WE THE MICROBIOLOGIST'S

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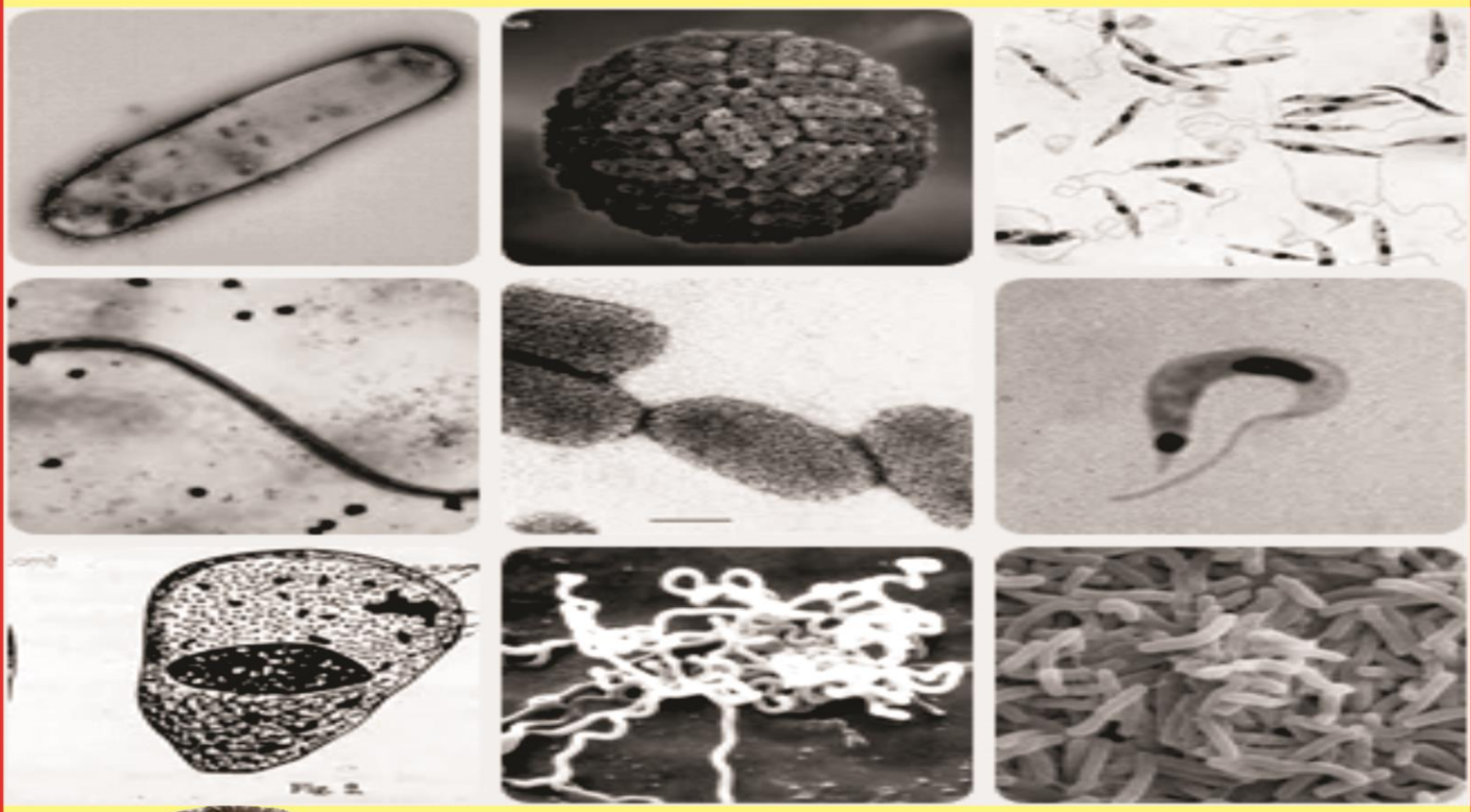

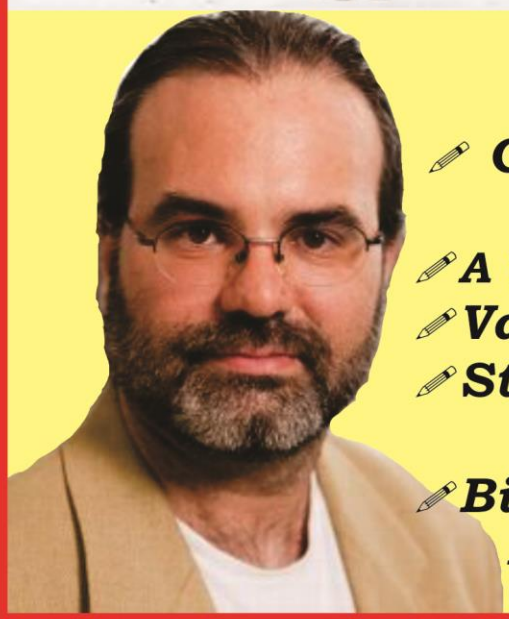

What's Inside...???

Editor's Message…..

At ***We The Microbiologist*** (WTM), ordinary people do extra ordinary things. Some climb bridges and buildings, some decipher bulky scientific reports and all of them do it just for the sake of scientific development. All of these are human efforts without any influence from super natural powers. Passion for science is their super power. This passion has given rise to a specialized magazine "***MICRIOGRAPHIA TODAY"*** to play a multi-faceted role in the field of science. This magazine demonstrates the intellectual concerns of the field, express the views and interests of individual writers, and provide them a platform for dialectic interactions among scholars.

It all started since 1st November 2011 with the vision and toil of Mr. Trinankur Bhattacharya and Mr. Saumyadip Sarkar to provide a growth opportunity for those aspirants who aspire for distinguished career in the field of Microbiology and Life Sciences. The main objective of the magazine is to create a breed of Innovative and dynamic professionals for academic and researches in the field of Microbiology, which are capable of making a difference in microbiological field and research sector. For past two years the magazine has been a mirror that reflects the field's collective and individual thought processes; a window that grants the readership access to different perspective.

For the creation of a successful magazine and the assurance of its long-term longevity require visionary thinking, determination, and sustained devotion which was given by all the members of WTM.

Thank you,

Mr. Swapnil Vichare,

Editor in Chief

swapnil@wethemicrobiologist.in

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Biofilms of Bacteria: A forgotten story in Veterinary medicine

Sophia I, Scientist, CIARI, Port Blair, Santhamani R, Ph.Ds scholar, Division of virology, IVRI, Izatnagar and Bagath, M, Scientist, NIANP, Bangalore

Abstract

Bacterial species encased themselves in a polysaccharide matrix to form biofilms. Biofilms in turn act as a protective sheath for bacteria preventing it from host immune system and antibiotics. The virulence mechanism of biofilms is mainly through differential gene expression. Biofilms are also suspected as one of the reasons for vaccine failure against bacterial pathogens.

Keywords: biofilms, bacteria, antibiotic, gene

Introduction

Biofilms are a collection of bacteria of same or different species in a surrounded by polysaccharide matrix. In short, it is the microbial community with altered genetic and physiological characteristics than their counterparts. Interestingly, the first bacteria observed as 'animalcules' in by Antonie van Leeuwenhoek itself was from a biofilm source of his dental plaque. But the world become aware of this microbial community in 1970s after Costerton *et al*., 1978 coined the term 'biofilm'. He defined biofilms as bacterial species which stick to each other in either live or inanimate substratum. Later, Percival *et al*., 2000 better explained it as microbes immobilized in extracellular polymer matrix acting as an independent functioning ecosystem, homeostatically regulated.

Steps In Biofilm Formation

1. Preconditioning / selection of suitable substratum

2. Bacterial cells deposition
3. Aggregation and micro colony formation by bacteria
4. Extracellular polymer production around biofilms
5. Diffusion of nutrients and waste products removal
6. Growth and maturation of biofilms
7. Detachment of micro colonies or plankton

Composition Of Biofilms

The basic unit of biofilm is the micro-colonies of homogenous and heterogeneous bacterial species. In a biofilm, the matrix material is composed of 85 % and microbial cells about 15 % .The extracellular polysaccharide (EPS) consist of polysaccharides, proteins (75-90%) nucleic acids, lipids, phospholipids & humic substances.

Role Of EPS In Biofilms

- Barrier function – inside the biofilms only diffusion transport is possible thus it retards biocides passage
- Protective effect
- Delays / prevents antimicrobials passage due to barrier effect or chemical interactions
- Facilitates cell-cell communication and community behavior
- Polysaccharides and proteins which act as fundamental structural elements of biofilms give them mechanical strength
- Lipids and nucleic acids which also constitutes biofilms add to the stability of biofilms
- Extracellular DNA and plasmid elements are required for initial biofilm establishment in organisms like *P. aeruginosa*

Different Mechanisms that Happen in Biofilms

Alteration in Gene Expression

The genes are differentially expressed in biofilms when compared to planktonic culture. During initial phase of biofilm formation, flagellar genes are expressed in order to aid the bacteria to reach the surface. After reaching the surface, there is repression of flagellar gene expression (Pratt and Kolter, 1998). Moreover, bacteria in biofilms mostly express genes corresponding to dormant lifecycle such as stress induced genes, genes for starvation, sporulation etc. in a biofilm, the bacterial cells are encased in polysaccharide matrix. Normally the genes for buildup of matrix such as PIA / PNAG (Ica ABCD IN G+ve bacteria), colonic acid (*E. coli*), alginate, glucose and mannose (*P. aeruginosa*) are expressed in biofilms (Friedman and Kolter, 2004). Furthermore, the function of 30-50% genes expressed in biofilms is unknown.

Phase Variation

Phase variation is defined as the random switching of phenotypes at frequencies that are much higher than classical mutation rates leading to increased virulence, immune evasion etc. In biofilms, bacteria adopt to dormant stage like small colony variants (SCV) through phase variation. In biofilms, where the bacteria live as a community there is increased frequency of horizontal gene transfer through mobile genetic elements like DNA fragments, mobile transposons etc leading to phase variation. For example in *Streptococcus epidermidis*, insertion of IS 256 into *ica gene* leads to phase variation in biofilm formation (Kiem *et al*., 2004).

Quorum Sensing In Biofilm Bacteria

It is a cell density dependant cell to cell signaling system in bacteria mediated by chemical molecules called auto inducers. These auto inducers increase in concentration w.r.t bacterial population. When the amount of auto inducers exceeds the threshold level, they bind to transcription regulators which in turn will either activate or repress gene expression. This method of quorum sensing plays an important role in extracellular polysaccharide formation around biofilm bacteria. For example *Vibrio cholerae* uses quorum sensing to produce EPS at high cell density and to secrete cholera toxin at the end with biofilm disaggregation and cell dispersal (Finkelstein *et al*., 1992).

Contribution To Disease Pathogenesis

The biofilm contribute effectively to disease pathogenesis by various mechanisms.

1. First of all, the neutrophils which act as first line of defense on encountering bacteria instead act as a matrix upon which biofilms are formed.
2. Generation of reactive oxygen species is high under biofilm conditions. Even if generated, the biofilm bacteria are highly resistant enough to neutralize it.
3. The antibodies produced against bacteria residing in biofilms are unable to penetrate interior and hence remain ineffective.
4. Furthermore, phenomena like phase variation and quorum sensing that occurs within biofilm enable bacteria to progress disease pathogenesis (Costerton *et al*., 1999).

Bacteria	***Disease in animals***	***Genes/factors involved in biofilm formation***	***References***
Actinobacillus pleuropneumoniae	Porcine pleuropneumoniae	pgaABCD operon, H-NS	Kaplan *et al*.,2004
Aeromonas hydrophila	Fish pathogen	N-acylhomoserine, AI-1 QS, Lateral flagella	Lynch *et al*., 2002, Gavin *et al*., 2002
Arcanobacterium pyogenes	Summer mastitis, sporadic abortion	ploSR	Jost & Billington., 2005
Bartonella henselae	Cat scratch fever	Pilins	Kyme *et al*., 2003
Bordetella sps.	Kennel cough	PGA, BvgAS signal transduction system	Irie *et al*., 2004
Brucella melitensis	Malta fever, abortion in goats	QS dependent regulator VjbR	Urueau *et al*., 2007
Burkholderia pseudomallei	Melioidosis	rpoE operon, pil A – type IV A pilin	Korbsrisate *et al*., 2005
Campylobacter jejuni		Flagella (flaAB), QS (luxS), LOS mutants, CprS –VE	Reeser *et al*.,2007, Svensson *et al*., 2009
Clostridium perfringens	Enterotoxemia in calves,lambs	Type IV pilus dependent gliding motility, catabolite control protein (CcpA)	Varga *et al*.,2008
Enterococcus faecalis	Oppurtunistic pathogen	Epa- enterococcal polysaccharide antigen	Teng *et al*., 2009
Erysipelothrix rhusiopathiae	Swine erysipelas	Outer surfaceproeins RspA, RspB	Shimoji *et al*.,2003
E. coli	Oppurtunistic pathogen	LPS, cellulose, capsule, PGA, colanic acid, AHLS, AI-2, Hha/TomB, MqsR/B3021,	Zogaj *et al*., 2001, Agladze *et al*.,2005, Wood *et al*., 2009 Puttamreddy *et al*.,2010

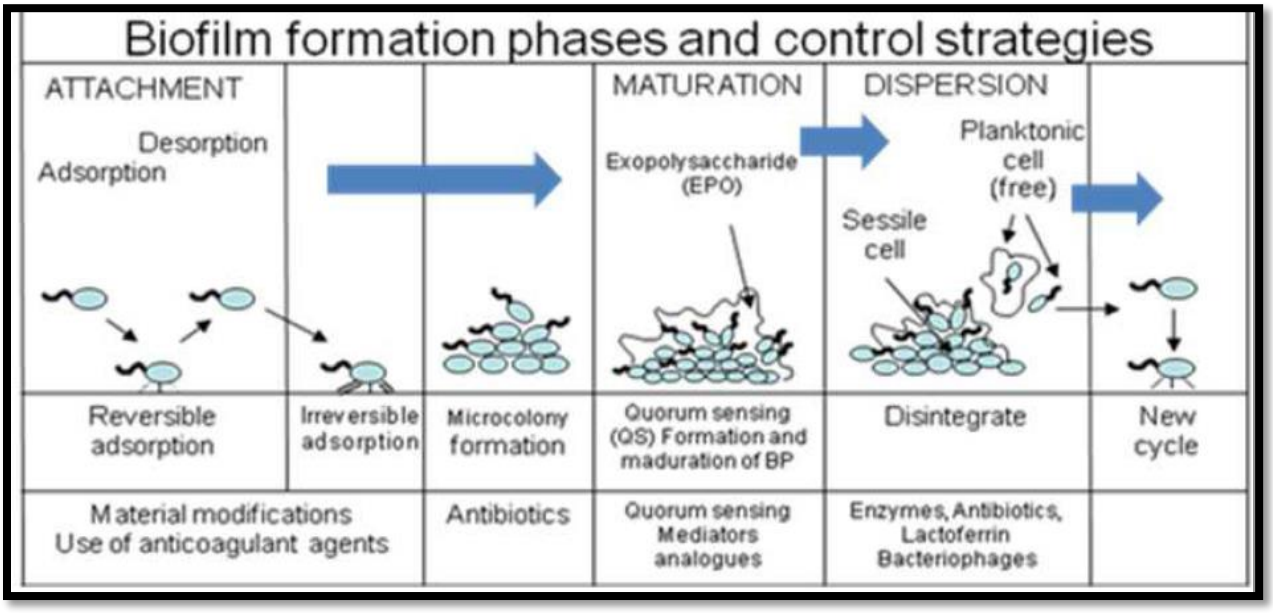
		small RNA- CsrB espP- autotransporter serine protease, ehxD – enterohemolysin translocator	
Francisella tularensis	Tularemia	Orphan response regulator	Durham- Colleran *et al*., 2010
Histophilus somni	Bovine pneumonia, reproductive tract infections	Filamentous hemagglutinin	Olson *et al*., 2002
Listeria monocytogenes	Abortions,	Agr peptide sensing system (agrD)	Riedel *et al*., 2009
Mycobacteria sps.	Tuberculosis, Johne'sdisease	GLPs –glycopeptidolipids, cell wall lipopeptide	Wu *et al*., 2009
Mycoplasma sps.	Mastitis, metritis, pneumonia, arthritis	Glucose-galactosecontaining EPS-I, EPS-II N-acetyl glucosamine	Daubenspeck *et al*., 2009
Salmonella sps.	Salmonellosis in humans, abortion	Cellulose, curli fimbriae	Romling,2005
Staphylococcus sps,	Mastitis, skin disease	ica ADBC, Bap (biofilm associated protein), IS257, MSCRAMMS genes	Melchior *et al*., 2009
Streptococcus sps.	Mastitis	Pili-type 2a	Konto-Ghiorghi *et al*., 2009
Yersinia pestis, Y.enterocolitica	Bubonic plague	hmsHFRS	Hinnebusch & Erickson, 2008
Pseudomonas aeruginosa	Oppurtunistic infection	Psl (polysaccharide synthesis locus), Pel (pellicle formation), alginate	Ryder *et al*., 2007

Increased Resistance To Antibiotics

Bacteria residing inside biofilms are more resistant to antibiotics than their planktonic counterparts due to i) Barrier and protective effect provided by extracellular polysaccharides ii) Majority of bacterial cells inside a biofilm enter into a stage of slow and dormant growth leading to a condition called persister due to variabity in nutrition availability. iii) Antibiotic degrading enzymes like ß lactamases and pumping out mechanisms are prevalent within the biofilm population. iv)The frequency of exchange of resistance genes and plasmids within a biofilm environment is more thus leading to resistance (Costerton *et al*., 1999).

Control Of Biofilms

Control strategies can be adopted at various stages of biofilm formation namely attachment, maturation and dispersal. The initial attachment of bacterial cells to surfaces can be prevented by coating the devices with antimicrobials etc (Lewis, 2001). During maturation phase, quorum sensing inhibitors can be used (Hentzer & Givskov, 2003). The dispersion of biofilms can be done with enzymes, antibiotics, lactoferrin and bacteriophages (Curtin & Donlan, 2006).



Need For Biofilm Vaccines

The need for biofilm vaccines arise when vaccination with either live or killed planktonic culture fail to elicit effective immune response. The reason may be due to variation in gene expression in biofilms compared to planktonic bacteria. Even if antibodies are formed, the extracellular polysaccharide layer of biofilm is too thick to allow the FC portion of antibodies to communicate with the surface phagocyte receptors (Riot *et al*., 1996). So a couple of strategies can be followed in production of biofilm vaccines i.e. either the abundantly expressed biofilm proteins or the extra cellular polysaccharide layer (EPS) of biofilms can themselves act as vaccine candidates. For example, *S. aureus* biofilms contain poly N-acetyl ß 1, 6 glucosamine (PNAG) as the major EPS constituent. Vaccine – challenge studies against PNAG revealed improved results compared to control in cattle (Perez *et al*., 2009). The immunity produced against PNAG is not serotype specific thus it can be used as a vaccine candidate against biofilm forming *S. aureus* isolates irrespective of the serotype. Moreover studies by Gouwet *et al.*, 2014 revealed that

BipA (Bordetella intermediate protein A) was the most abundant protein expressed in biofilms of *Bordetella pertussis*. Immunization with recombinant BipA in mice model reduced the bacterial load in respiratory tract suggesting that BipA can be used as vaccine candidate against whooping cough.

Conclusion

Though the role of biofilms in human disease pathology is well exploited, its role in important animal diseases like haemorrhagic septicemia, brucellosis, black quarter, mycoplasmosis have yet to study in detail. This deep insight is required to understand variation in gene expression in biofilms and also to improve the already existing vaccines which were only based on plank tonic bacterial culture.

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Vaccination failure in animals

Santhamani, R1 and Sophia, I2
Ph. D., scholar, 1 Division of Virology, 2 Division of Bacteriology and Mycology
Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

Introduction

Vaccine is a biological preparation containing weakened or killed disease causing microbes or its toxins, administered to produce the same immunity as the natural disease without causing the symptoms and protect the individual against the particular pathogen. Vaccines are generally used for prophylactic or therapeutic purposes. In spite of the availability of vaccines for almost all important livestock diseases, there are regular disease outbreaks resulting in high morbidity and mortality and ultimately huge economic losses. Here comes the neglected term "vaccination failure". Vaccination failure can be defined as the development of disease in an individual even after vaccination against the same pathogen. It can be classified into primary and secondary vaccine failures; the former denotes the failure to seroconvert like Measles vaccine failure due to maternal antibodies and in the later, protective level of antibodies are produced but the level declines due to lack of booster ex. Mumps vaccine failure (Bernard *et al*., 2008). The reasons for vaccine failure are briefly discussed here in order to take necessary steps and to prevent future outbreaks.

Causes of Vaccine Failure

Both the animal and vaccine factors contribute to vaccine failures.

Animal factors

a. Maternal Antibody

Maternal antibodies are passed from dam to the neonates through placenta or colostrum. They have the ability to neutralize the organisms present in the vaccine. The maternal antibodies are present in neonates for a period ranging from 4-8 wks in calves and 9 months in humans (Hasselquist *et al*., 2008).Window of susceptibility is a time duration in which maternal antibody levels are less enough to protect against disease and good enough to neutralize the administered vaccine and results in vaccine failure. It is a critical period, during which young ones are susceptible to diseases.

b. Immunosuppression

Immunosuppression is a condition wherein the non-cellular (antibody) and cellular components of the immune system are not functioning properly. Genetic disorders and many extrinsic factors are responsible for immunosuppression. The former causes an improper development of immune system and the later suppresses the optimum functioning of the immune system. Immunosuppressed animals can't be protected by vaccination. Some of the extrinsic causes are

1. Pathogens e.g. Infectious Bursal Disease virus, Mareks Disease virus, Chicken Anemia virus, Rinderpest virus, Bovine Viral Diarrhoea virus, etc. will damage the cells of immune system.
2. Mycotoxins (Aflatoxin) inhibit the activity of phagocytic cells like neutrophils and macrophages.

3. Age: Neonates posses' undeveloped immune system. In old animals, there is replacement of bone marrow with fat and as a result immune system is inhibited.
4. Nutritional deficiency: Vitamin B complex and vitamin C deficiency causes atrophy of immunocompetent organs like bone marrow, thymus and bursa in birds (Ghoshal *et al.*, 1990).
5. In stress conditions like pregnancy, extremes of cold and heat corticosteroids are produced which in turn inhibit the immune response.
6. Prolonged therapy: Cancer treatment inhibits the rapidly dividing cells of the body like bone marrow cells and causes anemia and immunosuppression. Prolonged steroid therapy in autoimmune diseases also causes immunosuppression.
7. Drugs: Treatment with chloramphenicol, furazolidone etc. result in bone marrow suppression and anemia.
8. Parasitic infestation makes the animal nutritionally deficient and immunosuppressant.

Vaccine Factors

1. Vaccine Titer: Reduced vaccine titer will produce insufficient immune response. But vaccines manufactured from reputed companies will have optimum titer.
2. Loss of immunogenic epitope during manufacturing process such as use of phenol in killed vaccine preparation was found to cause denaturation of proteins, so that it may damage the protein epitopes on the virus envelope.

3. Clinical disease may be caused by vaccine itself (in case of live vaccine), if reversion back to virulence, E.g. Live attenuated polio vaccination in human beings.
4. Storage: Live vaccines are sensitive to heat than cold and so cold chain maintenance is essential e.g. Live MD vaccine must be stored at 2-8°C in freeze dried form and -20 to 8°C in liquid form. Killed vaccines are sensitive to both cold and heat. So it should be stored at 2-8°. In developing countries like India, lack of maintenance of cold chain facilities at field level lead to several vaccine failures.
5. Administration of vaccines in drinking water especially for poultry, death of live virus due to mishandling, presence of water sanitizers and lack of skim milk powder will also result in vaccine failure.
6. Use of wrong diluents for diluting the freeze dried vaccine.
7. Use of antiseptics like alcohol for swabbing the skin before vaccine administration produces nil or insufficient antibody titer.
8. Antigenic variation: The protection afford by vaccination is incomplete if serotype or strain is different than field strain (ex. Massachusetts and Connecticut serotype of infectious bronchitis virus does not protect against Arkansas and Florida serotypes in poultry). Also if field strain is highly virulent and vaccine strain is highly attenuated there may be chance of inefficient immune response. Antigenic drift / shift in influenza virus causes change in immunogenic epitope. Antigenically different strains of foot and mouth disease virus can cause infection even after vaccination (Woolhous *et al.*, 1996). These diseases are difficult to prevent by vaccination (Ekkommonen *et al.*, 1997).

9. Vaccine interference: Administration of more than one vaccine at a time may interfere with protective immunity offered by the other. Without recommendation of manufacturers, we should not administer two vaccines simultaneously. At least 14 days interval should be there between two vaccinations.
10. Administration factors:
    a. Aerosol and drinking water administration of vaccines is followed in poultry. Here we can't assure that all birds got exposure to adequate dose of vaccine. "Misses" may be possible and incomplete protection may be accounted as vaccination failure.
    b. Inappropriate route of administration: Vaccine should be administered in recommended route for which it was prepared. Otherwise there may not be sufficient protection.
    c. Site of administration also plays a role in eliciting immune response.
    d. Inadequate dose
    e. Period between priming and booster dose: If priming and booster doses are not administered in recommended interval, there will not be appropriate protection.
    f. Vaccination at incubation period of disease ex. Rabies vaccination (Winkler *et al*., 1991).
    g. Period between vaccination and exposure to infection: After vaccination it will take at least 14 days to produce protective level of antibody, if animal got exposed before 14 days will succumb to disease.
    h. Too long period between vaccination and exposure.

Conclusion

For control and eradication of infectious diseases, vaccination program is an essential tool. But the success of vaccination (immunization) depends on factors like maternal antibodies, immune status of the animal, stress conditions like nutritional deficiency, dose and site of administration. No single vaccine is available to give complete protection in the field as it depends on animal, microbe factors, etc. At least to offer maximum protection, we have to follow the recommendations of manufacturer from storage to administration.

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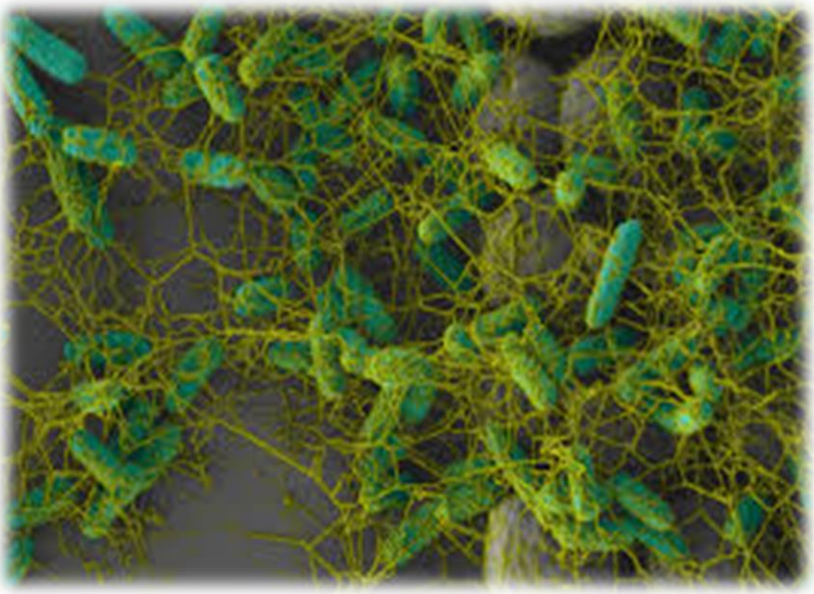
Stratagem to Combat Bacterial Colonization

Mrs. Megha Manoj Machhar,
Scientific Communicator, WTM
meghaapanpaalia@gmail.com

The fierce battle between bacterial infections and mankind has been one of the high points of concern and research for the modern biosciences. The discovery of Penicillin during World War II and other antibiotics thereafter offered a powerful tool to fight against these infections. However, due to widespread and constant use of these antibiotics and other environmental factors many bacterial strains have developed resistance and it is this class of antibiotic-resistant bacteria that have posed a great threat to human life. As per the statistics of World Health Organization (WHO) in 2013 approximately 480,000 new cases of Multidrug-resistant Tuberculosis (MDR-TB) have been reported and instances of extensively drug-resistant Tuberculosis (XDR-TB) have been identified in over 100 countries. A high percentage of hospital-acquired infection is caused by highly resistant bacteria such as methicillin-resistant *Staphylococcus aureus* or MDR Gram negative bacteria.



Prevalence of the most hazardous chronic infections is the ones that are caused by bacterial biofilms. A *biofilm* may be defined as a thin layer of microorganisms that adhere to the surface of a structure (organic or inorganic) together with the polymers that they secrete. These are complex multicellular communities. In general, bacteria have two life forms during their growth and proliferation. In one form they exist as single independent cells (planktonic) where as in the other form they are organized into aggregates known as biofilms. Infections caused by plank tonic bacteria are treatable whereas infections that result from bacterial biofilms are very difficult to treat with increased risk of worse clinical outcomes and deaths as they are impenetrable to both host immune response and antibiotics.

Several approaches have been undertaken to prevent biofilm formation. These include:

- Chemical approach- involves the use of antibiotics and biocides.
- Physical approach – involves the use of ultrasound, vibrations, and piezoelectric elements.
- Biological approaches – Involves the use of lytic bacteriophages and interference with interbacterial signaling and genetic programs that control biofilm formation.

Of several strategies being developed to inhibit bacterial adhesions, the most promising one is the attachment of polymer brushes to form antibacterial surfaces. A polymer brush is a layer of polymers attached with one end to a surface. A similar approach has been used by Shoghik Hakbyan, a chemist at Umeå University, Sweden, who in her doctoral thesis has studied ways to decrease bacterial colonization.

Shoghik Hakbyan used *hydrazones*, a small class of organic molecules, as antibacterial compounds. These molecules disarm bacteria without killing them.

Since bacteria stay alive, the evolutionary pressure for developing resistance is lower than for bactericidal compounds. She also found that these molecules form highly stable complexes with metal ions like gallium ions and this hydrazone-gallium ion complex suppresses the pathogenic action of bacteria this preventing biofilm formation.

The research also carried for studying surface modifications with different types of polymer brushes and testing their antibacterial properties. The results highlight the brushes contain equal amounts of positively and negatively charged units that strongly suppress biofilm formation and reduce the attachments of proteins along with bacteria.

The whole stratagem used the hydrazone-gallium complexes and have raised hope to combat these resistant microbes with profitable success.

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Neglected Tropical Diseases still Neglected

(Cover Story)

Saumyadip Sarkar,
Scientific Communicator, WTM
saumyadip.gis@gmail.com

World Health Organization has highlighted seventeen (17) neglected tropical diseases which are being neglected based on less common importance towards priority of disease prevention. WHO had highlighted this to spread awareness of the advent of the spread of this diseases in the tropical areas which are yet needed to be cured and also there are possibilities of proper treatments. In this cover story, you can find brief descriptions of 10 among 17 neglected tropical diseases with its wider importance highlighted.[1]

1. ***Dengue*** [2, 3]: A vector borne disease, where mosquito infected with one of the four dengue serotypes, causing mild fever to higher incapacitating fever affecting infants, young children as well as adults. The virus is not transmitted directly from person to person, and there is still no vaccine or specific medical treatment of dengue. The patients are advised to take adequate rest, to drink plenty of fluids and have drugs to reduce fever. Severity of this disease causing abdominal pain, bleeding while vomiting, breathing difficulty and fever. WHO reported that half of the population is now still at risk based on last update on May 2015. An estimated 500,000 people require hospitalization with severe dengue each year, where most of them are children, and about 2.5% of the affected individuals die. The additional control and prevention solely depends on the effective vector control measures.

2. ***Rabies*** [4, 5]: A zoonotic disease, infecting the central nervous system caused by lyssavirus. It is so common that it is endemic in every continents apart from Antarctica. A high score of over 3 billion people are known to be potentially threatened with this disease, with most incidence in Asia and Africa. The important fact lies although 100% vaccine preventable disease although there are escalating deaths in various parts of the world. A general perception towards the lack of awareness is among the policy makers of the rabies burden and acting over the prioritizing resources towards effective control. Cost effective way over mass-vaccination of the dogs is much significant although they are not monitored by the government nationals in even most endemic countries. There are several key scientific questions yet to be resolved with urgency of promoting research and development on the fundamental aspect of improved control tools rely on the national measures.

3. ***Trachoma*** [6, 7]: A disease causing infection leading to blindness, with reported 2.2million people of visual impairment, among whom 1.2million people are irreversibly blind. The infection is caused by the bacterium *Chlamydia trachomatis*, known to spread from direct contact with the eye or nasal discharges of the infected persons or some inanimate objects that carry the agents. Based on reports of 2012, 28 countries had implemented SAFE strategy (Surgery, Antibiotic treatment, Facial cleanliness, Environmental

improvement) worldwide with treatment reach of 48.8 million people. In the same year, several countries like Ghana, Morocco, Mayanmar, Gambia, Oman and Viet Nam reported WHO of successful elimination of Trachoma as public health problem. Global burden is still found of active trachoma in five countries: Ethiopia, India, Guinea, Sudan and Nigeria. WHO estimated of $2.9 billion annually on economic cost of trachoma in terms of lost productivity.

4. ***Buruli Ulcer*** [8]: A deliberating skin and soft tissue infection disease caused by the bacterium *Mycobacterium ulcerans*, although the exact mode of transimission is yet unknown. Thirty three (33) different countries have reported the burden of this disease including regions of Africa, South America and Western Pacific regions. The disease is curable with combination of antibiotics. Environmental and climatic changes provide an influence of number of cases each year. Based on the detection and confirmation of the disease there are four technical laboratory methods – IS2404 polymerase chain reaction (PCR), direct microscopy, histopathology and culture. WHO has also published a manual based on these four techniques to help researchers and health professionals. The negativity of prevention of this disease lies of the lack of knowledge of how Buruli ulcer is transmitted and hence preventive measures cannot be applied.

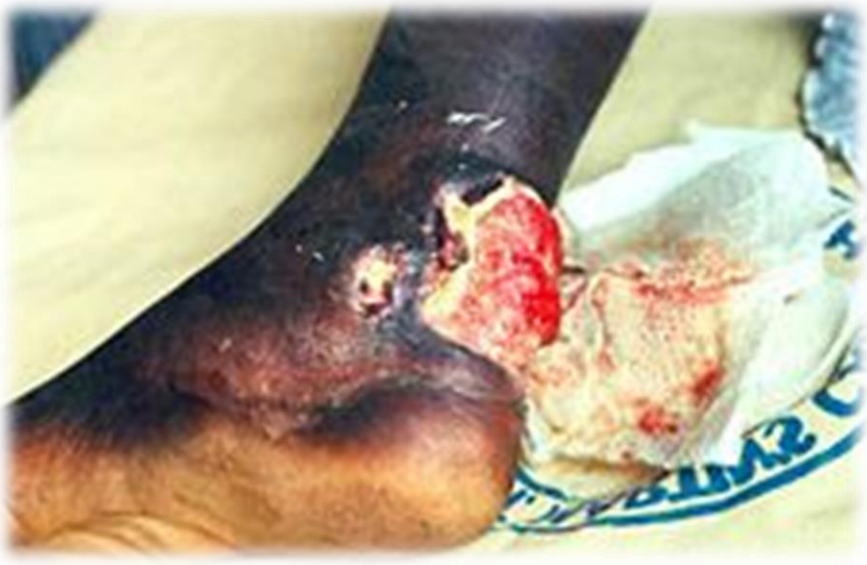

Fig: Buruli ulcer in an ankle of a patient from Ghana

5. ***Yaws*** [9]: A chronic infectious disease leading to deformities of nose and bones of leg, caused by the bacterium *Treponema pallidum*. It was considered long before that yaws can be eradicated as humans are the only known reservoir. Earlier only two countries: Equador and India were once endemic but currently there are 13 different nationals which need support of WHO's new eradication strategy. In 1960, WHO expert committee set two important criteria of eradication strategy which includes epidemiological eradication and complete eradication. As per WHO Weekly Epidemiological report on 17th of April, India appear to have eliminated yaws. The importance of yaws of being neglected tropical disease also due to lack of vaccination. Prevention strategy includes interruption of transmission with early diagnosis, treatment of individual patients with yaws and targeted treatment of affected populations.
6. ***Leprosy*** [10]: The causable agent of this disease is *Mycobacterium leprae* which multiplies slowly showing symptoms after 5-20years. It affects skin, mucousal layer of upper respiratory tract, peripheral nerves and eyes. It gets

transmitted through droplets and also from close contacts of affected individuals. The disease is curable and treatment is offered in the early stages. WHO has ascertained free treatment with Multidrug therapy (MDT) to all patients worldwide since 1995, and by year 2000 elimination of leprosy was achieved globally. Currently, leprosy control has improved implicitly with increased drug facilities, trained stuffs, increased health services for early detection of cases and possible efforts for increased treatments. Global statistics in 2013 has figured that around 96% of new leprosy cases were detected from 14 countries and 4% from rest of the world. Why still neglected? Since there are still high endemicity remain in many counties lying in south Asian countries, few countries of Africa and others like Brazil and Angola.

7. ***Chagas Disease*** [11]: The disease is named after the Brazilian physician Carlos Chagas, who discovered the disease in 1909. The causative agent is *Trypanosoma cruzi* which get transmitted to humans via insect vectors. The disease is mainly found in America, especially in the rural areas of Latin America. The treatment of the disease is possible and is effective highly in the acute stages. In U.S., the medication is only available through Centers for Disease Control and Prevention (CDC). The prevention strategy involves improved housing and spraying of insecticide to eliminate bugs or possible vectors of this disease. Moreover, screening of donated bloods is also mandatory action to prevent any spread of the disease. In U.S. and other associated regions where Chagas disease is found is not endemic and government is slowly undertaking the measures.

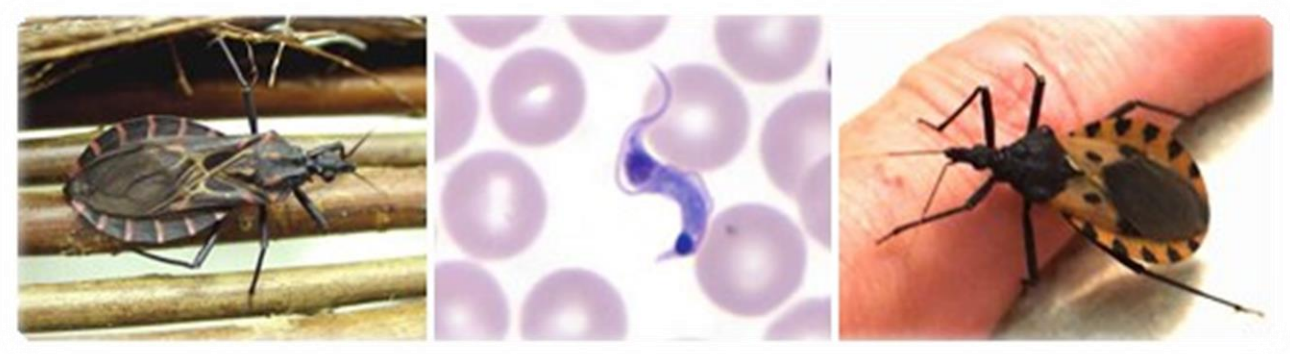

Fig: Insect vectors that transmit *Trypanosoma cruzi*, the causative agent of Chagas disease (Source: CDC)

8. ***Human African Trypanosomiasis*** *(sleeping sickness)*[12]: The disease is caused by the bite of an insect called Glossina, which is commonly known as tsetse fly. The disease is prevalent in the rural areas of Africa. If remain untreated, the subject may lead to death. It is 100% fatal. Many travellers also get affected if they venture through the place where this insect is common. The disease is not observed in urban areas, but many reports have been made to be observed in sub-urban regions. Based on the prevalence, in 1995 WHO expert committee had reported of about 60 million people were at risk with estimated 300,000 cases per year in Africa, where fewer cases were diagnosed and treated. Later in 2009, the reported cases were reduced to 10,000 and the estimated actual cases to be 30,000. The trend is maintained in 2010 with 7139 reported cases.
9. ***Leishmaniasis*** [13]: It is caused by the Leishmania parasite by the bite of phlebotomine sand flies. There are different types of Leishmaniasis, among which the common form is cutanious leishmaniasis causing skin sores, visceral leishmaniasis which affects internal organs like spleen, liver and bone marrow. Before treatment, the patients are screened on the type of lesihmania, the leishmania species that caused it, the severity of the case and patient's health. Cutanious leishmanisis heal on its own, even without treatment, but it takes longer time leaving back scars. Mucosal leishmaniasis

is prevented by adecuate treatment of cutanious infection. There are currently no vaccines or drugs available for this disease. Best way to prevent oneself is to protect themselves from sand fly bites. This also highlights the potential importance of this disease, yet neglected.

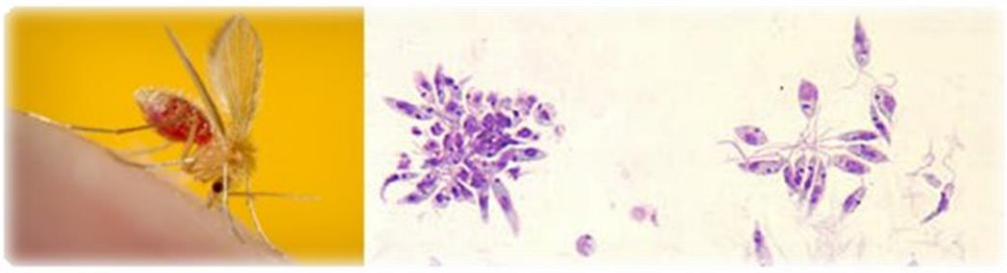

Fig: The sand flies that transmit the parasite are only about one third the size of typical mosquitoes or even smaller. On the left, an example of a vector sand fly (*Phlebotomus papatasi*) is shown; its blood meal is visible in its distended transparent abdomen. On the right, *Leishmania promastigotes* from a culture are shown. The flagellated promastigote stage of the parasite is found in sand flies and in cultures.

10. ***Lymphatic Filariasis*** [14, 15]: The disease commonly known as elephantiasis, a serious disfiguring disease. The infection is acquired in childhood, while the visible deformities observed later in life causing temporary or permanent disabilities. In some endemic countries Lymphatic filariasis remain as social and economic impact. There are three species of worms which cause these deformations named *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*, and the vector is mosquito. For treatment, Diethylcarbamazine (DEC) is a drug of choise in U.S., although now it has been banned from the country by Food and Drug Administration (FDA) committee. Patients are well advised for proper sanitation and maintain proper health care. The burden of the disease still remains.

Fig: Microfilaria of *Wuchereria bancrofti* in thick blood smear stained with Giemsa. Right: Microfilaria of *Brigia malayi* in a thick blood smear, staned with Giemsa. Center: Photograph of a female *Aedes aegypti* mosquito as she was in the process of obtaining a "blood meal". Laboratory strains of *Aedes aegypti* can be infected with Brugia. (Source: CDC)

In conclusion of the Neglected tropical diseases, are almost common in around 149 countries, affecting more than 1.4 million people allowing government economies to cost billions of dollars every year. The importance withholds the world wide eradication of these diseases with large scale prevention campaigns with educative information. In some situation, the tropical diseases get tied towards gender difference where females play bigger role in expectation for medical facilities, education and support from health officials. The most important criteria for the negligence is the public awareness rate where most people in developing countries stay backward toward information regarding preventive measures to stay away from these diseases. In future, together with WHO and other International Organizations may eradicate the burden of the disease with proper treatment and valuable information.

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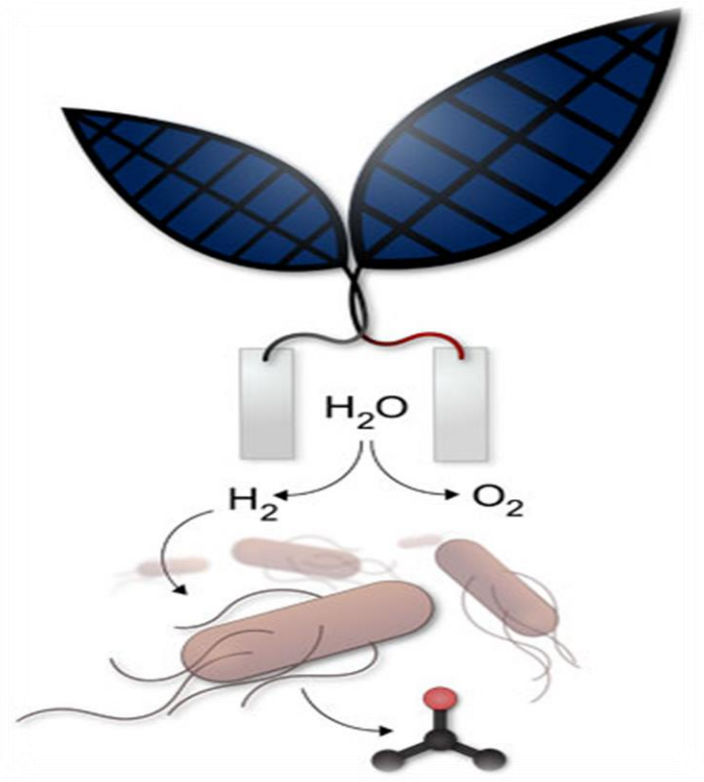
Bionic Leaf-Bacteria Assisted Renewable Energy Harvesting System

Energy is the basis of life. It is the ability of a system to perform work. This energy has two forms, one that is inexhaustible, the renewable energy sources like sun, wind and biomass and the second one is exhaustible,non renewable energy sources like coal, petroleum, natural gas. We are highly dependent on non renewable energy sources for every work we do like cooking, transportation, electricity, machinery, etc. With increasing population and growing industrialization of the world economy the non renewable energy sources are on the verge of depletion. It has become imperative to switch to renewable energy sources by implementing technologies that help harness energy from sun, wind and biomass.



One such step in this direction has been taken up by Adams professor of biochemistry and systems biology Pamela Silver at Harvard Medical School (HMS) and Patterson Rockwood professor of energy Daniel Nocera in the Faculty of Arts and Sciences (FAS). Their fruitful collaboration has led to the invention of an *artificial leaf* that can trap the solar energy from the sun (like any other living leaf) and convert into hydrogen fuel.

The process to harvest the renewable energy source from sunlight started in 2009 itself by chemist Daniel Nocera. He designed water splitting cobalt-phosphate catalyst system that used electricity to make hydrogen from ordinary water. But hydrogen so formed failed to be used as a practical fuel. Two years later Daniel Nocera in association with Pamela Silver came up with a hybrid system by pairing a machine and microbe, thus, developing the *bionic leaf* concept.

Bionic leaf converts solar energy into liquid fuel. It is a battery like system that feeds the microbes with the hydrogen from water split by special catalysts connected in a circuit with photovoltaic. The special catalysts incorporated disintegrate the water and the hydrogen generated is split into liquid fuel, isopropanol, by genetically engineered bacteria *Ralstonia eutropha*. It is a gram negative, non-spore forming, non-pathogenic, motile, facultative aerobe. It has a great potential for use in bioremediation. Its strains are model organisms for hydrogen oxidation because it can nurture on hydrogen as the solar energy source. Isopropanol can be used as fuel like ethanol and can be separated from water using salt.

In the process of bionic leaf development Daniel Nocera and Pamela Silver subjected the recombinant *Ralstonia eutropha* to fine metabolic adjustments by placing them in sealed jars filled nutrient free liquid, hydrogen and dissolved carbon dioxide. After few transfers the starving *R. eutropha* switched from its normal mode of growth to panic mode inducing itself to feed directly on hydrogen. The resulting colony was placed in jar with water splitter and a stainless steel electrode connected to photovoltaic array to provide current. After two days the bionic leaf began to grow and spit out isopropanol.

It has been observed that bionic leaf operates best at high voltages that help *R. eutropha* to thrive better in harsh conditions, while producing the desired

molecules. The prime objective of its developers is to work on increasing its efficiency. Improvement can come on:

1. Using mutant *R. eutropha* strains that might be more tolerant of harsh conditions, which could help produce more fuel.
2. Using entirely different microbial species that more efficiently convert carbon dioxide to fuels.
3. The electrode materials could be tweaked to minimize or remove challenges they present to the microbial population.

The newly developed artificial leaf mimics the process of photosynthesis. It uses the solar energy and the photovoltaic help split the water into hydrogen which is then used by *R. eutropha* to produce fuel. It follows the principle of reverse combustion where the waste product of fossil fuel burning, carbon dioxide, is converted into useful fuels. This system when fully developed with its efficiency enhanced and launched on commercial scale would be a boon to the world economy as it would help fight the most complex issue of global warming and also provide us with an alternative source of energy.

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Lokiarchaeota:

The Missing Link Microorganism Discovered

Mrs. Megha Manoj Macchar,
Scientific Communicator, WTM

Biosphere, the zone of life on Earth, is colonized by millions and trillions of different life forms. These varied life forms can be broadly classified as *prokaryotes* and *eukaryotes*, with prokaryotes outnumbering the eukaryotes. It is generally held that the first living organism has been formed around four billion years ago, with earliest forms being simple molecular groupings, having evolved from the existing inorganic substances-life from non-life! The prokaryotes, the first one to evolve, include *bacteria* and *archaea* –the single celled organisms. The origin of the complex eukaryotic life forms that include plants, animals, and fungi is considered to be one of the critical events in history of life on Earth.

The evolutionary studies state that the eukaryotic cell has evolved from the symbiotic association of bacteria and archaea, but how the evolution process took place still remains a mystery. Recent studies have suggested that eukaryotes are closely related to archaea but biologists have not been able to find an organism that form a link between the two, till date.

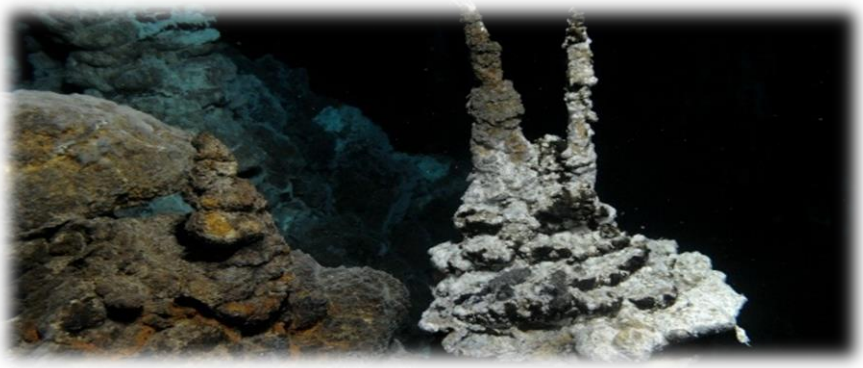

Image of a hydrothermal vent field along the Arctic Mid-Ocean Ridge, close to where 'Loki' was found.

Dr Thijs Ettema, Dr Guy and their colleagues from the University of Bergen, University of Uppsala and the University of Vienna have succeeded in finding that missing link. They have discovered a new group of Archaea-*Lokiarchaeota* from deep sea sediments.

Lokiarchaeota, the missing link in the origin of eukaryotes, derives its name from the adverse environment close to where it was discovered, Loki's Castle. It is a hydrothermal vent situated on the Mid-Atlantic ridge between Greenland and Norway, at a depth of 2352 meters.

According to Dr Ettema eukaryotes are probably a sister clad to the TACK Archaea, a superphyllum in Archaea that includes- Thaumarchaeota, Aigarchaeota, Crenarchaeota and Korarchaeota. He asserted that Lokiarchaeota fall within the TACK Archaea and represents the closet prokaryotic organism to the eukaryotic state.

Dr Ettema and his team sequenced the DNA sample taken from Loki's Castle and constructed one mostly complete genome (which they called as Lokiarchaeum) and two partial ones. The genes found in Lokiarchaeum suggest that it might have features exclusive to eukaryotes. It has five genes that code for *actin* (the protein molecule used by eukaryotes to build their internal cytoskeletons); genes that codes for molecules which help it to divide into two or to package unwanted molecules for recycling. It has huge number of genes that are supposed to code for eukaryotic enzymes- small GTPases. This microbe, thus, has shown some characteristics of cytoskeleton and phagocytosis, feature of eukaryotic cell type.

Some biologists have disagreed with this theory. They state that the above findings speculate Lokiarchaeaum to consist of characteristics specific to eukaryotic cell type but they have not reached on a thorough conclusion. The researchers are, thus, constantly working on the newly discovered microbe to collect more evidence to

support their hypothesis. They are also looking for Lokiarchaeum like organism in other locations including hot springs in Yellowstone National Park (US) and in New Zealand.

Identification and genetic analysis of neoteric organisms, like Lokiarchaeum, can bridge the gap between the sequenced minority and the non sequenced majority, thus, providing a better understanding of the biological history.

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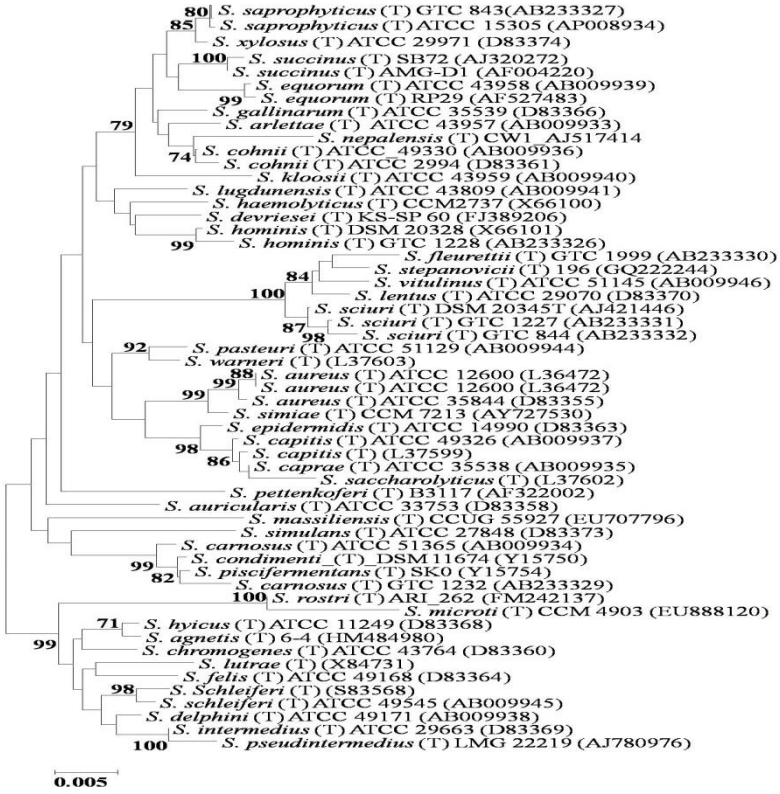
Staphylococcus

The superbug behind human infections

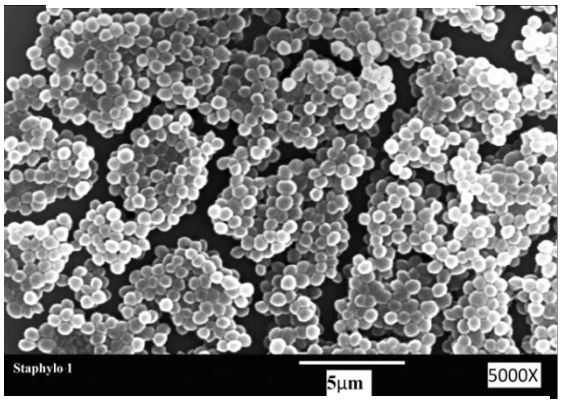
Staphylococcus: The Historical Background

Staphylococcus, most popularly known as Staph is gram-positive cocci that occur in clusters like grapes. For the 1st time in 1884, Rosenbach described and proposed the appropriate nomenclature of Staph, where *Staphylococcus aureus* forms a yellow and *Staphylococcus epidermidis* forms white colony. Till date, there are 57 validly described type species and 27 sub species, whose phylogeny is described in the figure. It is a member of family *Staphylococcaceae*, including *Gamella*, *Macrococcus* and *Salinicoccus* and members of the genus *Bacillus* in the family *Bacillaceae* as near relatives.



Phylogenetic tree showing all described type strains of Staph spp. through neighbor joining method. 0.005 indicates 0.5% divergence (own analysis)

Source: Microbelibrary.org, ASM



SEM micrograph of Staph showing grape like apperance (Own study)

Phenotypic traits

Gram-positive, cluster-forming coccus, nonmotile, catalase and oxidase positive, non-spore forming, facultative anaerobic, capable of fermenting glucose and mannitol producing lactic acid, coagulase positive with formation of golden yellow colony on agar by *S. aureus* to white colony by *S. epidermidis* and others. Most prevalently present as normal flora of humans associated with nasal passages, skin, oral cavity, gastrointestinal tract and mucous membranes. Pathogenic strains form suppurative infections, food poisoning and toxic shock syndrome. All the strains can grow at a temperature range of 15 to 45 degrees and at NaCl concentrations as high as 10-15%. *S. aureus* is famous in producing coagulase and perform haemolysis where *S. epidermidis* is unable to do so. All are perfectly spherical in shape with diameter of 1µm presented in below figure. The typical grape like appearance is due to the successive cell division in three perpendicular divisional plane, where the sister cells change their position while remaining attached to the parent cells and hence form a irregular cluster.

Ecology and behavior

Usually Staphs is mostly the normal flora of humans, pigs, cattle, rabbits, other animals and birds. Most strains of *S. epidermidis* are nonpathogenic and may even play a protective role in humans as normal flora. *S. epidermidis* may be a pathogen in the hospital environment. *S. aureus* always considered as a potential pathogen due to its highly invasive property. Recent studies indicated its presence in soil as typical fraction where it plays an important role in different biogeo-chemical cycling. The report also describes the apparent role of these strains in food products like meat, fish and poultry as a normal contaminant.

Genome description

Recent techniques of sequencing whole genome of the bacteria is found to be most promising in unraveling the genomic potential of the bacterium involved in pathogenesis and various ecological functions. NCBI taxonomy browser shows around 5500 registered bioproject with 32000 of biosample indicating its isolation from various habitats. Around 6000 Staph genomes have been assembled showing its unique property of invasiveness towards host tissues. Genome of the most common species, *Staphylococcus aureus* is found to be 2.8 Mb long with 2,600 ORFs, comprising 84.5% of the genome with a G+C content of about 33%, and a circular plasmid of 25Kb. Most of the genes for antibiotic resistance are located on plasmids, where

virulence factors are encoded by phages, plasmids, pathogenicy islands and cassette chromosome. It has been estimated that the resistance genes has been procured through horizontal gene transfer (HGT) from near relatives encoded within the Tn5 like transposons (Tn 1546).

S. aureus, a potential infectious invader of human host

S. aureus causes a variety of suppurative infections through production of toxins in humans. It causes boils and furuncules as superficial skin lesions like mastitis, phlebitis, meningitis, and urinary tract infections (UTI). *S. aureus* is found to be a major cause of nosocomial infection. Enterotoxins of *S. aureus* cause food poisoning and superantigens causes toxic shock syndrome in the blood stream.

Methicillin-resistant *S. aureus* (MRSA) have been known as the most devastating strains occure in hospital and recently emerged outside the hospital as community associated- MRSA((CA-MRSA) or superbug. The primary infection by the organism is indicated by formation of inflammation with elevated temperature, swelling, pus, and necrosis. More serious infections of the skin may occur, such as impetigo. In staphylococcal infections, the bacteria invade the blood stream resulting septicemia/bacteremia leading to infections in the lung, kidney, skeletal muscle etc.

The pathological virulence factors are:

- **surface proteins** for colonization of host tissues;
- **Invasins** for promoting bacterimia in tissues
- **Surface factors (Protein A)** for inhibition of inhibit phagocytic engulfment
- **Coagulase** for blood cells invasion
- **Membrane-damaging toxins** (hemolysins, leukotoxin, leukocidin, αβ toxins)
- Exotoxins (secreted outside) for cell lysis
- Wide **resistance to antimicrobial agents** (vancomycin, erythromycin, methicillin etc)

Treatment and Vaccines

Vancomycin or other alternative are the most promising primary treatment for hospital acquired infection caused by MRSA. Infections acquired outside hospitals are mostly treated with penicillinase resistant ß-lactams. Nosocomial and community level MRSA are resistant to

penicillins and cephalosporins. These infections have been treated with combination therapy using sulfa drugs and minocycline or rifampin. Vaccine therapies represent a new and innovative approach for treating global health problem of community and healthcare-associated *S. aureus* No vaccine still available against staphylococcal infections in humans, but vaccine based on fibronectin binding protein induces protective immunity in cattle and as well as in humans. Monoclonal antibodies against surface components (e.g., capsular polysaccharide orprotein A) are effective against bacterial adherence. Designing compounds that block the interactions and thus prevent bacterial colonization could also be a potential tool for Staph infections. There are many vaccines like **StaphVAX**, **TriStaph™**, **PentaStaph™** etc for effective treatment of the nosocomial infections of MRSA and other Staph species.

So, know your neighbor superbug and stay safe……..

A Talk with Dr. Dirk Linke and Dr. Jack Leo

Dr. Dirk Linke

Conducted by
Saumyadip Sarkar
Scientific Communicator, We The Microbiologist

Q) What bacteria can easily do, most of the eukaryotes does not. That is quite obvious for simple machinery that a bacterium bears. Although not yet simple and hence lot of bacterial mechanism is yet to decipher. It is our immense pleasure to have Dr. Dirk Linke and Dr. Jack Leo from the University of Oslo to share the story behind an identification of a protein that help the well known opportunistic pathogen *E. coli* to survive in high acidic conditions of stomach to reach the intestines. Before we start along, I would like to ask Dr.Linke and Dr. Leo about how you gave a thought to identify this previously unknown mechanism of the bacteria?

We did not specifically look for survival in acidic conditions. Like in many other cases in science, this finding was a fascinating by-product of other research. Originally, we investigated the binding of Intimin to the bacterial cell wall. Our hypothesis was that the binding would somehow stabilize the interaction of pathogenic *E. coli* with host cells. We only noticed the pH-dependence of the binding during the studies.

Q) Carrying the thought to action is a major challenge. Most of major hypothesis modifies and gives a result more than what we obviously expect. Would like to know from you while you set the experiments to go, what was your expectations form the results and what was the conclusion after your receive the result?

Our original hypothesis was that Intimin would bind to the cell wall, based on bioinformatics predicitons – the structure of parts of Intimin is clearly similar to other cell-wall binding proteins.

We then designed various assays to study the interaction in detail, using recombinant Intimin domains and purified cell walls. The pH dependence was noticed during these assays, when we varied buffer conditions to optimize binding.

Q) The protein you identified which was termed as "Intimin" is a part of new identification and may provide understanding to overcome major challenges in research field to control some of the opportunistic pathogens. What you define now about the complex structure that a bacterial cell surface bear and how this protein intimin providing an integral part of it?

Intimin was discovered in the late 1980s, and is a well known and intensely studied protein of so-called "attaching and effacing" pathogens. These include enteropathogenic*E. coli* and enterohaemorrhagic*E. coli*. The former causesrelatively mild diarrhoea, but the latter causes a much more serious disease. Intimin is required for the pathogenesis of both organisms. It mediates intimate attachment of the bacteria to the intestinal lining (hence the name). We also study the way Intimin reaches the cell surface. If we could disrupt that, or the way it binds to the cells lining the intestine, we could potentially use that knowledge to design new therapeutics for diarrhoeagenic infections.

Q) The cell membrane plays an important role in the infection property in the host. Intemin might now after this discovery change the meaning of infection. How this intemin plays role in stabilizing peptidoglycan in high acidic environment of the stomach?

Intimin consist of three separate regions: an extracellular part, a transmembrane domain and the cell-wall binding LysM-region which is on the inside of the outer membrane. The extracellular region is responsible for attaching to the host cell. This is connected to a region which resides within the outer membrane. This transmembrane domain has two functions. Firstly, it anchors the protein in the outer membrane, and secondly, it is responsible for transporting the extracellular part of the protein to the cell surface. The third region, which binds to the peptidoglycan of the cell wall, is the one we investigated for this study. Our assumption is that binding to the peptidoglycan helps to keep the outer membrane and cell wall together under stress conditions, such as in the stomach. We want to emphasize here that this is currently just a hypothesis. We plan many further experiments to investigate this in more detail.

Q) Can you share us how the team "The Bacterial Cell Envelope" set goals for the specific objectives?

This is a dynamic process – each new finding leads to further experiments. As described above, the finding of a Intimin domain similar to other cell-wall binding proteins led to the detailed measurements of this interaction. The pH dependence that was found in the process will now probably lead to cell biology experiments, and so on. The essential thing is to keep an open mind for unexpected results, and to apply a broad range of methods. Collaborations with colleagues from other fields are key to success. In this regard, we benefitted a lot from a long-term funding scheme in Germany (before we recently relocated to Oslo, Norway). In this funding scheme, called Collaborative Research Center, high-level science on a particular subject is funded. Such a center would consist of 15-20 individual research groups working on related topics. The SFB766, which investigates the bacterial cell envelope, is such a research center. This study involved three individual research groups within the SFB766. One group worked on the cellular biology of Intimin, another on the structure of peptidoglycan, and we investigated the biochemical and structural aspects. Only by combining different types of methodology and expertise we can push the boundaries of science and make new discoveries.

Figure caption: Linke Group: from left to right - Gupta Udatha, Dirk Linke, Jack Leo, Agnieszka Wrobel, Anja Winther, Marcin Michalik, Daniel Hatlem (missing: Marcella Orwick Rydmark, Nandini Chauhan, Kheshav Bhusal). Photograph by Marcin Michalik.

Q) Crawling ahead of the research, we would like to know both of your life apart from research? Can you share your words, to let us know the readers how scientists enjoy life along with their research?

Comment: Jack Leo's main hobby outside science is amateur theatre. He has been doing this for over 15 years, both as an actor and a director. He is currently working on a production of Shakespeare's Macbeth, were he will play the protagonist. Dirk Linke enjoys hiking in nature (for which Norway is a great place to be!), cooking, and reading books.

MRSA – Who can stop me?

Dr. M. Malathi, Postgraduate, Department of Microbiology, Chengalpattu Medical College, Chengalpattu, Tamil Nadu
drmalathi13@gmail.com

Introduction:

MRSA - Methicillin resistant staphylococcus aureus.

Staphylococcus aureus is a gram-positive cocci bacterium. They are the most common cause of skin infections, respiratory infections and food poisoning. Depending upon the virulence and susceptibility of the host, they can cause simple skin infections to life threatening complications. Since being the major cause of common infections, it's been treated promptly with antibiotics. With the advent of newer broad spectrum antibiotics and it's over the counter availability, the problem of resistance started emerging. Dating back to history, the discovery of "Penicillin" by Alexander Fleming was the major breakthrough in the antibiotic era. In due course of time, the microorganisms were found evading the attack of penicillin and causing severe infections. This evading mechanism by microorganisms is called resistance. It gained public health importance by being a major cause for hospital-acquired infections. These nosocomial infections, as otherwise called pose stiff competition to researchers and treating physicians in controlling them. The sad fact is these microbes have developed resistance to almost all present day antibiotics from narrow to broad spectrum. The resistance moved from penicillin to aminoglycosides, cephalosporins, fluoroquinolones and lastly to carbapenems.Once they develop resistance to broad spectrum, the treatment options are dwindling. Hence these organisms are often referred as "Super

Bugs".**The sad fact being is that these strains moved from health care (i.e. hospitals) to community**.

Historical perspective:

In 1970, the Surgeon-General of the United States of America announced that it was "time to close the book on infectious diseases, declare the war against pestilence won, and shift national resources to such chronic problems as cancer and heart disease"[1], however morbidity and mortality figures have consistently been alarming for the past two decades and made his opinionopen to debate. The magnitude of problems related to infectious diseases are increasing due to new and re-emerging communicable diseases like Influenza and Ebola virusescapable of causing pandemic and epidemic warranting an effective intervention to control them. Although threats posed by the dangerous and exotic microbes are of great concern, the "iceberg" phenomenon is seen in common pathogenic microorganisms. Much importance has been given to understanding the mechanisms of major shift and drift of pathogens, but antibiotic resistance, which is considered an equally potential threat to mankind, received less attention and importance.

Mechanisms of antibiotic (or) antimicrobial resistance:

Antibiotic resistance refers to mechanisms adapted or inherited by microbes to evade antibiotics in vivo.

Should these superbugs be considered as potential threats?

Yes!

One such bug is called as Methicillin-resistant *Staphylococcus aureus*, MRSA; a term occupying the scientific journals and newspapers over the recent years.

MRSA is a bacterium that is resistant to penicillin, cephalosporins and many more antibiotics[2]. MRSA causes skin infections, localized abscess, systemic infections, pneumonia and surgical site infections and spreads via direct contact with an infected wound, or by sharing personal items like towels or razors. It also spreads through health personnel who do not observe proper personal hygiene and sanitation. The spread is more likely in crowded places like schools, daycare centers, prisons, and among military personnel.

Some known mechanisms of antibiotic resistance[3]include:

1) Beta lactamase production (Penicillinase)
2) Altered Penicillin binding proteins (PBPs)
3) Active secretion of antibiotic from the microbial cell (Efflux effect)

Each mechanism is discussed as follows.

Beta lactamases:

Penicillins, cephalosporins, carbapenems, monobactams are the group of antibiotics possessing a beta lactam ring in their structure. This beta lactam ring is responsible for the antibiotic activity as they inhibit bacterial cell wall synthesis, which is lethal to bacteria. But the bacteria started producing certain enzymes, which can cleave this beta lactam ring and make them inactive, hence they are called as beta-lactamases. Beta lactamases against corresponding antibiotics gets the name as penicillinase, cephalosporinase and carbapenemases.

These beta lactamases are also classified based upon their capacity as follows

Beta lactamases	Resistant to	Sensitive to
Extended spectrum beta lactamases (ESBL)	Penicillins, Cephalosporins, Monobactams	• Carbapenems, • Cephamycins (cefoxitin, cefotetan, cefometazole) • Beta lactam + lactamase inhibitor combination (Amoxicillin + Clavulanic acid, Piperacillin + Tazobactam)
Inhibitor resistant beta lactamases	Resistant to beta lactam inhibitors (sulbactam, clavulanic acid)	• Piperacillin + Tazobactam • Cephamycins • Carbapenems
Amp C strains	Cephamycins	• Carbapenems
Carbapenemases	Carbapenems	• Non beta lactam especially Vancomycin (aminoglycosides / quinolones) • Tigecycline • Linezolid (But the resistance to non beta lactams is especially high in this group)

Yet another classification of beta lactamases is there based on the gene involved (like New Delhi Beta Metallo lactamase-1[*Ndm-1*],*Shv-18* and so on)

"The organisms which harbor carbapenemases are potential threats and constitutes the major super bugs"

Methicillin is penicillinase resistant beta lactam antibiotic. Initially methicillin is used to treat infections caused by staphylococcus aureus. But later it developed resistance to methicillin. But it doesn't mean MRSA is resistant only to methicillin. **It is an umbrella term; MRSA is resistant to penicillins, cephalosporins and carbapenems**.

Penicillin Binding Proteins (PBPs)

These proteins can also play a role in antibiotic resistance.

PBPs are important constituents necessary for synthesis of bacterial cell wall (peptidoglycan). Beta lactam antibiotic are structurally similar to certain cell wall components, to which PBP binds[4]. Once beta lactam antibiotic binds PBP, beta lactam amide bond ruptures to form a covalent bond with PBP (serine residue) at its active site. This reaction is irreversible and destroys the action of PBP, thereby inhibiting cell wall synthesis.

*mecA*gene encodes a protein called PBP-2A; this protein has low affinity to beta lactam antibiotic. So the antibiotic cannot inhibit cell synthesis. *mecA gene* is a part of staphylococcal cassette chromosome (SCC*mec*)

Efflux effect:

These efflux pumps are examples of active transport mechanism (ATP dependent), known for transporting the antibiotic out of the cell. Apart from this, efflux pumps are known to pump out metabolites and toxins playing a role in virulence.

Antibiotics can regulate the expression of these pumps. The genes are either encoded in plasmid or chromosome so it confers either acquired or innate defense.

The genes responsible for resistance are

- Plasmid encoded (mostly)
- Nuclear DNA (some)
- Episomal

Bacteria can be intrinsically resistant or it can acquire by **horizontal gene transfer** (transformation, transduction, conjugation, transposons)
Horizontal gene transfer is the important mechanism of acquiring and spreading the resistance.

Epidemiology:

It has been reported that the incidence of MRSA, in an endemic country like India is between 25 to 50% based on geographic location[5]. Studies have also shown that one in three people, (33%) carry *Staphylococcus*spp. in their nose, usually without any illness but these could be constant sources of infection to other people. Two in hundred people, (2%) carry MRSA strain[2]. In another study which compiled data from 15 Indian tertiary care centersover a period of two years, the overall prevalence of MRSA was 41%[6]. However, there is no proper statistical data in many tertiary care centers about the prevalence of MRSA as well as protocol for screening MRSA carrier state.

In epidemiological point of view, the one more disturbing statistic with regards to MRSA is, Asia is among the regions with the highest prevalence rates of healthcare-associated methicillin-resistant Staphylococcus aureus (HA-MRSA) and community-associated methicillin-resistant S. aureus (CA-MRSA) in the world[7].

CA-MRSA is another potential threat, because the so-called resistant strains have been isolated from community itself, which were initially constrained to the health care associated areas. Thus increasing the burden of treating those resistant strains.

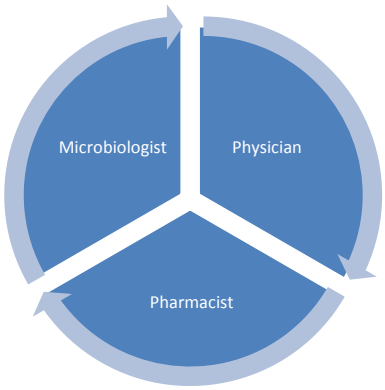
Molecular epidemiology:

The genes responsible for causing these CA-MRSA, HA-MRSA has been studeied in detail by genome wide approach from clinical isolates in this study[7]. Two pandemic HA-MRSA clones, namely multilocus sequence type (ST) 239 and ST5, are disseminated internationally in Asia, whereas the molecular epidemiology of CA-MRSA in Asia is characterized by clonal heterogeneity, similar to that in Europe[7]

Steps in fighting this battle of antibiotic resistance:

Hand washing with suitable disinfectants especially at all health care settings; Screening for MRSA carriers among health care workers on a periodic basis; MRSA screening for pre-operative patients; Readily available resources for MRSA screening in microbiological laboratory; Immediate reporting of MRSA cases for proper action to clinicians; Separate statistical data should be maintained for estimating the prevalence of MRSA; Strict guidelines for using higher end antibiotics for empirical treatments; Appropriate samples testing using uniform standard protocol method, for example, cefoxitin disc diffusion method according to Clinical Laboratory Standard Institute (CLSI) guidelines; Last but not the least, Inter-disciplinary approach (microbiologist, medicine, pharmacist)

Figure 1.1: **Antibiotic Policy Makers**



Finally, an integral part of the intervention in the fight against the spread of MRSA which is often overlooked is the active participation and coordination of stakeholders in health care delivery including physicians, clinical microbiologists and pharmacists (Figure 1.1); this type of collaborative effort should be made mandatory in all health care set-upsin order to ensurean antibiotic policy that appropriately checkmates prevalence and drug resistance patterns and effectively combats evolving MRSA strains.

Conclusion:

This review is to highlight the potential threat of MRSA towards health care. To conclude, inappropriate therapy, including self-medication with over-the-counter antimicrobial agents, is a common response to infectious diseases in our society. The care should be taken on starting higher end antibiotics for empirical infections. As evident from above data, the high antibiotic selective pressure among the overcrowded inhabitants creates an environment that is suitable for the rapid development and efficient spread of numerous multidrug-resistant pathogens.

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