INTRODUCTION

Post-burns microbial infections remain a major problem in burns. Soon after burns, however small, the burnt region can become susceptible to infection; small burns can be easily controlled by antibiotics but when it is second or third degree burns, it can cause major problems and the survival of patients depends on the severity, type and extent of infections. The infection can include fungal, viral and more importantly bacterial. The former two types of microbes may not be as much life threatening as the infection of opportunistic pathogenic bacteria which can thrive due to reduced immunity of the skin against these pathogens. Also orally given antibiotics may not reach at the sites of infection due to damage to the blood vessels especially capillaries. Another major problem is the infection by drug resistant bacterial species, especially methicillin resistant Staphylococcus aureus (MRSA) and multiple drug resistant Pseudomonas aeruginosa [1].

PREVALENCE OF MICROBES IN BURNS

Although a large number of microbes have been identified invading the burnt skin the occurrence and types of infection depends on the presence of pathogen in that environment. Most commons are: Pseudomonas aeruginosa, Staphylococcus aureus, Enterobacter faecalis, Escherichia coli, Candida albicans and Klebsiella, Acinetobacter, Proteus and Shigella spp. Among these P. aeruginosa appears to be most ubiquitous and most notorious being multiple resistant to various antibiotics [2,3].

Treatment of P. aeruginosa infection by piperacillin-tazobactum and tobramycin showed that the second antibiotic was more effective (3.3% resistant microbes) over the first (36.1%) [4]. In another study the Gram positive (G+ve) organisms were the most common causative agents of blood stream infection (BSI) in patients with burns (64%) followed by Gram negative (G-ve) bacteria (22.1%) and fungi (11.5%). Furthermore, it took only 5 days for the G+ve bacterial species to infect BSI in contrast to G-ve (12 days) and fungi (13 days) [5]. A recent study of antibiotic susceptibility revealed that methicillin resistant MRSA incidence was 1.7%, E coli the next resistant to cephalosporin (8%), Klebsiella spp. (5%) and carbapenem resistant P. aeruginosa and Acinetobacter spp. were 26% and 3% respectively [6]. The impact of hospital length of stay also played the roles and found that the distribution of G-ve bacteria

Abstract

Soon after burn accident, as the protective parts of the skin become damaged and exposed to air the opportunistic pathogens start invasion at the burn sites. All three kind of microbes, viruses, bacteria and fungi, target the exposed part of the burn and due to the loss of immunity in those regions pathogens can thrive and create hindrance in the healing process; also if the infection not controlled soon it can spread in body and the survival of patients depends on the severity, the type, extent and persistence of infection. Out of the three types of microbes, it is the bacterial species which play the most important roles in inflicting problems in burns. Many different types of bacterial infections have been identified in different places, areas and parts of the world but Pseudomonas aeruginosa and Acinetobacter baumannii are most ubiquitous and the former, being resistant to a number of antibiotics, creates the biggest problem. Two bacterial mutant strains of the species Staphylococcus aureus, the multi-drug resistant (methicillin resistant MRSA), and vancomycin resistant strain (VRSA) are causing additional problems in controlling the bacterial infections. Attempts to find new antibiotics proving limited success and it appear that a race between antibiotic search and development of resistance bacteria are the ongoing processes.

These multi-drug resistant bacterial species not only create problems with burn patients but significant headache for other diseases all over the world especially in hospitals. Third World countries are especially more vulnerable with this kind of problems. Thus there is an urgent need to put more resources and efforts to discover new antibiotics as soon as possible, also find alternative treatments to eliminate microbial pathogenic infections of all types. In this review two new novel techniques and one existing but unpopular treatment are being proposed and discussed as alternative to antibiotic applications.
and resistant to antibiotic types as was follows: *P. aeruginosa* increased with hospital length of stay (LOS), was 0-7 days: 8% vs >28 days: 55%. On the other hand clinical culture of *H. influenzae* mostly occurred within the first 7 days: 36% vs >28 days: 0.7%.

*Enterobacteriaceae* isolation was highest between 7-14 days of hospitalizations, 7-14 days: 62% vs >28 days 38% concluding that antibiotic resistance was directly proportional to hospital LOS with a net increase of G-ve from 6% (LOS 0-7) to 44% (LOS >28 days) [7].

As the data collected coming from different parts of the world, it can be concluded that although the burn infections can occur by a variety of microbes, *P. aeruginosa* prevails most commonly among them. Secondly the percentage variations from region to region most likely are due to the quality of sanitation, general cleanliness and disinfectants used. Hospital environment also plays role and the longer the patient stays in the hospital the greater is their number.

Indiscriminate use of antibiotic to eradicate bacterial infections is another major problem now emerging globally and MRSA is a good example. Combining the two i.e. the emergence and prevalence of MRSA strain and the multiple resistance *P. aeruginosa* to antibiotics has been creating a huge burden on the health service to save the lives of burnt patients. This problem is more severe in the Third World countries as an example in Gabon, the death rate from burn infection had reached to 54.8% and the majority of death was due to septicemia caused by *P. aeruginosa* infection [8].

Vancomycin, one of the valuable antibiotics to treat MRSA to be used only in emergency, now *S. aureus* resistant to this antibiotic (VRSA) has emerged [9]. Additional worry is the newly reported prevalence of MRSA in milk and dairy products [10].

No doubt, due to these worrying information attempts to discover new effective antibiotics, able specially to kill *P. aeruginosa*, MRSA and VRSA is continuing. Until then it is then that alternative solution(s) for controlling these opportunistic multi-resistant bacterial species must be found. This review describes and discusses a number of alternative solutions to treat burn infections.

**BACTERIOPHAGE THERAPY**

Bacteriophage therapy, commonly known as phage therapy, has been in use from as early as 1921 in Tbilisi, Georgia and in Poland, and in 1930s and 1940s it was widely used globally specially in Eastern European countries and in the former Soviet Union [11]. In a detail Medline citation search from 1966-1996, the Polish and Soviet people administered cocktail of phages orally, topically and systematically to treat a wide variety of pathogens in both adults and children. The treatment included of suppurative wound infections, gastroenteritis, sepsis, osteomyelitis, dermatitis, empyema and pneumonia. The pathogens treated were *Staphylococcus, Streptococcus, Klebsiella, Escherichia coli, Proteus, Pseudomonas, Shigella and Salmonella* spp and the success rate was 80-90% with rare reversible gastrointestinal or allergic side effects [12,13]. From this valuable information on phage therapy it can be predicted that this treatment, alternative to antibiotics, may soon leave the ground for enhanced applications.

My support for phage therapy in the publication [14] was with the following arguments: (i) unlike antibiotic therapy which requires continuous regular and timely applications, phages do not stop working so long their targets, in this case bacterial population sensitive to that phage, has been wiped out; this is because phages invade their host, in crease in number, lyse them, come out, attack other cells and the cycle continue until the host has developed its resistance. In Table 1 [14] is presented 21 bacterial genera for which phages have been isolated and stored; this include the two important pathogens *S. aureus* for which four different phages and *P. aeruginosa* for which fifteen different phages are available (ii) this treatment gives an extra advantage over the possibility that if bacteria became resistant to one type of phage, alternatives are available (iii) no complicated media are required to propagate phages (iv) they are quick to grow purify, store and be used (v) due to their specificity, development of multiple resistant bacteria to phages, unlike for antibiotics, is less likely to be generated (vi) unlike antibiotic which can disrupt the normal bacterial flora, phage being specific no damage to indigenous flora is expected (vii) and more importantly as phages have been in use for almost a century, and no serious adverse effects noted, is suggestive that this treatment can be applied with fair amount of safety (viii) there is no evidence that human cells ever being infected and harmed by phages [14] (Table 2).

Interest in phage therapy has been remaining calm for a number years but a new surge of desire has recently developed and a number of excellent research publications can be found supporting the use of bacteriophages to treat a variety of bacterial infections especially when the infected pathogens cannot be treated by available antibiotic [15-21].

**PRECAUTIONARY MEASURES FOR THE PHAGE TREATMENT**

It is likely that prolonged phage treatment may end up bacterial cells developing resistant to that phage. One mechanism the bacterial cells can develop resistance is by mutating the gene responsible to synthesize the protein(s) for the phage receptor(s). The pathogen developing such mutation most likely stays sensitive to other phages; one good example is *E. coli* developing resistance for phage lambda, they still remain sensitive to say T phages; the nature has provided alternative arsenals for such combat. Some phages can undergo two types of life cycles: lytic and lysogenic. I suggest that if there is choice, lytic phages should be preferable over lysogenic types to be used in post burn infections.

**ACTIONS REQUIRED BEFORE PHAGE THERAPY**

Before the phage treatment is made common it is important to care that the viruses selected are not capable of inserting their genes in the human chromosome, do not carry genes for bacterial virulence, not able to synthesize any toxin to affect human, and do not have homologous genes with antibiotic resistance genes in target bacteria. Also exhaustive test must be carried out to confirm that phages employed do not show any adverse side effects however small either in short or long run on the patients treated. For animal experiments, the blood tests are carried out before employing phage therapy to avoid any likelihood of the phages entering its stream and showing any adverse effect.
Proper purification of phages is also highly recommended.

**EFFECTIVE TREATMENT**

For effective treatment, it is recommended that as soon as infective pathogens identified, a cocktail of phages, specific to the targeted bacterial species be introduced (may be by spray) at the site of burns. Swabs to be taken at regular intervals to measure the reduction in the bacterial population as well purified colonies to be tested for development of any resistance against any phage.

**TREATMENT OF BURN INFECTIONS BY FENTON REAGENT**

When ferrous sulphate (FeSO₄) and hydrogen peroxide (H₂O₂) are mixed it gives rise of hydroxyl radicals (OH) via Fenton reaction. This reaction is based on the Haber and Weiss proposal, Fe²⁺ + H₂O₂ → OH⁻ + H₂O[22]. Can Fenton’s reagent be applied to treat bacterial infections, especially the pathogenic microbes which have either natural resistance for a number of antibiotics such as *P. aeruginosa* or MRSA or VRSA? We carried out a set of experiments to determine (i) percentage kill of a number of bacterial species and rate of killing of *P. aeruginosa* in culture condition by Fenton reagents at varying concentrations of the reagents. The results were encouraging and argued that an alternative method may be available to treat opportunistic microbial infections in burn wounds[23].

Method to determine the effectiveness of Fenton reagent against various species of bacteria, standard microbiological practice, was employed. It was found that various species of bacteria were killed with various rates when samples taken every 5 minutes for up to 25 minutes. At the, concentration of H₂O₂ 0.06% and of FeSO₄ 1.0 mM *E. faecalis* was inactivated by 64%, *S. enteritis*, 70%, *S. aureus*, 77%, *E. coli*, 78%, *P. vulgaris*, 82.6%, *P. retgeri*, 89.8%, *K. pneumoniae*, 95.6%, *A. calcoaceticus*, 98.6% and *P. aeruginosa*, 99.9%. Two striking information is that Fenton reagent is fast acting and secondly *P. aeruginosa* is most sensitive amongst those tested. Subsequently this bacteria was exposed to FeSO₄ 0.2mM, and H₂O₂ 0.06% and the percentage inactivation was over 3 logs in 25 minutes and when the 0.03% H₂O₂ and 0.2 mM FeSO₄ used the percentage cell inactivation reduced to 3 logs after 175 minutes, remained constant for up to 320 minutes and then started rising. The conclusion was that the concentration of Fenton reagent had killed cells of *P. aeruginosa* up to 5 hours and then the radical formation stopped due to decaying of the starting materials and the growth of residual cells started [23] (Figure 1.2).

Unlike antibiotics which are specific to kill (or stop growth) mostly only bacterial species, ROS can indiscriminately kill microbes including viruses, bacteria and fungi; hence gives an additional advantage over antibiotics to eradicate most invading microbial infections. Additional benefits include its fast reactivity leading to cell death, no evidence of any adverse side effects, no known development of resistance against the agent (exceptional is certain anti-oxidants producing mutants), and comparatively cheaper than several conventional treatments. It is also easier to administer due to high solubility in water, effective at relatively low concentrations and less storage problem. Of course, to apply this treatment on human it is essential that a thorough safety investigation under ethical guidance must be carried out. Support of this treatment is present[24-26].

**TREATMENT OF BURNS INFECTION BY ULTRAVIOLET LIGHT A AND ITS SENSITIZERS**

Unlike ultraviolet light A (UVA) which has weak effects, its C band (UVC) inflicts a stronger lethal effect on all kinds of living organism, primarily due to its action on DNA. However, in 1976 it was shown that UVA can become a powerful antimicrobial agent if combined with H₂O₂[27]. The rate of synergistic action was measured on phages T7 and *E. coli* exposed to sub lethal concentrations of these agents, they sustained lethal effects with varying degrees (0.001% and 55 Jm⁻²s⁻² with 99.95% inactivation of T7 in 15 min). Subsequently a study showed that photolysis of H₂O₂ generated O₂[28]. In subsequent studies a number of other biological agents were identified to be sensitized by UVA light; these are β-phenyl pyruvic acid, phenylalanine, tyrosine, tryptophan, histidine and L-mandelate[29-32]. Considering the importance of synergic action of UVA + H₂O₂ (HUVA) it was proposed that this combination may be used in leprosy therapy especially in the Third World countries where UVA prevails with high rates[33].

In this review is presented that this powerful tool (UVA + any of the sensitizers identified) may be used to combat a variety of skin infections including the post-burns. Other benefits of this treatment are that water is the end product of H₂O₂ decay and required at very low concentrations hence it should be fairly safe to be used. Also to be noted that H₂O₂ is already in use for certain cosmetic preparations such as in hair dyes[34]. Also HUVA therapy may be considered parallel to the treatment of psoriasis by PUVA (8-methoxypsoralen + UVA) which has been in use from an unknown period[35]. The only difference is that in HUVA therapy psoralen is being replaced by H₂O₂ also is used at concentrations which are probably much safer than psoralen in PUVA therapy. However, should high concentration of H₂O₂ is required and this may react with the body cells causing lysis through lipid peroxidation, or induction of mutation by ROS, the treatment will have to be balanced between advantages and disadvantages and also animal studies must first be carried out before applying this therapy on human. Finally, if H₂O₂ is not considered suitable, alternative sensitizers (indicated above) are available.

**CONCLUSION**

Burn accident, although prevails globally but it is more common in the Third World countries and the post burn bacterial infections can cause a major crisis especially in those regions where the arsenal of antibiotics cannot reach specially to eradicate MRSA, *P. aeruginosa* and other local pathogens such as *Acinetobacter spps*, or that it becomes difficult for average income people and the poor villagers to purchase the expensive specialized antibiotic for such treatment. Lack of proper safety measures against kitchen fire and the vulnerability of huts they live in can be added risk. Other problem includes that the burnt patients may not be able to reach proper burn unit soon. Furthermore, the clinic they go may not have adequate facilities to treat burn patients and when infection starts, it may not have the required cocktail of antibiotics to treat a battery of infections.
Although a good number and varieties of anti-bacterial antibiotics are available, it appears that there is a race going on between the discovery of new antibiotics and the development of resistance against them. A good example is the vancomycin and the development VRSA [9]. If the MRSA will become resistance to vancomycin, we can imagine the crisis it will create.

Thus requirements to be considered to treat burn infections (or any infection) are: (i) there is need to facilitate more research and hence more funds be available to search for new antibiotics targeting specially to multi-drug resistant bacterial species (ii) more social and press media and TV to be used to publicize about the indiscriminate use of antibiotics (iii) develop alternative treatments to antibiotics use (iv) animal model studies to be carried out to determine every safety measures before applying them on human (v) ethical permission to be taken before research on live animals and testing of the new method on human carried out (vi) make it sure that the cost of alternative treatment is kept low to be affordable by the poor people specially in the Third World countries.

In this review two novel techniques have been presented; although the information on these alternative treatments is limited and requires considerable research both in vitro and in vivo, nevertheless it has additional benefit that the arsenal used can attack indiscriminately to other microbes else than bacterial species. Also they are cheaper than conventional antibiotics and can be transported equally easily; also the agents mostly have much longer shelf life.

The author presents this appeal to the medical scientists and industries working for and searching new antibiotics, to give a careful thinking on the applications of these alternative and plausible treatments and promote them to enter into practice.

REFERENCES


28. Ahmad SI. Synergistic action of near ultraviolet radiation and


33. Ahmad SI. Control of skin infections by a combined action of ultraviolet A (from sun or UVA lamp) and hydrogen peroxide (HUVA therapy), with special emphasis on leprosy. Med Hypotheses. 2001; 57: 404-406.
