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Editorial

Contents

Mini review section – Extraction of DNA, RNA, and protein is the basic method used in molecular biology. In the past, the process of extraction and purification of nucleic acids used to be complicated, time-consuming, labor-intensive, and limited in terms of overall throughput. Currently, there are many specialized methods that can be used to extract pure biomolecules, such as solution-based and column-based protocols. Automated systems designed for medium-to-large laboratories have grown in demand over recent years. It is an alternative to labor-intensive manual methods.

Current Trends section - Due to an alarming rise in the occurrence of antibiotic resistant bacterial strains, identification of new antimicrobial compounds has become one of the frontier areas in biomedical research. The focus is now being shifted to the use of antimicrobial ingredients, other than antibiotics, present in disinfectants, antiseptics and sanitizers, for the occurrence of resistant strains. **A large group of low molecular weight natural compounds that exhibit antimicrobial activity has been isolated from animals and plants during the past two decades. Among them, cationic peptides are the most widespread.** These peptides have been found to play a major role as cutaneous antimicrobial and immunomodulating agents, known as antimicrobial peptides (AMPs) or host defence peptides (HDPs).

In Profile Scientist – "Fanny Angelina Hesse" Without Fanny's introduction of agar the study of bacteria, the understanding of disease, and even the clinical diagnosis of disease would not have made the rapid progress we have seen in the last hundred years.

Bug of the month – H9N2 is the most common subtype of influenza viruses in Chinese chickens and thus causes great economic loss for the poultry industry, even under the long-term vaccination programs. The crucial role of H9N2 viruses due to the wide host range, adaptation to both poultry and mammals and extensive gene reassortment. In China, which is regarded as a breeding ground of avian influenza viruses, the H9N2 virus has been detected in multiple avian species, including chicken, duck, quail, pheasant, partridge, pigeon, silky chicken, chukar and egret.

Did You Know? - Melatonin is a highly important antioxidant. Melatonin is an efficient neutralizer of free radicals. Melatonin plays an important role in various physiological processes including the regulation of circadian and endocrine rhythms, aging, the stimulation of immune functions and the prevention of the adverse effects of antibiotics, including renal failure. These effects of melatonin are related to scavenging of a variety of toxic oxygen and nitrogen based reactants and stimulation of antioxidative enzymes.

Best Practices - It has become a well known and documented fact for over a century that raw fruits and vegetables have served as vehicles of transmission of human pathogens. Contamination of fruits and vegetables and thus the spread of pathogens and diseases is more common in developing countries as compared to developed countries due to various underlying factors, including the continued use of untreated wastewater and manure as fertilizers for the production of fruits and vegetables which is a major contributing factor to contamination that causes many foodborne disease outbreaks.

Nucleic acid Extraction: The past and the present - issue II

After conventional method we will discuss the types of solid-phase nucleic acid extraction. Solid-phase nucleic acid purification can be found in most of the commercial extraction kits available in market. It allows quick and efficient purification compared to conventional methods. Solid-phase purification is normally performed by using a spin column, operated under centrifugal force. This method can purify nucleic acid rapidly compared to conventional methods. Silica matrices, glass particles, diatomaceous earth, and anion-exchange carriers are examples that have been utilized in solid-phase extraction method as solid support.

Solid-phase Nucleic Acid Extraction:

Many of the problems that are associated with liquid liquid extraction such as incomplete phase separation can be prevented. Solid phase system will absorb nucleic acid in the extraction process depending on the pH and salt content of the buffer. The absorption process is based on the following principles: hydrogen-binding interaction with a hydrophilic matrix under chaotropic conditions, ionic exchange under aqueous conditions by means of an anion exchanger, and affinity and size exclusion mechanisms.

Four key steps involved in solid-phase extraction are cell lysis, nucleic acids adsorption, washing, and elution.

The initial step in a solid phase extraction process is to condition the column for sample adsorption. Column conditioning can be done by using a buffer at a particular pH to convert the surface or functional groups on the solid into a particular chemical form. Next, the sample which has been degraded by using lysis buffer is applied to the column. The desired nucleic acid will absorb to the column with the aid of high pH and salt concentration of the binding solution. Other compounds, such as protein may have strong specific bond with the column surface as well. These contaminants can be removed in the washing step by using washing buffer containing a competitive agent. For the elution step, TE buffer or water is introduced to release the desired nucleic acid from the column, so that it can be collected in a purified state. Normally, rapid centrifugation, vacuum filtration, or column separation is required during the washing and elution steps of purification process.

A mixed-bed solid phase nucleic acid extraction and its use in the isolation of nucleic acid have been disclosed. The mixed-bed solid phases of this invention are the mixtures of at least two different solid phases, can be solid or semisolid, porous or non-porous. Each solid phase can bind to the target nucleic acid under different solution conditions and release the nucleic acid under similar elution conditions.

(1) Silica Matrices:

The basis for most of the products related to nucleic acid purification is the unique properties of silica matrices for selective DNA binding. The principle of silica matrices purification is based on the high affinity of the negatively charged DNA backbone towards the positively charged silica particles. Sodium plays a role as a cation bridge that attracts the negatively charged oxygen in the phosphate backbone of nucleic acid. Sodium cations break the hydrogen bonds between the hydrogen in water and the negatively charged oxygen ions in silica under high salt conditions ($pH \leq 7$).

The DNA is tightly bound, and extensive washing removes all contaminations. The purified DNA molecules can be eluted under low ionic strength ($pH \geq 7$) later by using TE buffer or distilled water.

Besides silica matrices, nitrocellulose and polyamide membranes such as nylon matrices are also known to bind with nucleic acids, but with less specificity. These materials are often used as solid-phase nucleic acid transfer and hybridization matrices. Polyamide matrices are more durable than nitrocellulose and are known to bind nucleic acids irreversibly. Nucleic acids can be immobilized on polyamide matrices in low ionic strength buffer.

EX-RNA™ SPINTUBE-CR (Cat No 1108150100) is a simple and efficient system for extraction and purification of total RNA from fresh samples.

Fig 1: EX-RNA™ SPINTUBE-CR KIT

(2) Glass Particle:

Glass particles, powder and beads are useful for nucleic acid purification. For example, DNA isolation from agarose gels involved the use of chaotropic salts to facilitate binding of DNA to common silicate glass, flint glass, and

borosilicate glass (glass fiber filter). The adsorption of nucleic acid onto the glass substrate occurs most likely based on the mechanism and principle that similar to adsorption chromatography. Nucleic acid purification can also be done on silica gel and glass mixture. This invention has discovered that a mixture of silica gel and glass particles can be used to separate nucleic acid from other substances in the presence of chaotropic salts solution.

(3) Diatomaceous Earth:

Diatomaceous earth, which is also known as kieselguhr or diatomite, has silica content as high as 94%. It has been used for filtration and in chromatography and it is useful for the purification of plasmid and other DNA by immobilizing DNA onto its particles in the presence of a chaotropic agent. The resulting diatomaceous earth-bound DNA is then washed with an alcohol-containing buffer. The alcohol–containing buffer is then discarded and DNA is eluted out in a low salt buffer or in distilled water.

(4) Magnetic Bead Based Nucleic Acid Purification:

Magnetic separation is a simple and efficient way which is used in purification of nucleic acid nowadays. Many magnetic carriers are now commercially available. Particles having a magnetic charge may be removed by using a permanent magnet in the application of a magnetic field. Often, magnetic carriers with immobilized affinity ligands or prepared from biopolymer showing affinity to the target nucleic acid are used for the isolation process. For example, magnetic particles that are produced from different synthetic polymers, biopolymers, porous glass or magnetic particles based on inorganic magnetic materials such as surface modified iron oxide. Materials with a large surface area are preferred to be used in the binding of nucleic acids. Magnetic particulate materials such as beads are more preferable to be a support in isolation process because of their larger binding capacity. The nucleic acid binding process may be assisted by the nucleic acid "wrapping around" the support. A magnet can be applied to the side of the vessel, which contains the sample mixture for aggregating the particles near the wall of the vessel and pouring away the remainder of the sample.

Particles having magnetic or paramagnetic properties are employed in an invention where they are encapsulated in a polymer such as magnetizable cellulose. In the presence of certain concentrations of salt and polyalkylene glycol, magnetizable cellulose can bind to nucleic acids. Small nucleic acid required higher salt concentrations for strong binding to the magnetizable cellulose particles. Therefore, salt concentration can be selectively manipulated to release nucleic acid bound to magnetizable cellulose on the basis of size. The magnetizable cellulose which bound with nucleic acid will be washed with suitable wash buffer before they are contacted with a suitable elution buffer to separate out the desired nucleic acid with cellulose. Separation of magnetizable cellulose from supernatant during all the purification steps can be done by applying a magnetic field to draw down or draw them to the side of the vessel. The magnetizable cellulose used in this invention has an iron oxide content of up to around 90% by weight of the total mass of the cellulose. The magnetic component of cellulose can also be substituted by other magnetic compounds such as ferrous oxide or nickel oxide.

An extraction kit based on the principle of magnetic bead based nucleic acid purification is commercially available in the market. EX-RNA™ MAG (Cat No 1108120048) is a simple and efficient system for extraction and purification of total RNA from fresh samples.

Fig 2: EX-RNA™-MAG Kit

The special part of this kit is that the reagents provided are intended for use with magnetic tools. This magnetic tool is recommended if working in microtube format i.e. a magnetic stand (Cat no.: 20221130060). It is a practical device for performing separations based on magnetic particle technology.

Fig 3: MagStand

The kit does not require any organic solvents and eliminates the need for repeated centrifugation, vacuum filtration or column separation. The protocol is based on a modified alkaline lysis procedure followed by binding of the nucleic acid to magnetic particles. The magnetic tool is used to capture magnetic particles with the bound nucleic acid and contaminants are removed by washing with wash buffer provided. The nucleic acid is then eluted from the magnetic particles with the elution buffer.

The automated nucleic acid extraction system has also been developed (amplichain EX 32).

Fig 4 .: amplichain EX 32 automated nucleic acid extraction system.

This uses the same extraction kit as manual method. It combines the speed and efficiency of DNA/RNA purification with the convenient handling of magnetic particles. A magnetic rod protected by a rod cover is used for the capture of magnetic particles. It enters a vessel containing the samples and attracts the magnetic particles. Then, the magnetic rod cover is positioned above another vessel and the magnetic particles are released.

Nucleic acid purification by using zirconia bead is another type of magnetic bead based purification. These microspherical paramagnetic beads have a large available binding surface and can be dispersed in solution. This characteristic allowed thorough nucleic acid binding, washing, and elution. The total nucleic acid isolation kit, which uses this technology for the nucleic acid purification, makes use of the mechanical disruption of samples with zirconia beads in a guanidinium thiocyanate-based solution that not only releases nucleic acid but also inactivate nuclease in the sample matrix. After the lysis step, dilution of samples is done by using isopropanol. Paramagenetic beads are added to the samples for the nucleic acid binding purpose. The mixture of beads and nucleic acid are immobilized on magnets and washed to remove protein and contaminants.

Removal of residual binding solution is done with a second wash solution and finally the nucleic acid is eluted in low-salt buffer.

Solid-phase reversible immobilization paramagenetic bead-based technology has been utilized for a PCR purification system to deliver quality DNA. It requires simple protocol without centrifugation and filtration. PCR amplicons bind to paramagenetic particles which draw them out of solution, allowing contaminants such as dNTPs, primers, and salts to be rinsed away.

Magnetic oligo (dT) bead is an alternative to other oligo (dT) matrices for the purification of poly(A)+ RNA from total RNA sample. The poly(A)+ RNA can be extracted by introducing magnetic beads coated with oligo (dT). RNA with a poly-A tail attach to the oligo (dT). The beads will then be drawn to the bottom of a tube removing mRNA directly from total RNA. The magnetic beads which are specially treated minimize the nonspecific binding of other nucleic acids and ensure the purity of mRNA.

Conclusion

Since the first DNA isolation was successfully done by Friedrich Miescher in 1869 and the initial DNA extraction developed from density gradient centrifugation strategies by Meselson and Stahl in 1958, many techniques for biomolecules purification has been developed. From guanidinium thiocyanate-phenol-chloroform extraction to the column-technology that is widely used in DNA and RNA extraction, biomolecules extraction has helped researchers and scientists in manipulating subsequent molecular biology analysis in order to have a better understanding in the biological materials of the earth.

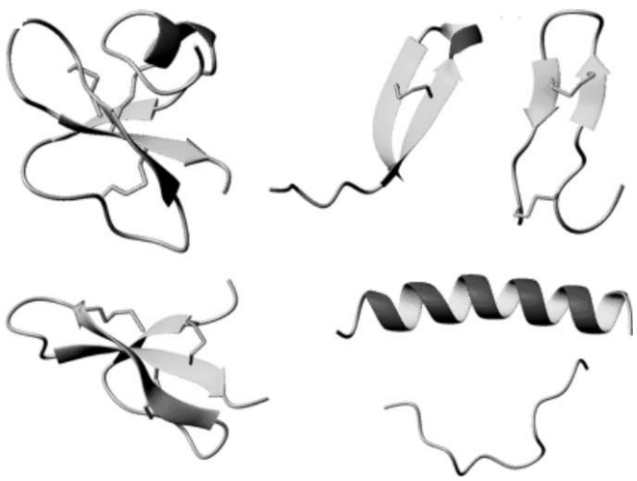
The automated nucleic acid extraction system has been developed due to the influence of rapid growth of automation technology nowadays. Automating nucleic acid extraction process is potentially beneficial for a number of reasons including to reduce working time, decrease labour costs, increase worker safety and at the same time provides opportunity in increasing reproducibility and quality of results. However, improvement of the weaknesses for some of the instruments needs to be conducted all the time. In the meantime, an all-in-one biomolecules extraction system, or the invention of a miniature and portable extraction system can become a prospective development in the future.

Antimicrobial Peptides (AMP) and PHMB

Due to an alarming rise in the occurrence of antibiotic resistant bacterial strains, identification of new antimicrobial compounds has become one of the frontier areas in biomedical research. The emergence of antibiotic-resistant strains is linked with the overuse and misuse of antibiotics and is long-established, widelystudied problem. The focus is now being shifted to the use of antimicrobial ingredients, other than antibiotics, present in disinfectants, antiseptics and sanitizers, for the occurrence of resistant strains. It is important to understand that, by reducing the number of infection outbreaks through effective hygiene, the number of antibiotic courses prescribed can be lowered, which can in turn reduce the impact of antibiotic resistance.

As a promising approach for the reduction of bacterial pathogens in wounds, the innate immune system has aroused new scientific interest. It is well known that the innate immunity is triggered immediately after microbial infection to produce antimicrobial compounds. Based on this fact recent studies show that several metabolites of unusual structure and exhibiting biological activities are expressed in many vertebrate, invertebrate and bacterial species. Some of these bioactive metabolites have biomedical potential. **A large group of low molecular weight natural compounds that exhibit antimicrobial activity has been isolated from animals and plants during the past two decades. Among them, cationic peptides are the most widespread. These** peptides have been found to play a major role as cutaneous antimicrobial and immunomodulating agents, known as antimicrobial peptides (AMPs) or host defence peptides (HDPs). AMPs form part of the ancient, nonspecific innate immune system, which is the principal defense system for the majority of living organisms. In many cases, their primaryrole is in the killing of invading. pathogenic organisms. The value of antimicrobial peptides in innate immunity lies in their ability to function without either high specificity or memory, and their small size makes them easy to synthesize. **In spite of the astonishing diversity in structure and chemical nature displayed by these molecules, all of them present antimicrobial activity, a condition that has led researchers to consider them as "natural antibiotics" and as such a new and innovative alternative to chemical antibiotics with a promising future as biotechnological tools. A resulting new generation of anti microbial peptides (AMPs) with higher specific activity and wider microbe-range of action could be constructed, and hopefully endogenously expressed in genetically-modified organisms.** The knowledge acquired in the past two decades and the discovery of antimicrobial peptides (AMPs) make them the basic element of a novel generation of drugs for the treatment of bacterial and fungal infections. AMPs show remarkable specificity for prokaryotes with low toxicity for eukaryotic cells. This is a characteristic that has favored their investigation and exploitation as potential new natural drugs.

Antimicrobial peptides are a unique and diverse group of molecules, which are divided into subgroups on the basis of their amino acid composition and structure. Antimicrobial peptides generally have between 12 and 100 amino acids. They are defined as molecules less than 10 kDa in mass which show antimicrobial properties. These evolutionarily conserved peptides are usuallypositively charged and are amphiphilic (have both a hydrophobic and hydrophilic side) in nature, that enables the molecule to be soluble in aqueous environments yet also enter lipid-rich membranes. The major classes of antimicrobial peptides include (i) á-helices, (ii) â-sheet and small proteins, (iii) peptides with thio-ether rings, (iv) peptides with an over representation of one or two amino acids, (v) lipopeptides, and (vi) macrocyclic cystine not peptides. The most prominent structures are amphiphilic peptides with two to four-strands, amphipathic-helices, loop structures, and extended structures.

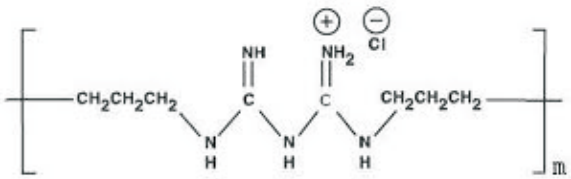


Various Antimicrobial Peptides

Unlike the majority of conventional antibiotics it appears as though antimicrobial peptides may also have the ability to enhance immunity by functioning as immunomodulators. In addition to important antimicrobial properties, growing evidence indicates that AMPs alter the host immune response through receptor-dependent interactions. Cathelicidins and defensins are major groups of epidermal AMPs. AMPs have been demonstrated to kill Gram positive and Gram negative bacteria (including strains that are resistant to conventional antibiotics), mycobacteria, enveloped viruses, fungi and even transformed or cancerous cells. Examples of antimicrobal peptides include magainins, alamethicin, pexiganan or MSI-78, human antimicrobial peptide, LL-37, defensin, protegrins etc. In addition to the wide spectrum of antimicrobial activities reported for these molecules, a number of naturally occurring peptides and their derivatives have been developed as novel anti-infective therapies for conditions as diverse as oral mucositis, lung infections associated with cystic fibrosis (CF), cancer, viral and topical skin infections. Pexiganan has been shown to be useful to treat infection related diabetic foot ulcer. AMPs have been shown to be important in such diverse functions as angiogenesis, wound healing, and chemotaxis.

On the other hand, the antiseptic/antimicrobial compound polyhexamethylene biguanide or PHMB belongs to the biguanide group. Biguanides are important class of cationic surface active antimicrobial agents, which are used as antiseptics & disinfectants. PHMB is a polymeric biguanide. It is a polycationic linear polymer comprising of a hydrophobic backbone with multi-cationic grouping separated by

hexamethylene chain. The basic molecular chain of PHMB can be repeated from 2 to 30 times, with increasing polymer chain length correlating with increasing antiseptic/antimicrobial efficacy. Polyhexamethylene biguanides (PHMB) are mixtures of polymeric biguanides with an average polymer length (*n*) of 5, but containing high ($n > 15$, mol. wt 3300) and low molecular weight material ($n = 2$, mol. wt 400). Studies involving discrete molecular weight fractions of PHMB have shown that antimicrobial activity of PHMB increases with increasing polymer length.



PHMB

PHMB is a heterodisperse mixture of polymers and is asynthetic compound which is structurally similar to naturally occurring antimicrobial peptides (AMPs). The structural similarities between AMPs and PHMB mean that the latter can enter bacterial cell membranes and kill bacteria in a similar way to AMPs (Moore and Gray, 2007). The primary targets appear to be the outer and cytoplasmic membranes. PHMB is thought to adhere to and disrupt target cell membranes, causing them to leak potassium ions and other cytosolic components which results in bacterial cell death. There is also evidence that following penetration into target cells, PHMB binds to DNA and other nucleic damaging or inactivating bacterial DNA.

PHMB is chemically stable and non volatile compound. It has very low surface activity, having a surface tension essentially identical to water. PHMB consequently can be readily water rinsed from surfaces & do not have residual streaks or tackiness. It is odorless, non foaming, clear & colorless. PHMB is effective & stable over a wide pH range (4-10). It is easy to handle & apply. PHMB is clear and colourless, soluble in water, glycols & alcohol but insoluble in non polar solvents like petroleum, ethers or toluene. In medicine PHMB was introduced by Willenegger in 1994 as an antiseptic in abdominal surgery. PHMB has good tissue compatibility based on its activity against the acid lipids contained within the bacterial cell membranes and minor effect on the neutral lipids of human cell membranes. This helps to prevent damage to the surrounding healthy tissue. PHMB containing wound rinsing solutions show superior wound healing due to its antimicrobial efficacy as well as its ability to maintain the moist environment. *In-vitro* and *in-vivo* studies into the effectiveness of PHMB in wound care have demonstrated that the product may also have other benefits in wound management. Daeschlein *et al.,* (2007) reported that the product may reduce pain and malodour, while Mueller and Krebsbach (2008) found that its use reduced fibrin slough and prevented the buildup of necrotic tissue and so promoted connective tissue regeneration. Wiegand *et al.,* (2008) demonstrated that PHMB can have a positive effect on tissue proliferation. Use of PHMB, in the form of gels, wound irrigants and dressings, is ideal in care and management of wounds including chronic wounds, burns and diabetic foot ulcers. Over the past years end-use (ready to use) wound care products containing PHMB have been successfully launched including wound rinsing solutions, wound gels and dressings. Special features qualify PHMB for growing and effective application in wound care management.

PHMB has been successfully tested for wound irrigation, wound care dressings (Surgical & non surgical), diabetic foot ulcer management, pre-operative antiseptic for surgery, sanitizer, and preservative in topical ophthalmic preparations, inter-operative irrigation, peri-operative cleansing products, treatment of fungal infections, contact lens cleaning solutions and swimming pool cleaners.

Similarities between AMPs and PHMB

1. Both are structurally similar
2. AMPs generally contain 12 to 100 amino acids while the basic molecular chain of PHMB can be repeated 2 to 30 times
3. Both are cationic in nature
4. Both are low molecular weight compounds (molecular weight of AMPs is not more than 10 KD while molecular weight of PHMB varies from 400 to 3300 depending on the number of polymers it contains)
5. Both have the novel non specific mode of action against pathogens. They target the outer and cytoplasmic membranes of pathogens and kill the pathogen by disrupting membranes, interfering with metabolism and targeting the cytoplasmic components
6. Both require a short contact time to induce killing of pathogens
7. Both have broad spectrum of activity against pathogens
8. In contrast to conventional antibiotics both PHMB and AMPs do not appear to induce resistance in pathogens
9. Both are safe and non cytotoxic to human cells
10. Both play an important role in wound repair and healing
11. Both are excellent candidates for development as novel therapeutic agents

Both AMPs and PHMB are antiseptics/antimicrobials that are gaining importance as alternatives to conventional antibiotics. AMPs and PHMB are exciting candidates as new antiseptic/antimicrobial agents due to their broad antimicrobial spectra, highly selective toxicities, and the difficulty for bacteria to develop resistance to these peptides. These compounds bind to bacterial cell membrane and induce cell lysis by destroying membrane integrity, in a similar way that penicillin and cephalosporin antibiotics do. Antiseptics/antimicrobial compounds have been in use for much longer than antibiotics yet resistance to antiseptics/antimicrobial compounds present much less of a problem. Antiseptics such as PHMB are an alternative to antibiotic prophylaxis and are less likely to generate resistance.

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Fanny Hesse

Name: Fanny Angelina Hesse (also known as 'Lina')
DOB: 22 June 1850
DOD: 1st December 1934 (Age 84)
From: New York, USA
Area of work: Bacteriology

Without Fanny's introduction of agar the study of bacteria, the understanding of disease, and even the clinical diagnosis of disease would not have made the rapid progress we have seen in the last hundred years.

Polenta, potato, gelatin, and coagulated egg white….were some of the materials used as solid surfaces on which to grow and study bacteria in the early days of bacteriology. All of these had significant disadvantages, so when laboratory technician Fanny Hesse suggested the use of agar, it revolutionized this burgeoning new area of science.

Fanny was born in 1850 in New York, daughter of a successful Dutch immigrant. Little is known of her early life but in 1872, Fanny met her future husband Walther, then serving as a ship's surgeon on a German passenger liner. The couple was married on 16th May 1874 and Fanny moved to Dresden to be with her husband.

Walther was a country doctor who was passionate about hygiene and public health for workers, particularly the conditions of workers in the local mines. In 1881 he took a sabbatical to study in the Berlin labs of Robert Koch, the 'father of bacteriology', to investigate airborne microorganisms.

On top of her duties running the household and the education of their sons, Fanny became Walther's assistant. She worked as an unpaid technician, preparing bacterial growth media (normally beef broth), cleaning equipment, and using her considerable artistic talents to produce beautiful water colour illustrations for his publications.

To study airborne microbes, Walther was using tubes lined with the growth media made by Fanny. Unfortunately, the gelatin used to solidify this media melted at 37°C and would liquify on warm days. Similarly, some bacteria broke down the gelatin to liquid. These issues plagued their experiments and were a source of great frustration.

Fanny suggested replacing gelatine with the seaweed extract agar-agar. She had been using agar for years in the preparation of fruit and vegetable jellies using recipes from her mother, who had in turn obtained the formula from some Dutch friends who had lived in Java, Indonesia.

Agar solved their problems: solid up to 90°C, transparent, indigestible by microorganisms, and sterilizable, it was perfect for growing and studying bacteria. Walther told Koch and he immediately saw the benefits. In his 1882 paper, Koch states, "The tubercule bacilli… grow, for example, on a gelatinous mass which was prepared with agar-agar, which remains solid at blood temperature…" No mention of either of the Hesses was made in that paper.

Walther himself published many papers in his lifetime, not only on bacteriology but on hygiene and public health issues. His last publication in 1908 described method of culturing intestinal bacteria from typhoid fever patients. Fanny painted beautiful and highly accurate images of the magnified colonies on agar plates during different growth phases using water colours; work that was only possible if she had a thorough understanding of both bacteriology and microscopy. Despite her contribution to this and many more of Walther's papers she was never included as author or acknowledged in his work.

Agar has become almost as important to bacteriology as the petridish, which would be invented just a few years later. Despite this, when Fanny died in 1934, few bacteriologists knew of her death. In their 1939 paper on Fanny's life, Hitchens and Leikind suggested that "plain agar" be referred to as "Frau Hesse's medium" to acknowledge her forgotten "service to science and to humanity." This is yet to happen, but it is never too late.

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Jokes

Two Children Were Waiting In The Doctor's Waiting Room.
The Little Girl Started Crying.
Little Boy Asked Her: "Why Are You Crying?"
The Girl Said: "I'm Here For Blood Test And The Doctor Is Going To Cut My Finger"
The Little Boy Too Started Crying.
Girl: "Now Why Are You Crying?"
Boy: "I'm Here For the Urine Test"

Banta Asked To Santa.
Banta: "When You Kiss Your Wife?"
Santa: "I Kiss My Wife Before I Go To Office Every Day And You?"
Banta: "I Kiss Your Wife After You Go To Office Everyday"
Santa: "Ha Ha Ha, I Am First"

Doctor To A Rich Man: "Do You Prefer A Local Anesthesia?"
Rich Man: "Nop, I Would Rather Prefer An Imported One"

Professor To Students: "I Want You To Write An Essay With The Following Elements:
1. Religion
2. Royalty
3. Sex And
4. Mystery
After Two Minutes
Pappu Shouts: "Done"
Proffesor: "Let Me See"
Pappu Had Written: "Oh My God, Says The Queen, I Am Pregnant Yet… I Don't Know Who Did It"

I Was In The Restaurant Yesterday When I Suddenly Realized,
I Desperately Needed To Pass Gas. The Music Was Really, Really Loud,
So I I Timed My Gas With The Beat Of The Music.
After A Couple Of Songs, I Started To Feel Better. I Finished My Coffee,
And Noticed That Everybody Was Staring At Me.
Then I Suddenly Remembered That I Was Listening To My iPod…..

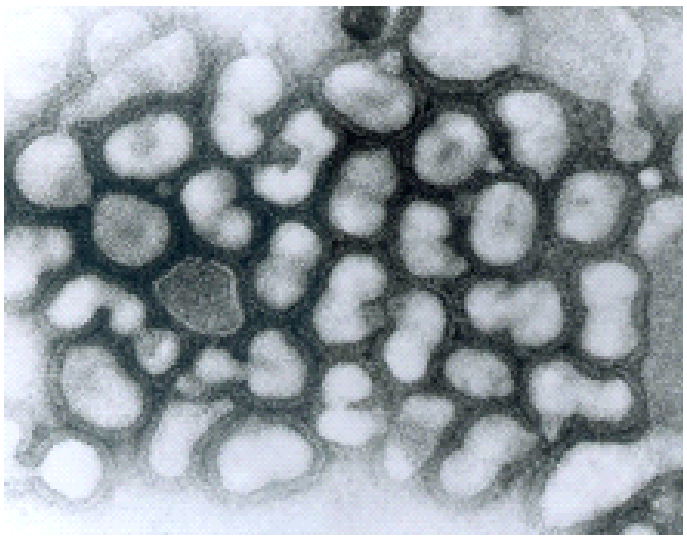
Height Of A True Bad Luck.
A Guy And A Girl Met Last Time For Their Break-Up..
Girl's Father Caught Them.
Now They Are A Married Couple.

A Bar Opened Opposite A Church!
The Church Prayed Daily Against The Bar Business
Days Later The Bar Was Struck By Lightning & Caught Fire Which Destroyed It.
Bar Owner Sued The Church Authorities For The Cause Of Its Destruction,
As It Was An Action Because Of Their Prayer, The Church Denied All Responsibility!
So, The Judge Commented,
"It's Difficult To Decide The Case
Because
Here We Have A Bar Owner Who Believes In The Power Of Prayer
&
An Entire Church That Doesn't Believe In It !"

Once An Indian And An American Both Were Friends.
They Both Went Into A Chocolate Store.
Everybody Is Busy In The Store So American Steal 3 Chocolates And Put Those In The Pocket.
Both Came Out From The Store Then American Said: "Man, I'm The Best Thief Ever, I Stole 3 Chocolates And No One Saw Me, You Can't Beat That."
Indian Replied: "This Is Nothing, You Wanna See Something Better, Lets Go Back To The Shop And I Will Show You Real Stealing."
So They Went To The Counter And Indian Said To The Shop Boy: "Do You Wanna See Magic?"
Shop Boy Replied: "Yes, Of Course."
Indian Said: "Give Me One Chocolate Bar."
Shop Boy Gave Him One, And He Ate It.
Indian Asked For The Second, And He Ate That As Well.
Indian Asked For The Third, And Finished That One Too.
The Shop Boy Asked: "But Where Is The Magic?"
Indian Replied: "Check In My Friends Pocket, And You'll Find Them."
You Can't Beat An Indian.

Difference Between Thoughts Of Imran Khan And Imraan Hashmi.
Imran Khan: "If You Give Me 100 Young Boys, I Can Change The Nation"
Imraan Hashmi: "If You Give Me 100 Young Girl, I Can Create Another Nation"

Influenza A virus subtype H9N2



Influenza A virus subtype H9N2 (A/H9N2) is a subtype of the species Influenza A virus (bird flu virus).

H9N2 viruses are an LPAIV subtype found worldwide in wild birds and are endemic in poultry in many areas of Eurasia and Africa. Compared to H5 and H7 viruses they are somewhat neglected, however, recent evidence, summarised in this review, suggests they could potentially have a major role in the emergence of the next influenza pandemic, either directly as an H9N2 subtype virus, or through the donation of internal genes to a pandemic virus.

Global surveillance of H9N2, has a problem when compared to HPAIV viruses in that LPAIV H9N2 is not a notifiable pathogen and causes relatively few overt human infections. In many resource-limited regions surveillance is performed sporadically, or not at all. It is likely that H9N2 viruses are present or even endemic in more countries, particularly in low- and middle-income countries in Africa and Asia, than is outlined below. For example, poultry adapted strains of the virus usually spread short distances (rather than by long distance flyways), therefore the isolation of the virus in Uganda in West Africa, most related (though not very closely related) to viruses from the Arabian Peninsula, ~2000km away, suggests that it is likely countries in-between also contain intermediately related H9N2 viruses which are yet to be isolated.

H9N2 viruses are nearly uniformly low pathogenicity in experimental settings when tested by IVPI, however, in the field they often exhibit moderate-to-high morbidity and mortality. For example, there are many reports of mortality rates more commonly associated with HPAIV outbreaks. This is usually associated with confounding factors such as co-infection with bacterial or viral pathogens, and other factors such as poor nutrition and housing. However, certain strains do also show high morbidity and mortality in controlled in vivo experiments.

Furthermore, when an HPAIV-like polybasic cleavage site was engineered into an H9 virus, an HPAIV phenotype was not observed in an H9N2 virus background. However, when the polybasic H9 HA was combined with the remaining genes from an HPAIV strain the reassortant virus did develop an HPAIV phenotype. This implies H9N2 virus internal genes may not be compatible with an HPAIV phenotype in some cases.

Four routes of transmission are widely described for influenza viruses: droplet, aerosol, faecal-oral and direct contact. Droplet transmission describes exhaled particles >10 µm which are deposited into the upper respiratory tract, whereas aerosol droplets are typically less than 5 µm and can reach the lower respiratory tract. Contact transmission relies on the transfer of particles to mucous membranes directly, or via a fomite intermediate. For a successful transmission event to occur, enough virus must persist long enough in the external environment to reach the target tissue. Transmission is therefore determined via several viral, host, and environmental aspects, including: (i) The major site of viral replication and viral titres shed; (ii) The distance and frequency between contacts and (iii) Environmental conditions and virus stability. In wild aquatic birds such as ducks and gulls AIVs generally exhibit gastrointestinal tropism and are thought to be spread primarily through the oral-faecal route. In poultry adapted AIVs, there exists some heterogeneity in tropism and transmission routes. HPAIV, such as H5N1, have a systemic distribution and are probably transmitted by a combination of the oral-faecal route and airborne transmission, whereas, LPAIVs in chickens tend to show more respiratory tropism, though some strains also show gastrointestinal tropism. One of the key molecular markers that facilitates adaptation of an AIV from wild aquatic birds to poultry is the deletion of amino acids from the stalk domain of NA, which have been shown to mediate the switch to respiratory tropism in chickens. There is good evidence to suggest that many LPAIV strains transmit by the airborne route, the oral-faecal route and the waterborne route. However, the favoured mechanism of transmission between individuals varies by host species and viral strain.

Many studies have implicated direct contact as an important transmission route for H9N2 viruses in chickens, although indirect routes such as aerosol and faecal-oral have been shown to be important for some strains and many viruses show primarily a respiratory tropism. However, some H9N2 strains have been shown to have an extended tropism for the kidneys or oviducts. Both in the field and experimentally poultry adapted H9N2 viruses are mostly detected from buccal rather

than cloacal swabs. Additionally, inoculation of some H9N2 viruses into the respiratory tract is 40 times more effective than gastrointestinal inoculation at initiating infection. However, many of these routes appear to be environmentally contextual, for example, at LBMs communal water sources have been implicated as the major route of transmission of endemic H5N1 and H9N2 viruses. Together these studies indicate that for H9N2 and other enzootic poultry adapted H9N2 viruses, respiratory and contact transmission are likely the primary routes of transmission and that respiratory transmission may partly arise initially as an adaptation to poultry which clearly has implication for zoonotic transmission.

The increase in H9N2 isolation rates due to greater screening of patients with influenza-like illness indicates that mild, or even symptomatic, human H9N2 cases may be relatively common. This possibility is supported by an extensive body of serological evidence showing particularly high seropositivity rates amongst poultry workers in many enzootic countries including India, Cambodia, China, Vietnam, Egypt, Hong Kong, Iran, Thailand and Pakistan (reviewed in). Serological assays looking at H9 exposure suffer several limitations such as H9-antigenic cross-reactivity with other HA subtypes, however, in recent studies this limitation has been overcome through a number of approaches such as concurrent sero-typing against multiple human and avian HA subtypes, meta-analysis, and longitudinal studies of poultry workers. Furthermore, there is a single study which has managed to isolate a virus from an asymptomatic poultry worker in Pakistan. Overall this suggests that although H9N2 infections may be fairly common, they are mostly mild or asymptomatic and do not transmit any further than the initial zoonotic infection implying poor adaption of H9N2 viruses to mammals.

Due to the economic damage caused by enzootic H9N2, many countries including China, Israel, South Korea, Morocco, Pakistan, Egypt, Iran and UAE have adopted vaccination at either a national or local level as a key approach for preventing H9N2 disease in poultry. The most common vaccines in use are traditional inactivated vaccines, similar to those used in human seasonal vaccines. H9N2 viruses exhibit a wide antigenic variability, both between, and within lineages. Unlike human vaccines, H9N2 vaccines are generally not as regularly assessed for their efficacy against antigenically drifted viruses and consequently are far less often updated. Therefore, in many regions H9N2 viruses continue to infect and cause disease in vaccinated poultry with tentative evidence suggesting that sub-optimal use of vaccination may be driving antigenic drift and/or clade replacement, and theoretically zoonotic potential and pathogenicity. Because of this, there is a real need for: (i) better understanding of the molecular determinants of H9 antigenicity, (ii) better understanding of antigenic drift and the consequences upon viral fitness and zoonotic potential and (iii) next generation vaccines that protect against multiple strains and antigenically drifted variants.

Stamping out, which involves culling of potentially infected birds and birds presenting influenza-related morbidity has occasionally been used as a first line of defence against H9N2 in countries without a history of the virus. This was the case during early outbreaks in Korea and the recent outbreaks in Russia and Ghana. However, once the virus becomes endemic in a country, stamping out becomes uneconomical and unfeasible, therefore vaccination is commonly used beyond this point. Stamping out is more often used during HPAIV outbreaks due to their status as notifiable diseases, regardless of a countries history with outbreaks/endemicity.

Other than vaccination and stamping out, several other interventions have been successfully used in the field to halt or reduce avian influenza virus spread in poultry and subsequent zoonotic infection. As discussed above LBMs are a hotspot for influenza infection due to the convergence of a high density of different poultry species from across a wide geographic range. LBMs were identified early on as the main sources of AIV outbreaks in the late 1990s in China and Hong Kong and several interventions were utilised such as temporary closures, periodic rest days, and overnight market depopulation, as well as basic increases in biosecurity and hygiene practises. A detailed review of the effectiveness of these practises has previously been performed by Offeddu and colleagues, who concluded that these practises, particularly LBM closure, were effective at both halting the spread of AIV between birds, as well as having a knock-on effect at reducing zoonotic AIV cases. A second detailed review by Fournié and colleagues indicated that individual as well as community-wide habits which expose humans to AIVs and risk of zoonotic infection are highly heterogeneous and may require control strategies tailored to individual communities

Melatonin is effective against polycystic kidney disease

Melatonin is a hormone produced in the pineal gland. Melatonin is produced of tryptophan and serotonin and is metabolized to 6-hydroxyl melatonin in the liver. Melatonin is a highly important antioxidant. Free radicals damages cells. Melatonin is an efficient neutralizer of free radicals . Melatonin plays an important role in various physiological processes including the regulation of circadian and endocrine rhythms, aging, the stimulation of immune functions and the prevention of the adverse effects of antibiotics, including renal failure. Melatonin reduces the oxidative induced brain, heart, kidney, and liver damage in rats. These effects of melatonin are related to scavenging of a variety of toxic oxygen and nitrogen based reactants and stimulation of antioxidative enzymes . Liver and kidney are metabolically highly active in xenobiotic metabolism and excretion, they have, compared to other organs, a greater load of free radical activity and thus are more prone to oxidative damage. Nephrotoxicity is an important side effect of contrast media, aminoglycosides, chemotherapy. In vivo and in vitro melatonin has been found to protect tissues against oxidative damage generated by a variety of toxic agents and metabolic processes, including chemotherapy induced toxicity and ischemia reperfusion injury in kidney, liver and brain. Melatonin has recently been found to protect against Adriamycin induced nephrotoxicity, aminoglycosides induced nephrotoxicity, and contrast media induced nephrotoxicity. Studies indicated that pretreatment with melatonin improves dramatically the histological and functional damage in this experimental model . In summary the studies showed that melatonin administration attenuated oxidative stress, inflammation, and kidney function and structure in rats. If proven effective, melatonin would be an attractive adjunctive therapy, since it is a natural, inexpensive, widely available, orally administered and relatively safe product administration attenuated oxidative stress, inflammation, and kidney function and structure in rats. If proven effective, melatonin would be an attractive adjunctive therapy, since it is a natural, inexpensive, widely available, orally administered and relatively safe product.

Conclusion

Recent studies shown that melatonin administration, attenuated oxidative stress, inflammation, and restored renal function and structure in rats. Melatonin could be an attractive adjunctive therapy, since it is a natural, inexpensive, widely available, orally administered and relatively safe product.

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BEST PRACTICES – FRUITS AND VEGETABLES AS VEHICLES OF PATHOGEN TRANSMISSION

It has become a well known and documented fact for over a century that raw fruits and vegetables have served as vehicles of transmission of human pathogens. Contamination of fruits and vegetables and thus the spread of pathogens and diseases is more common in developing countries as compared to developed countries due to various underlying factors, including the continued use of untreated wastewater and manure as fertilizers for the production of fruits and vegetables which is a major contributing factor to contamination that causes many foodborne disease outbreaks.

Bacteria such as *Clostridium botulinum*, *Bacillus cereus* and *Listeria monocytogenes*, all capable of causing infections, are normal inhabitants of many soils, whereas Salmonella, Shigella, *Escherichia coli* and Campylobacter reside in the intestinal tracts of animals, including humans and are more likely to contaminate raw fruits and vegetables through direct or indirect contact with feces, sewage, untreated irrigation water or surface water. Post harvest handling may also be responsible for contamination with bacteria, viruses and parasites at the site of transport and vending.

Other factors, that may also contribute to the increase in diseases associated with fruits and vegetables include application of improperly composted manures to soils in which fruits and vegetables are grown, changes in packaging technology viz; the use of modified or controlled atmosphere and vacuum packaging, extended time between harvesting and consumption, and changing food consumption patterns. Increased global trade in raw fruits and vegetables, as well as increased travel in general, could also increase the risk of produce-associated diseases. Finally, the susceptibility of the public to food borne diseases, at least in more developed countries, is changing due to increased number of people who are elderly, immunocompromised or have chronic diseases. This change in social demographics is likely to lead to increased risk of illness associated with the consumption of raw produce that otherwise may contain levels of pathogens innocuous to healthy individuals.

Microbes commonly present on the surface of raw fruits and vegetables may comprise of chance contaminant from soil or dust, or bacteria or fungi that have grown and colonized by utilizing nutrients exuded from plant tissues. Bacterial groups commonly found on plant vegetation are those that test positive as for coliforms or fecal coliforms.

Fruits which have to be peeled off before consumption like mangoes, oranges and bananas seem lesser significant in the transmission of microbes as compared to fruits which are consumed whole. However, care must be taken to ensure that pathogens present on the rind/skin of these fruits are not transferred to the edible part, and thus consumed. Its a preferred practice to wash the fruit prior to peeling, to mitigate

the numbers of microbes. Alternatively, microorganisms that may get trapped on the inner leaves of certain vegetables can be particularly difficult to remove by routine cleansing practices.

In these cases, in order to minimize the potential risk to acquire infection or an intoxication associated with raw fruits and vegetables, key sources of contamination from the environment to the table must be identified and in turn specific measures and interventions to prevent and / or minimize the risk of contamination must be considered and appropriately implemented. In cases where the possibility of contamination cannot be excluded, the application of the most effective decontamination processes should be considered. Application of good hygienic practice during production, transport and processing, combined with Hazard Analysis Critical Control Point (HACCP) system, will definitely reduce the contamination of fruits and vegetables and reduce the risk of illness associated with these foods.

A Simple domestic practice of washing raw fruits and vegetables in hot water or water containing detergent or permanganate salts aids to remove a portion of pathogens and spoilage microbes that may be present. Washing fruits and vegetables in potable water or rinsing in potable water would aide in removing microorganisms. Additional 10-fold or 100-fold reductions can sometimes be achieved by treatment with disinfectants. It is noteworthy to mention that viruses and protozoan cysts on fruits and vegetables usually exhibit higher resistance to the effect of disinfectants than do bacteria or fungi.

A wide variety of treatments are known to be partially effective in removing disease causing microbes from the surface of whole and cut raw fruits and vegetables or from contact surfaces during handling. These treatments should be considered as methods of disinfection, causing reductions in populations of microorganisms but not always yielding fruits and vegetables completely free of pathogens.

Each type of disinfectant has its own efficacy in killing or eliminating microbial cells. Effectiveness depends on the nature of the cells as well as the characteristics of fruits and vegetable tissues and juices. Some disinfectants are best suited for use in disinfectant direct contact washes, while others may be apt for equipments or containers used to process, store or transport fruits and vegetables.

Common disinfecting treatments include the use of:

Chlorine: Liquid chlorine and hypochlorites are moderately effective disinfectants for surfaces that may come in contact with fruits and vegetables during harvest, processing, and for whole and cut fruits and vegetables. To disinfect produce, chlorine used at concentrations of 50 – 200 ppm with a contact time of 1 – 2 minutes is sufficient.

Chlorine Dioxide (ClO_2): The oxidizing power of ClO_2 is about 2.5 times that of chlorine, and its activity is not affected by pH. This compound must be generated on site since it can be explosive when concentrated. Its mechanism of action involves disruption of cell protein synthesis and membrane permeability. Its used in varied concentrations depending on the purpose and the material for which the equipment is being disinfected.

Bromine: Bromine has had limited use either alone or in combination with chlorine compounds in water treatment programmes. Bromine is selective in its activity against microbes and is effective against a few pathogens at a concentration of 200 ppm with a contact time of 15 minutes.

Iodine: Iodine compounds are widely used for sanitizing food processing equipment and surfaces. Iodophores are less corrosive than chlorine at low temperatures. Iodophores are most effective at pH 2 – 5 and at concentrations ranging from 6-13 ppm.

Other disinfectants include:

Trisodium phosphate (TSP): Is known to be effective in removing Salmonella from poultry and red meats.

Quaternary ammonium compounds: Are cationic surfactants, used largely to sanitize floors, walls, drains, and equipment and other food-contact surfaces in fruit and vegetable processing plants. This group of disinfectant has potential for application to surfaces of uncut fruits and vegetables which subsequently would have their peel, rind or skin removed prior to consumption.

Acids: Organic acids naturally found in or applied to fruits and vegetables behave primarily as fungistats, bacteriostats. Also washes and sprays containing organic acids, particularly lactic acid, have been successfully used to disinfect beef, lamb, pork and poultry carcasses.

While every effort must be made to prevent contamination of fruits and vegetables during the phases of production, transport, processing and handling, much improvement is still required in many parts of the globe if hygienic production of fruits and vegetables is to be ensured.

Reference:

www.WHO.int

Highlights of the coming issue

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