully... If I gave you two cats, and another two cats and another two, how many would you have?"

Johnny: "Seven."

Teacher: "Let me put it to you differently. If I gave you two apples, and another two apples and another two, how many would you have?"

Johnny: "Six."

Teacher: "Good. Now if I gave you two cats, and another two cats and another two, how many would you have?" Johnny: "Seven!"

Teacher: "Johnny, where in the heck do you get seven from?!"

Johnny: "Because I've already got a freaking cat!"

A husband asks his wife, "Will you marry after I die?" The wife responds, "No, I will live with my sister." The wife asks him back, "Will you marry after I die?" The husband responds, "No, I will also live with your sister.

Is Google male or female?

A: Female, because it doesn't let you finish a sentence before making a suggestion.

A doctor and a lawyer are talking at a party. Their conversation is constantly interrupted by people describing their ailments and asking the doctor for free medical advice. After an hour of this, the exasperated doctor asks the lawyer, "What do you do to stop people from asking you for legal advice when you're out of the office?" "I give it to them," replies the lawyer, "and then I send them a bill." The doctor is shocked, but agrees to give it a try. The next day, still feeling slightly guilty, the doctor prepares the bills. When he goes to place them in his mailbox, he finds a bill from the lawyer.

### **Brainteasers**



 A doctor gave a microbiologist a juice glass, a dinner plate, water, a match, and a lemon wedge. The doctor poured enough water onto the plate to cover it. The doctor told the microbiologist, "If you can get the water on the plate into this glass without touching or moving this plate, I will give you \$100. You can only use the match and lemon to do this."

A few minutes later, the microbiologist walked away with \$100 in her pocket. How did the microbiologist get the water into the glass?

- 2. Inside each set of the following words, there is a pair of smaller words. By putting "&" between them, you'll make a familiar phrase. For example, "Thighbone/Swallowtail" conceals "High & Low."
  - 1. Skyrocketing/Trolleyman
  - 2. Thermometer/Apoplexy
  - 3. Delaware/Bordering
  - 4. Surprised/Trashiness
  - 5. Throughout/Stumblebum
- 3. What do the following words have in common?
  - Assess Banana Dresser Grammar Potato Revive Uneven Voodoo

#### Solutions

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

- 2. 1. Rock & Roll
  - 2. Mom & Pop
  - 3. Law & Order
  - 4. Rise & Shine 5. Rough & Tumble
- 3. If you take the first letter and move it to the end and read the word backwards, you will have the same word.

### Bug of the Month

# Azotobacter vinelandii



Azotobacter vinelandii is an aerobic soil-dwelling organism with a wide variety of metabolic capabilities which include the ability to fix atmospheric nitrogen by converting it to ammonia. Like *Klebsiella pneumoniae* it fixes nitrogen in the free-living state and does not enter into symbioses with plants; a process typified by the symbiosis between members of the genus *Rhizobium* and a variety of leguminous plants. Two features of the biology of *Azotobacter* make it of particular interest to scientists studying the nitrogen fixation process.

Firstly, *Azotobacter vinelandii* is capable of synthesising not only the molybdenum-containing nitrogenase enzyme that typifies most diazotrophs including *Klebsiella pneumoniae* and *Rhizobium leguminosarum*, but also two alternative nitrogenases; one in which vanadium replaces molybdenum and a second which contains neither transition metal but only iron. This ability to carry out the chemistry of nitrogen reduction at sites that do not contain molybdenum is of particular importance to chemists and biochemists investigating the mechanism of biological nitrogen fixation. The alternative nitrogenases are encoded by distinct structural genes, *vnfHDGK* and *anfHDGK*: the *vnfG* and *anfG* genes encoding an extra small subunit not found in molybdenum nitrogenase. However many of the same ancillary genes e.g. *nifUSVWZ* and *nifM* are used in biosynthesis of all three enzymes.

Synthesis of the alternative nitrogenases is regulated by availability of the appropriate metals i.e. molybdenum or vanadium, and expression of each set of genes is controlled by a specific regulatory protein, the products of the *nifA*, *vnfA* and *anfA* genes. Interest in this regulation has focussed research on the mechanisms whereby *Azotobacter* transports molybdate into the cell and distinguishes it from similar molecules such as

sulphate. This has led to the dissection of the molybdate transport genes, *modEABC* and *modG* of *Azotobacter* that have homologues in many other bacteria.



Secondly, *Azotobacter* has evolved a number of physiological mechanisms to allow it to fix nitrogen aerobically despite the inherent oxygen-sensitivity of nitrogenase. It has uniquely high rates of respiration coupled with specific cytochromes to ensure that nitrogenase experiences an essentially anoxic environment despite the fact that energy is being derived from aerobic metabolism. It can also synthesise a protective 2Fe-2S protein which can bind to nitrogenase in conditions of oxygen stress to form an oxygen-stable complex that is inactive but protected from damage.

Current studies are focussed on the transcriptional regulatory proteins NifA and NifL (Ray Dixon), the complex mechanisms underlying the regulation of the three different nitrogenase systems (Martin Drummond), and the molybdenum transport proteins of *Azotobacter*. More details of current research projects in this area can be obtained by reference to individual researchers who work with *Azotobacter*.

## Garlic Oil May Reduce Adverse Effects of Chemotherapy and Radiotherapy

Garlic oil could be used as complementary medicine for cancer patients receiving Chemotherapy / Radiotherapy, to ease the adverse effects of these treatments, reveals a study published in the Journal of Food Science.



Chemo and radiation therapy are effective against the cancer, but they also have many side effects that adversely affect the patient's body. Side effects such as anemia (causing fatigue, paleness, shortness of breath, and a fast heartbeat) and blood clotting problems (causing small red spots on the skin, bloody or black bowel movements or vomit, or bleeding from the nose or gums) are often seen with chemotherapy because these drugs and radiation adversely affect the body's production of healthy blood cells including RBC, WBC, and platelets.

With the incidence of cancer cases rising globally, it has become imperative to find natural ways to reduce the adverse effects of the two major methods for cancer treatment, chemotherapy and radiation therapy.

Anticancer effects of garlic and its products have already been demonstrated by various studies but not much information is

available on whether garlic benefits people undergoing chemo/ radiotherapy.

So, researchers at School of Public Health, Shandong University, China, set out to investigate the effects of garlic oil on the adverse effects of chemo/radiotherapy in mice with cancerous tumors.

In the chemotherapy test, the tumor-bearing mice were treated with cyclophosphamide (CTX) or CTX plus garlic oil for 14 days, while the mice received a single 5 Gy radiation or radiation plus garlic oil in radiotherapy test.

The findings showed that garlic oil did not enhance the sensitivity of cancer cells to chemotherapy or radiotherapy, but they did have the following positive results with garlic oil co-treatment -

CTX / radiation plus garlic oil **suppressed the decrease** of the peripheral total white blood cells (WBCs) count induced by chemo/radiation.

Added garlic oil treatment significantly **inhibited the decrease** of the DNA contents and the micronuclei ratio of the bone marrow. Micronuclei are small nuclei in the DNA that are essential for reproduction.

The garlic oil treatment also **suppressed the reduction** of the endogenous spleen colonies induced by CTX/radiation. Spleen colonies are stem cells located in the bone marrow that is responsible for the formation of RBC, WBC, and platelets and have the capability of self-renewal.

The findings thus support the idea that garlic oil does help reduce the adverse effects of chemotherapy and radiotherapy.

However, further research is needed to find out if garlic oil could reduce the adverse effects induced by other chemotherapeutic drugs.

#### **Reference:**

http://www.ncbi.nlm.nih.gov/pubmed/23772706

### **Best Practices**

# **Blood Spills Disinfection in Healthcare**

#### **OSHA Blood borne Pathogen Standard**

OSHA promulgated a standard entitled "Occupational Exposure to Blood borne Pathogens" to eliminate or minimize occupational exposure to blood borne pathogens. One component of this requirement is that all equipment and environmental and working surfaces be cleaned and decontaminated with an appropriate disinfectant after contact with blood or other potentially infectious materials. Even though the OSHA standard does not specify the type of disinfectant or procedure, the OSHA original compliance document suggested that a germicide must be tuberculocidal to kill the HBV.

To follow the OSHA compliance document a tuberculocidal disinfectant (e.g., chlorine) would be needed to clean a blood spill. OSHA amended its policy and stated that EPA-registered disinfectants labeled as effective against HIV and HBV would be considered as appropriate disinfectants ". . . provided such surfaces have not become contaminated with agent(s) or volumes of or concentrations of agent(s) for which higher level disinfection is recommended." When bloodborne pathogens other than HBV or HIV are of concern, OSHA continues to require use of EPA-registered tuberculocidal disinfectants or hypochlorite solution (diluted 1:10 or 1:100 with water). Studies demonstrate that, in the presence of large blood spills, a 1:10 final dilution of EPA-registered hypochlorite solution initially should be used to inactivate blood borne viruses to minimize risk for infection to health-care personnel from percutaneous injury during cleanup.

#### **Organic and Inorganic Matter**

Organic matter in the form of serum, blood, pus, or faecal or lubricant material can interfere with the antimicrobial activity of disinfectants in at least two ways. Most commonly, interference occurs by a chemical reaction between the germicide and the organic matter resulting in a complex that is less germicidal or non-germicidal, leaving less of the active germicide available for attacking microorganisms.

Chlorine and iodine disinfectants, in particular, are prone to such interaction. Alternatively, organic material can protect microorganisms from attack by acting as a physical barrier.

The effects of inorganic contaminants on the sterilization process were studied. These and other studies show the protection by inorganic contaminants of microorganisms to all sterilization processes results from occlusion in salt crystals. This further emphasizes the importance of meticulous cleaning of medical devices before any sterilization or disinfection procedure because both organic and inorganic soils are easily removed by washing.

If a sharps injury is possible, the surface initially should be decontaminated then cleaned and disinfected (1:10 final concentration). Extreme care always should be taken to prevent percutaneous injury. At least 500 ppm available chlorine for 10 minutes is recommended for decontaminating CPR training manikins. Full strength bleach has been recommended for self-disinfection of needles and syringes used for illicit-drug injection when needle-exchange programs are not available. The difference in the recommended concentrations of bleach reflects the difficulty of cleaning the interior of needles and syringes and the use of needles and syringes for parenteral injection.

The use of powders, composed of a mixture of a chlorinereleasing agent with highly absorbent resin, for disinfecting spills of body fluids has been evaluated by laboratory tests and hospital ward trials. The inclusion of acrylic resin particles in formulations markedly increases the volume of fluid that can be soaked up because the resin can absorb 200–300 times its own weight of fluid, depending on the fluid consistency. When experimental formulations containing 1%, 5%, and 10% available chlorine were evaluated by a standardized surface test, those containing 10% demonstrated bactericidal activity. One problem with chlorine-releasing granules is that they can generate chlorine fumes when applied to urine.

Health services should have management systems in place for dealing with blood and body substance spills. Protocols should be included in procedural manuals, and emphasised in ongoing education or training programs.

## The basic principles of blood and body fluid/substance spills management are:

- standard precautions apply, including use of personal protective equipment (PPE), as applicable
- spills should be cleared up before the area is cleaned (adding cleaning liquids to spills increases the size of the spill and should be avoided)
- generation of aerosols from spilled material should be avoided.

Using these basic principles, the management of spills should be flexible enough to cope with different types of spills, taking into account the following factors:

- the nature (type) of the spill (for example, sputum, vomit, faeces, urine, blood or laboratory culture)
- the pathogens most likely to be involved in these different types of spills for example, stool samples may contain viruses, bacteria or protozoan pathogens, whereas sputum may contain *Mycobacterium tuberculosis*
- the size of the spill for example, spot (few drops), small (<10 cm)="" or="" large="">10cm)
- the type of surface for example, carpet or impervious flooring
- the location involved that is, whether the spill occurs in a contained area (such as a microbiology laboratory), or in a public or clinical area of a health service, in a public location or within a community premises
- whether there is any likelihood of bare skin contact with the soiled (contaminated) surface.

#### Cleaning spills – equipment

Standard cleaning equipment, including a mop, cleaning bucket and cleaning agents, should be readily available for spills management. It should also be stored in an area known to all. This is particularly important in clinical areas.

To help manage spills in areas where cleaning materials may not be readily available, a disposable 'spills kit' could be used, containing a large (10 L) reusable plastic container or bucket with fitted lid, containing the following items:

- appropriate leak-proof bags and containers for disposal of waste material
- a designated, sturdy scraper and pan for spills (similar to a 'pooper scooper')

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### **Best Practices**

## HYGIENE SCIENCES

- about five sachets of a granular formulation containing 10,000 ppm available chlorine or equivalent (each sachet should contain sufficient granules to cover a 10-cm diameter spill)
- disposable rubber gloves suitable for cleaning (vinyl gloves are not recommended for handling blood)
- eye protection (disposable or reusable)
- a plastic apron
- a respiratory protection device, for protection against inhalation of powder from the disinfectant granules or aerosols (which may be generated from high-risk spills during the cleaning process).
- destroyed by incineration
- immersed in sodium hydroxide or sodium hypochlorite for 1 hour, rinsed and placed in a pan of clean water, and sterilised on an 18-minute cycle.

Single-use items in the spills kit should be replaced after each use of the spills kit.



With all spills management protocols, it is essential that the affected area is left clean and dry.

Sodium hydroxide (caustic soda) spills kits should be available for areas at risk for higher-risk Creutzfeldt–Jakob disease (CJD) spills, such as in neurosurgery units, mortuaries and laboratories.

#### Cleaning spills - procedures

In clinical areas, blood and body fluid/substance spills should be dealt with as soon as possible. In operating rooms, or in circumstances where medical procedures are under way, spills should be attended to as soon as it is safe to do so.

Care should be taken to thoroughly clean and dry areas where there is any possibility of bare skin contact with the surface (for example, on an examination couch).

PPE should be used for all cleaning procedures, and disposed of or sent for cleaning after use. Hands should be washed and dried after cleaning.

Where a spill occurs on a carpet, shampoo as soon as possible. Do not use disinfectant. Steam cleaning may be used instead. Wash hands thoroughly after cleaning is completed.

#### **Cleaning spots or small spills**

Spots or drops of blood or other small spills (up to 10 cm) can easily be managed by wiping the area immediately with paper towels, and then cleaning with warm water and detergent, followed by rinsing and drying the area. Dry the area, as wet areas attract contaminants. A hospital-grade disinfectant can be used on the spill area after cleaning.



#### **Cleaning large spills**

Where large spills (more than 10 cm) have occurred in a 'wet' area, such as a bathroom or toilet area, the spill should be carefully washed off into the sewerage system using copious amounts of water and the area flushed with warm water and detergent.

Large blood spills that have occurred in 'dry' areas (such as clinical areas) should be contained and generation of aerosols should be avoided.

Granular formulations that produce high available chlorine concentrations can contain the spilled material and are useful for preventing aerosols. A scraper and pan should be used to remove the absorbed material. The area of the spill should then be cleaned with a mop, and bucket of warm water and detergent. The bucket and mop should be thoroughly cleaned after use and stored dry.

#### Sodium hypochlorite (bleach)

Hypochlorites are corrosive to metals and must be rinsed off after 10 minutes and the area dried.

#### Cleaning spills that contain Creutzfeldt–Jakob disease prions

If a spill of tissue that is definitely or potentially infected with CJD prions occurs (for example, brain tissue), the contaminated item should either be:

The items should then be cleaned following routine cleaning and sterilisation procedures.

Surface spills should be cleaned up using paper towels before the surface is wiped with either sodium hydroxide or sodium hypochlorite, left for 1 hour (if possible, or as long as possible, with the area cordoned off), the solution wiped off and the surface cleaned by following routine cleaning procedures.

#### **References:**

https://www.cdc.gov/infectioncontrol/pdf/guidelines/disinfection-guidelines.pdf

https://www2.health.vic.gov.au/public-health/infectiousdiseases/infection-control-guidelines/manage-blood-bodyfluid-spills

### IOURNAL OF HYGIENE SCIENCES

## In Focus



# DETECT ENUMERATE IDENTIFY





- ✓ Spectrophotometric /Turbidimetric Technology
- ✓ 98% Correlation with Standard Plate Culture
- ✓ Identifies Urinary Pathogens Causing ~97% of Infections
- ✓ Facilitates Culture Report with DST within 24 Hours
- ✓ Optimizes Lab Work by Screening Out Negative Samples
- ✓ Simple Procedure Adaptable by almost all Laboratories
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• Pre & post-surgery skin cleaning & antisepsis : Use undiluted • Surgical, post operative, non surgical dressing : Use undiluted, once day/alternate • Antisepsis during minor incisions, scopy, catheterization, first aid, cuts, bites, stings etc : Use undiluted • Chronic wound management (diabetic foot, pressure and arterial/ venous leg ulcers) : Use undiluted • First aid : Use undiluted \*\*AMPs- Antimicrobial Peptides

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