The use of topical sodium hypochlorite in the management of *Pseudomonas aeruginosa* burn wound infection

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Declaration

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Date: 5 May 2011

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Proposal for MMed Thesis

Supervisor: Prof H Rode

The use of sodium hypochlorite management of *Pseudomonas aeruginosa* burn wound infection

Background

The skin is an active immune organ that is the major epithelial barrier between the body and a hostile environment.[1-4] Thermal injury causes instant coagulative necrosis which rapidly becomes a suitable site for bacterial colonization and proliferation due to its exclusion from the systemic circulation and impaired local immune responses.[1,2,5] Despite significant advances in burn care, infection is a common cause of death in burn patients and is responsible for 75% of all deaths in patients with burns exceeding 40% total burn surface area (TBSA).[1,6,7]

Pseudomonas aeruginosa is an opportunistic, aerobic Gram-negative bacterium that does not ferment glucose and is ubiquitous in the environment. It rarely causes serious infection in otherwise healthy individuals.[8-11] *Pseudomonas aeruginosa* is intrinsically resistant to several antibiotics because of the low permeability of its outer membrane, the constitutive expression of various efflux pumps, and the production of antibiotic deactivating enzymes.[7,8]

Pseudomonas aeruginosa is one of the commonest and most dangerous organisms in burn patient infections [9,10] It has a remarkable capacity to develop resistance against antimicrobial agents.[8,11]

The prevalence of multi-resistant *P. aeruginosa* is increasing.[9,11-13] The increased use of antibiotics including aminoglycosides and carbapenems, has led to the emergence of these multi-resistant organisms.

The introduction of topical antimicrobial agents dramatically reduced the deaths due to pseudomonas burn wound infection.[14] Resistance of pathogens, especially *Pseudomonas*

aeruginosa, to these topical agents is well described in burn units[15]. A bactericidal agent that is effective, non-toxic to the patient, does not lead to the development of resistance, and which is cost effective, is vital in managing burn wound infection.

As in most burn units across the globe, *Pseudomonas aeruginosa* burn wound infection is a major cause of morbidity and mortality in the burns unit of the Red Cross War Memorial Children's Hospital. However the incidence of *Pseudomonas aeruginosa* infection in our burns unit has not been documented previously. In addition, the morbidity and cost implications have not been analyzed. The local resistance patterns of *Pseudomonas aeruginosa* in our unit, is also unknown.

The bactericidal action of sodium hypochlorite has been known since the 1880's and it has been used in clinical practice for more than 70 years.[3] Its bactericidal action is directly related to its concentration and duration of exposure.[316] Sodium hypochlorite is the salt of hypochlorus acid. The salt in water splits into Na^+ and ClO^-. A substantial portion hydrolyses into sodium hydroxide and hypoclorous acid. These are of the most powerful oxidizing agents known, and is thought to exert its bactericidal effect by oxidizing essential enzymes in the bacteria.[3,17]

The toxic effects of sodium hypochlorite include impairment of wound healing, and are confined to a restricted range of concentrations. Since the bactericidal activity of sodium hypochlorite is also related to its concentration and duration of exposure, it is fundamental to use the optimum concentration that has both adequate bactericidal capabilities as well as low toxicity.[3,15,16]

In their landmark study in 1991, Heggers *et al* looked at the optimum concentration of sodium hypochlorite for adequate bactericidal capabilities with acceptable toxicity. They studied the effects of sodium hypochlorite at five, ten, fifteen and thirty minute intervals. They found that a sodium hypochlorite concentration of 0.025% was bactericidal within thirty minutes. The 0.025% sodium hypochlorite solution was buffered by adding 0.3 N sodium dihydrogen phosphate-disodium monohydrogen phosphate (NaH_2PO_4-Na_2HPO_4). This resulted in a solution with an osmolality of 354mOsm/L and a pH of 7.5. The buffered 0.025% sodium hypochlorite solution had no influence on fibroblast cytoarchitecture or viability.[16]

Currently, 0.025% Sodium Hypochlorite is used at the Red Cross War Memorial Hospital's Burns Unit in the topical management of *Pseudomonas aeruginosa* burn wound infection. The use of this 0.025% sodium hypochlorite is based on work done on a buffered solution of sodium hypochlorite used in the burns unit in Galveston, Texas in the United States of America. This buffered solution is not available at our institution and an un-buffered sodium hypochlorite solution is currently being used. (Appendix 1)

The actual antibacterial properties as well as the stability of this un-buffered solution over time have not been investigated. Furthermore, the effects on fibroblast proliferation and wound healing, of the un-buffered sodium hypochlorite solution are not known. The shelf life of this solution is based on the shelf life of Eusol.

The aim of the study is:

1. To undertake a meta-analysis of *Pseudomonas aeruginosa* burn wound infection.
2. To undertake a retrospective audit of *Pseudomonas aeruginosa* burn wound infection at the Red Cross War Memorial Children's Hospital.
3. To investigate the local effects of sodium hypochlorite solutions in *Pseudomonas aeruginosa* burn wound infection with regards to efficacy and toxicity.

Methods

1) A meta-analysis of the current literature on *Pseudomonas aeruginosa* burn wound infection will be undertaken to determine the incidence of *Pseudomonas aeruginosa* burn wound infection, as well as the susceptibility of *Pseudomonas aeruginosa* to antimicrobial agents in burn units.

2) A retrospective audit of all patients who were diagnosed and treated for Pseudomonas wound infection in the burns unit at the Red Cross War Memorial Children's Hospital in Cape Town between April 2007 and January 2010 will be undertaken. Attention will be paid to %total burn surface area (%TBSA), length of hospital stay before diagnosis, management, bacteriological resistance, and complications of pseudomonas burn wound infection. The time frame for the review has been chosen as sodium hypochlorite has been used in the management of patients with pseudomonas burn wound infection since

April 2007. The review will include data up to hospital discharge. No follow-up is necessary as patients are only discharged from hospital if the infected wounds have healed completely with adequate skin cover.

3) The effects of un-buffered sodium hypochlorite on fibroblast proliferation will be studied as follows:

   a) The toxic effects of sodium hypochlorite on fibroblast proliferation will be studied in fibroblast cultures.
   b) The minimum bactericidal concentration (MBC) of sodium hypochlorite to *Pseudomonas aeruginosa*, *Staphylococcus aureus* and β-haemolytic streptococcus (*Streptococcus pyogenes)* isolates will be studied using a modified broth dilution method.
   c) The biochemical properties and pH of the un-buffered 0.025% Sodium Hypochlorite solution will be analyzed using the standard biochemistry methods.

   The above effects of sodium hypochlorite will be studied using serial dilutions of freshly prepared solution un-buffered sodium hypochlorite solutions as well as solutions stored for 24-, 48-, 72- and 96-hours at room temperature.

Ethics, Cost and Safety

- The proposed study will adhere to the guidelines of the 2008 Helsinki Declaration.
- There will be strict protection of patient confidentiality.
- All laboratory tests will be done by the investigator, under supervision of a qualified laboratory technologist, in a registered laboratory, adhering to all health and safety standard operating procedures.
- Costs of laboratory tests will be funded by the department of surgery research fund.
- The proposed study was approved by the University of Cape Town Department of Surgery Research Committee as well as the University of Cape Town Health Sciences Faculty Research Ethics Committee.

Part 2

Literature Review

Review of the literature

1. Introduction

In South Africa, burn injuries affect 3,2% of people annually and approximately 50% of these patients are less than twenty years of age. [2] Approximately two million fires are reported in the United States of America annually, resulting in 1.2 million people sustaining burn injuries. Burns are amongst the commonest causes of traumatic death in children under four years of age in South Africa and the third most common in children up to eighteen years of age. 75% of burns occur at home, and mostly over weekends. [2]. One hundred thousand patients will require hospital admission for serious burn injuries and approximately 5000 deaths are reported annually in the USA, as result of thermal injury.[18]

The outcomes of patients with burn wound injury have improved over the last forty years, especially in experienced and dedicated burns units. One of the complications of thermal injury is burn wound infection. This is due to impaired systemic and local immune responses, as well as the exclusion of the eschar from the circulation.

Pseudomonas aeruginosa is a Gram negative organism that is regarded as one of the commonest pathogens in burn wound infection. It causes invasive infection and is associated with significant morbidity in burn patients, including delay in wound healing or non-healing of wounds, skin graft and allograft loss, hypertrophic scar formation as well prolonged hospital stay. It has a remarkable ability to acquire resistance to antimicrobial agents.

The use of topical antimicrobial agents in the management of burn wounds has been associated with less burn wound infections and improved outcomes. Unfortunately resistance of *Pseudomonas aeruginosa* to topical antimicrobial agents used in burn units is well described. A topical antimicrobial agent that is non-toxic to the patient, does not lead to the development of resistance and is cost effective is vital in the management of burn wound infection.

Sodium hypochlorite has been used for is bactericidal properties for more than seventy years and no resistance of bacteria to sodium hypochlorite has been reported. The toxic effects of

sodium hypochlorite on wound healing are confined to a restricted range of concentrations. But as the bactericidal activity of sodium hypochlorite is also related to its concentration and time of exposure, it is fundamental to use the optimum concentration that has both adequate bactericidal capabilities as well as minimal toxicity.[3,15,16]

Sodium hypochlorite has been used in the topical management of *Pseudomonas aeruginosa* burn wound infection in several burn units including the unit in Galveston, Texas in the United States of America.

2. The skin and thermal injury

The skin is an active immune organ that is the major epithelial barrier between the body and a hostile environment.[1-4] It is vital for body fluid homeostasis and thermoregulation. It has neurosensory as well as metabolic functions, such as the production of vitamin D. The skin is also one of the largest organs in the body in terms of overall size and weight. The skin of an adult male weighs between 6 and 10 kilograms and the average skin surface area is between 1,5 and 2 square meters.[18]

Thermal injury to the skin causes instant coagulative necrosis which rapidly becomes a suitable site for bacterial colonization and proliferation due to its exclusion from the systemic circulation and impaired local immune responses.[2,4,5,18] Severe burns cause a major physiological insult on the patient which leads to, amongst others, an impaired immune response. This destruction of the skin barrier and concomitant depression of local and systemic host cellular and humeral immune responses predisposes the significantly burned patient to infectious complications.[19] Patients with burns have higher rates of ventilator associated pneumonia and central venous catheter associated bloodstream infections.[6,20]

Full thickness burns are defined as burns where the entire epidermis and dermis are destroyed by thermal injury. Full thickness burn wounds cannot re-epithelialize and will need skin grafting to heal.[21]

Deep partial thickness burns are defined as thermal injury that destroys the skin deep into the dermal layer, only some dermal cells remain. Re-epithelializing is extremely slow and can take months to heal.[21]

In superficial partial thickness burns, the thermal injury damages the epidermis and only a small amount of dermis. The dermis contains blood vessels, nerve endings, sweat glands and hair follicles. This injury is extremely painful and blistering is common.[21]

Superficial burns damage only the outer layer of the epidermis. The skin does not blister, but swelling may occur. These burns are pink or red and painful.[21]

The burn wound surface of full-thickness and deep partial-thickness burn wounds is a protein rich environment consisting of avascular necrotic tissue. This is a favorable culture medium for organisms.[1,2] The avascularity of the eschar results in impaired migration of host immune cells and also restricts delivery of systemically administered antibiotics to the area. Although burn wound surfaces are sterile immediately after injury, all burn wounds eventually become colonized with microorganisms.[14,22] The nature and extent of thermal injury along with the types and amounts of organisms colonizing the burn wound appear to influence the risk of invasive burn wound infection.[14]

Gram positive organisms that survive the thermal insult such as staphylococci located deep within the sweat glands and hair follicles heavily colonize the wound surface within the first forty eight hours, unless topical antimicrobial agents are used. After about five to seven days, the wounds become colonized by other Gram positive and Gram negative organisms as well as yeasts that are derived from the patient's gastrointestinal tract, or from the hospital environment. Gram negative organisms are most commonly the cause of invasive infection.[22]

Table 1: Microorganisms most commonly associated with invasive burn mound infection[18]

Group	Species
Gram-positive organisms	*Staphylococcus aureus*
	Methicillin-resistant *S. aureus*
	Coagulase negative staphylococci
	Enterococcus spp.
	Vancomycin-resistant enterococci
Gram-negative organisms	*Pseudomonas aeruginosa*
	Escherichia coli
	Klebsiella pneumoniae
	Serratia marcescens
	Enterobacter spp.
	Proteus spp.
	Acinetobacter spp.
	Bacteroides spp.
Fungi	*Candida* spp.
	Aspergellus spp.
	Fusarium spp.
Virusses	Herpes simplex virus
	Varicella-zoster virus
	Cytomegalovirus

The survival rates for burn patients have improved significantly over the last few decades due to advances in intensive care, and establishment of specialized burn centers. Advances in fluid management, nutritional support, pulmonary care and ventilation strategies, burn wound care and infection control practices have also contributed to the improved outcomes in severely burned patients.[23] Mortality rates for burns patients admitted to an adult burns unit in Cape Town from 1997 to 2005 was 26% (range 18% per year to 32% per year).[24]

Early surgical excision and grafting of burn wounds has resulted in improved outcomes. [25] Full-thickness and deep partial-thickness burn wounds are excised as soon as possible, once the patient is hemodynamically stabilized. The primary aim of early excision and grafting is the removal of the dead tissue that stimulates an overwhelming systemic inflammatory response syndrome and acts as a culture medium for organisms. Further prevention of infection is obtained by temporary or permanent closure of the burn wounds with allografts or split-thickness skin grafts. It also shortens the period of wound inflammation which in turn reduces the development of hypertrophic scarring.[26]

Despite significant advances in burn care, infection is a common cause of death in burn patients and is responsible for 75% of all deaths in patients with burns exceeding 40% total burn surface area (TBSA).[1,6,7] Wound infection can result in wound progression from partial thickness to a full thickness wound.[1] *Staphylococcus aureus* and *Pseudomonas aeruginosa* have become the most commonly isolated organisms in most burn units.[7,14,21,2227-32]

3. *Pseudomonas aeruginosa* as burn wound pathogen

Pseudomonas aeruginosa is an opportunistic, aerobic Gram-negative bacterium that does not ferment glucose and is ubiquitous in the environment. It rarely causes serious infection in otherwise healthy individuals.[12,33-35] Reservoirs of *Pseudomonas aeruginosa* in health care setting are listed in table 2.[36] Most strains involved in infections are both invasive and toxigenic, as a result of surface virulence factors (allowing bacterial attachment, colonization and invasion) and secreted virulence factors (which damage tissues or trigger the production of cytokines).[11,34] This organism causes serious infection in critically ill patients like patients with burns. *Pseudomonas aeruginosa* is one of the commonest and most dangerous organisms in burn patient infections.[9,10] It has a remarkable capacity to develop resistance to antimicrobial agents. [8,11] Only 15%-25% of pseudomonas colonizing burn wounds arises from the patient's lower gastrointestinal tract, therefore, 75-85% of the organisms colonizing burn wounds are nosocomial organisms.[4]

Table 2: Environmental reservoirs of Pseudomonas aeruginosa in hospitals[36]

Taps sinks sluices
Showers
Disinfectants, sanitizers, antiseptics, bar soaps
Mops, buckets
Baths
Shaving, toothbrushes
Hydrotherapy pools
Flower vases
Ice makers
Medication e.g. eye drops, multi-dose vials, mouthwash
Urometers
Endoscopes, endoscope washers
Infant feeding bottles
Toys

The virulence of organisms colonizing burn wounds is another important factor in the development of burn wound infection. *Pseudomonas aeruginosa* produces a number of cell-

associated and extracellular virulence factors that mediate processes like adhesion, nutrient acquisition, immune system evasion, leukocyte killing, tissue destruction and blood stream invasion.[37]

Pseudomonas aeruginosa is intrinsically resistant to several antibiotics because of the low permeability of its outer membrane, the constitutive expression of various efflux pumps, and the production of antibiotic deactivating enzymes.[11,33] It also has a remarkable ability to acquire new mechanisms of resistance to antibiotics.[11,38] Mechanisms of resistance to antibiotics include amongst others:[11]

- β-Lactam antibiotics:
    - the production of Extended Spectrum Beta-Lactamases (ESBL)
    - Enzymatic inactivation
- Carbapenems:
    - Reduced intracellular carbapenem concentration due to loss of expression of outer membrane porins and the induction of an efflux pump
    - Carbapenem hydrolysis by carbapenemases
- Fluoroquinolones,
    - Efflux pumps
- Aminoglycosides
    - Enzymatic inactivation

Biofilms are complex communities of surface-attached aggregates of microorganisms embedded in a self-secreted extracellular polysaccharide matrix, or slime. They provide the organisms with a protected dynamic environment.[39,40] The ability of *Pseudomonas aeruginosa* to form biofilm is important for the bacterium to persist in environmental niches such as pipes and taps.[36] Biofilms act as efficient barriers against antimicrobial agents and the host immune system, resulting in persistent colonization and infection.[39,40] Recent work has confirmed the importance of biofilms in the pathogenesis of burn wound infection.[18]

A multi-resistant *Pseudomonas aeruginosa* is defined as an organism resistant to three classes of anti-pseudomonal antimicrobials. The prevalence of multi-resistant *Pseudomonas aeruginosa* isolates is increasing.[9,11-13] In a study investigating the incidence of multi-resistant *Pseudomonas aeruginosa* infections in a hospital in Rome, the incidence increased

from 9,7 per 1000 hospital admissions to 24,7 per 1000 hospital admissions over a ten year period.[41] The increased use of antibiotics including aminoglycosides and carbapenems has led to the emergence of these resistant organisms. Exposure to a single antibiotic may select for mutants with cross-resistance to many unrelated antibiotics.[9,11] Quinolones appear to be particularly prone to select for cross resistance to B-lactams and aminoglycosides.[11,139,11-13] There is a strong correlation between antibiotic consumption and resistant rates for *Pseudomonas aeruginosa*. The emergence of resistance during therapy occurs frequently.[25,29,30,35]

4. Topical antimicrobials in burn wound management

Before the development of effective topical antimicrobial therapy for burn wounds, Pseudomonas burn wound sepsis was considered to be the cause of a significant number of deaths in burned patients.[4,14,17] The introduction of topical antimicrobial agents dramatically reduced the deaths due to pseudomonas burn wound infection.[1,2,14,14,42] Topical antimicrobial treatment limits bacterial proliferation and penetration into the eschar. This leads to decreased need for systemic antibiotic usage and therefore to lower resistant rates in the burns ward.[1,2,11,40] None of the current available topical agents sterilize the burn wound, and sepsis can therefore develop in the wounds of any burned patient, despite topical treatment.[1] The likelihood of such infection increases with increasing burn size.[1]

Topical agents currently used in the treatment of burn wounds include silver, iodine, chlorhexidene and mupurocin.[1,2] Resistance of pathogens, especially *Pseudomonas aeruginosa*, to these topical agents, is well described in burn units.[15] A bactericidal agent that is effective, non-toxic to the patient, does not lead to the development of resistance and cost effective is vital in managing burn wound infection.

5. The clinical use of sodium hypochlorite

The bactericidal action of sodium hypochlorite has been known since the 1880's and it has been used in clinical practice for more than seventy years.[3] Its bactericidal action is directly related to its concentration and duration of exposure.[3,16] Hypochlorous acid, the active substance in sodium hypochlorite, is one of the most powerful oxidizing agents known, and it thought to exert its bactericidal effect by oxidizing essential enzymes in the bacteria.[3,17]

There have been no reports of the development of resistance to sodium hypochlorite by bacteria or yeasts, which make it a valuable topical agent to use in burn wound infection.[3,16]

The toxic effects of sodium hypochlorite on wound healing are confined to a restricted range of concentrations. But as the bactericidal activity of sodium hypochlorite is also related to its concentration and time of exposure, it is fundamental to use the optimum concentration that has both adequate bactericidal capabilities as well as minimal toxicity.[3,15,16]

In their landmark study in 1991, Heggers *et al* looked at the optimum concentration of sodium hypochlorite for adequate bactericidal capabilities with acceptable toxicity at five, ten, fifteen and thirty minute intervals. They found that a sodium hypochlorite concentration of 0.025% was bactericidal within thirty minutes. The buffered 0.025% sodium hypochlorite solution had no influence on fibroblast cytoarchitecture and viability[16]. They concluded that a 0.025% sodium hypochlorite solution is safe and effective the topical management of infected burn wounds.

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Part 3

Aims of this study

Aims of this study

1. Introduction

As in most burn units across the globe, *Pseudomonas aeruginosa* burn wound infection is a major cause of morbidity in the burns unit of the Red Cross War Memorial Children's Hospital. However, the incidence of *Pseudomonas aeruginosa* infection in our burns unit is not known. The local resistance patterns of *Pseudomonas aeruginosa* in our unit have not recently been studied and the morbidity and cost implications have not been analyzed.

0.025% Sodium hypochlorite is currently used at the Red Cross War Memorial Hospital's burns unit in the topical management of *Pseudomonas aeruginosa* burn wound infection. The use of this 0.025% sodium hypochlorite is based on work done on a buffered solution of sodium hypochlorite used in the burns unit in Galveston, Texas in the United States of America. This buffered solution is not available at our institution and an un-buffered sodium hypochlorite solution is currently being used.

The actual antibacterial properties as well as stability of this un-buffered solution over time have not been investigated. The effects on fibroblast proliferation of the un-buffered sodium hypochlorite solution are not known. The shelf life of this solution is based on the shelf life of Eusol.

2. Aims

This study aimed to investigate the incidence of *Pseudomonas aeruginosa* burn wound infection as well as resistance patterns in burn units around the globe. The incidence, complications and cost implications of *Pseudomonas aeruginosa* burn wound infection at the burns unit of the Red Cross War Memorial Children's Hospital was investigated and compared to the current literature.

The un-buffered sodium hypochlorite solution currently used at the Red Cross War Memorial Children's Hospital was analyzed to establish the optimum solution for the topical use of *Pseudomonas aeruginosa* burn wound infection that would be both bactericidal as well as

non-toxic to wound healing. A shelf-life for this optimum un-buffered sodium hypochlorite solution was also established.

3. Methods

This study was conducted in three parts:

1. A meta-analysis of the current literature on *Pseudomonas aeruginosa* burn wound infection.
2. A retrospective audit of all patients with clinically significant *Pseudomonas aeruginosa* burn wound infection managed at the Red Cross War Memorial Children's Hospital burns unit from April 2007 to January 2010.
3. *In vitro* studies of dilutions of un-buffered sodium hypochlorite solution:
   - *3.1.* To determine the effects of dilutions of sodium hypochlorite on fibroblast proliferation, used as surrogate marker for the effects on wound healing.
   - *3.2.* To determine the minimum bactericidal concentration (MBC) of the un-buffered sodium hypochlorite solution.
   - *3.3.* To determine the biochemical properties of the un-buffered sodium hypochlorite solution.

4. Conclusion

The results of this study will influence the current topical management of *Pseudomonas aeruginosa* burn wound infection at the Red Cross War Memorial children's Hospital as the optimum concentration of an un-buffered sodium hypochlorite solution will be determined. Knowledge regarding local resistance patterns is invaluable in the day-to-day management of patients in the unit. The morbidity and cost implications will aid to allocate appropriate resources to improve outcomes in these patients.

Part 4

Research Study

Chapter 1

Meta-analysis of *Pseudomonas aeruginosa* burn wound infection

1. Introduction

Burns are of the commonest and most devastating forms of trauma leading to physical, as well as psychological disability.[1] Thermal injury causes coagulative necrosis of the skin which rapidly becomes a culture medium for organisms.[1,2,6,16] These organisms can cause invasive burn wound infection, that lead to complications like non-healing of wounds, skin graft and allograft loss, hypertrophic scarring.[1] Together with impaired local and systemic immune responses, invasive burn wound infection can lead to septiceamia, multi-organ failure and death. [18] Gram-negative organisms are most often associated with invasive burn wound infection and its complications.[14]

Since topical antimicrobial agents were introduced in the management of burn wounds, deaths due to pseudomonas burn wound infection has declined significantly.[14] Topical antimicrobial treatment limits bacterial proliferation and penetration into the eschar. This leads to decreased need for systemic antibiotic usage and therefore to lower resistance rates in the burns ward.[1,2,14,42] None of the current available topical agents sterilize the burn wound, and sepsis can therefore develop in the wounds of any burned patient, despite topical treatment.

Pseudomonas aeruginosa is an aerobic Gram-negative bacterium that is commonly isolated in patients with invasive burn wound infections. Together with *Staphylococcus aureus*, it has become the most commonly isolated organisms in most burn units.[12,31,33,34] It has a remarkable capacity to develop resistance against systemic as well as topical antimicrobials.[10,36]

A meta-analysis of the current literature on *Pseudomonas aeruginosa* burn wound infection was undertaken to investigate the incidence of *Pseudomonas aeruginosa* burn wound infection, as well as the susceptibility of *Pseudomonas aeruginosa* to antimicrobial agents in burn units.

2. Methods

A PUBMED search was done using the keywords "pseudomonas infection burns". Of the 353 articles published in the English language literature from 1 January 1995 to 31 October 2010 found, only twenty three articles stating the incidence and resistance patterns of *Pseudomonas aeruginosa* infections in burns units were identified Papers looking at *Pseudomonas aeruginosa* burn wound colonization, infection and burn wound sepsis were included in the review..

Articles that only looked at the incidence of blood stream infections or septicaemia, and those involving only ICU patients were excluded[43-46]. Papers just looking at the incidence of resistant strains against various anti-pseudomonal agents, not looking at the overall incidence of pseudomonas infections in the burns units, were also excluded from this review.

Definitions of burn wound colonization, infection and sepsis:

The presence of bacteria on the burn wound surface is not always associated with invasive burn wound infection. If there are organisms cultured from the burn wound surface, without clinical or histological evidence of wound infection, it is known as colonization.

Several sampling techniques for microbiological investigations are available for burn wounds ranging from surface swabs to full thickness eschar biopsies. Surface cultures are useful to diagnose bacteria colonizing the wound, but not even quantitative cultures can indicate the presence of invasive burn wound infection. A low quantitative count can be a good indication that the wound is colonized rather than infected, but a high quantitative culture correlates poorly with invasive infection.[17]

Burn wound biopsies are the most reliable means of diagnosing invasive burn wound infection. Using a scalpel, the eschar as well as underlying viable tissue is biopsied and half sent for culture and the other half sent for histologic examination.[17]

Different authors in this review have defined burn wound infection using several clinical and bacteriological criteria. Mousa *et al*[21] defined burn wound infection using the following clinical criteria: suppuration, discoloration, violaceous and oedematous wound margin.

Rogers *et al*[28] used clinical, histological as well as the following bacteriological criteria: $>10^5$ colony forming units (cfu's) per cm^2 or $> 10^5$ cfu's on a tissue culture. Oncul *et al*[31] used the following criteria to define burn wound infection: local pain or tenderness, oedema, erythaema, change in eschar as well as histological evidence of infection.

Burn wound sepsis was defined by these authors as burn wound infection with evidence of systemic sepsis like leukocytosis, fever, positive blood cultures etc.

Definition of incidence of infection or colonization:

In the literature reviewed, there were two methods of reporting the incidence of *Pseudomonas aeruginosa* infections or wound colonization in burns units. Some authors took the number of microbiology samples sent for microscopy and culture from the burns unit over a period of time, and identified the number of positive cultures from those samples. They then took the number of samples positive for *Pseudomonas aeruginosa* and calculate the incidence of *Pseudomonas aeruginosa* infections as a percentage thereof.

Example:
Incidence of *Pseudomonas aeruginosa* infection (%) = Number of samples positive for *Pseudomonas aeruginosa* / Total number of positive samples X 100

Other authors expressed the incidence of Pseudomonas *aeruginosa* in their unit as the number of patients admitted in the unit over a study period who developed *Pseudomonas aeruginosa* infections.

Example:
Incidence of *Pseudomonas aeruginosa* infections (%) = Number of patients who developed *Pseudomonas aeruginosa* infections / Total number of patients admitted to the burns unit X 100

3. Results

A total of thirteen articles met the criteria to be included in this review. Only two articles exclusively looked at a paediatric burn population.[28,47] Eleven of the thirteen studies included in the review expressed the incidence of *Pseudomonas aeruginosa* as a percentage of positive

samples and two studies expressed it as patients developing *Pseudomonas aeruginosa* infections. Four studies looked at burn wound infection with or without burn wound sepsis[21,2830,31] whereas nine studies looked at the presence of organisms on wound samples, therefore including colonization, infection and burn wound sepsis[7,14,22,27,29,32,47,48,49].

Table 3: Articles included in literature review

Author	Year	No of Patients	% TBSA	LOS **	P/S ***	Infection Colonization	Incidence
Mousa, HAL *et al*[21]	1997	127	45%	NS****	S	Infection	19%
Lari, A *et al*[48]	1998	2122	NS	NS	S	Colonization	74%
Revathi, G *et al*[27]	1998	600	NS	NS	S	Colonization	36%
*Rodgers, GL *et al*[28]	2000	70	15%	NS	P	Infection	5.7%
Lari, A *et al*[29]	2000	582	NS	NS	S	Colonization	73%
Song, W *et al*[49]	2001	2190	30%	NS	S	Colonization	45.7%
Oncul, O *et al*[30]	2002	63	43%	39days	P	Infection	9%
Nasser, S *et al*[22]	2003	70	30%	NS	S	Colonization	21,6%
Singh, NP *et al*[32]	2003	759	NS	NS	S	Colonization	31%
*Geyik, MF *et al*[47]	2003	610	>20%	12days	S	Colonization	65%
Erol, S *et al*[14]	2004	51	22.9%	NS	S	Colonization	16.2%
De Macedo, JLS *et al*[7]	2005	203	15%	NS	S	Colonization	11.4%
Oncul, O *et al*[31]	2009	168	30%	26	S	Infection	57%

*Studies in dedicated paediatric burns units
** LOS = Length of Stay in Hospital
*** P = Incidence of *Pseudomonas aeruginosa* expressed as number of patients developing infection
***S= incidence of *Pseudomonas aeruginosa* expressed as number of positive samples
****NS = Not Stated

The mean incidence of positive samples for *Pseudomonas aeruginosa* in the reviewed series reporting on the number of positive swabs was 43% (range 11.4-74%). The incidences of the two studies reporting on the number of patients who develop *Pseudomonas aeruginosa* infections was 5.7 and 9%.[28,31] The mean incidence of *Pseudomonas aeruginosa* wound infection in the four studies reporting wound infection was 22.7% (range 5.7-57%) The mean incidence of positive samples in the studies looking at wound contamination was 41.1% (range 11.4-74%) It is interesting to note that two studies from the same burns unit done two years apart reported remarkably similar incidences of *Pseudomonas aeruginosa* in their unit (73% and 74%).[29,48]

Four studies from the thirteen reviewed studied the change in bacterial colonization of burn wounds during hospital admission. All four of these studies took samples of burn wounds on the day of hospital admission, as well as at various intervals. Erol *et al*[14] investigated wound colonization at day one, seven, fourteen and twenty one. Nasser *et al*[22] reported on the difference in organisms colonizing burn wounds during the first five days from admission, as well as colonization from day six onwards. Lari *et al*[48] investigated the frequency of positive cultures on the first, third and seventh day of admission. Oncul *et al*[31] reported on the frequency of *Pseudomonas* aeruginosa on burn wound cultures on day one and day seven of admission. All of these studies showed an increase incidence of *Pseudomonas aeruginosa* infections the longer patients stayed in hospital.[14,22,29,31]

Table 4: Incidence of *Pseudomonas aeruginosa* at different time intervals

	Day 1	**Day 7**	**Day 14**
Erol *et al*[14]	2%	13.8%	16.9%
Nasser *et al*[22]	10.7%	27.2% (Day 6)	
Lari *et al*[48]	35.5%	73.5%	
Oncul *et al*[31]	9%	79%	

Only two studies investigated an exclusive paediatric burn population. One of the two studies from dedicated paediatric burns units reported the incidence of *Pseudomonas aeruginosa* wound colonization as the % positive samples (65%) and one reported the incidence as % of patients developing *Pseudomonas aeruginosa* infections (5.7%)

Table 5: Audits from dedicated paediatric burns units

	%TBSA	**Length of Stay**	**Incidence**	**Infection / Colonization**
Rodgers, GL *et al*[28]	15%		5.7%*	Infection
Geyik, MF *et al*[47]	>20%	12 days	65%*	Colonization

*Incidence reported as patients developing *Pseudomonas aeruginosa* infection

**Incidence reported as percentage swabs

Five of the studies in the reviewed literature reported on the susceptibility of *Pseudomonas aeruginosa* to antimicrobials.[7,27,32,49] In the study done by De Marcedo *et al*[7], *Pseudomonas aeruginosa* isolates were sensitive to amoxicillin-clavuanic acid (13.3%), aztreonam (24%),

cefoxithin (6.7%), ceftriaxone (51.1%), piperacillin-tazobactam (53%), imipenem (60%), gentamycin (48.9%), amikacin (86.7%), co-trimoxazole (8.9%) and cefipime (48.9%). Singh *et al*[32] tested susceptibilities of isolates to amikacin (48%), gentamycin (41%), ciprofloxacin (11%), piperacillin (31%) and cefotaxime (44%). The susceptibilities of *Pseudomonas aeruginosa* isolates reported by Songh *et al*[49] were as follows: Piperacillin (36%), ceftazadine (46%), aztreonam (63%), gentamycin (20%), amikacin (69%) and ciprofloxacin (59%). Revathi *et al*[27] found that the isolates in their unit were susceptible to amikacin (72.7%), gentamycin (52.9%), norfloxacin (9.7%), cefotaxime (60.8%), ciprofloxacin (17.1%), tobramycin (71.8%), piperacillin-tazobactam (59.9%) and ceftazadine (82.8%).

Table 5: Reported susceptibility of *Pseudomonas aeruginosa* to antimicrobials

Reference:	De Marcedo *et al*[7]	Song *et al*[49]	Singh *et al*[32]	Revathi *et al*[27]	Oncul *et al*[30]
Ciprofloxacin	53.3%	59%	11%	17.1%	54%
Ceftazidime	51.1%	46%	48%	82.8%	
Cefepime	48.9%				
Tobramycin				71.8%	
Gentamycin	48.9%	20%	31%	52.9%	
Amikacin	86.7%	69%	48%	72.7%	72%
Piperacillin-Tazobactam	53.3%	36%	31%	59.9%	65%
Imipenem	60%	48%			

4. Discussion

The incidence of *Pseudomonas aeruginosa* infection and colonization varied considerably between the different articles, with de Macedo *et al*[7] reporting an incidence of wound colonization as low as 11.4% and Lari *et al*[48] reporting an incidence of 74%. However the susceptibilities to antimicrobials are more constant amongst the different units. The high incidence of resistance against anti-pseudomonal agents is worrying.

None of the studies reporting on susceptibility of *Pseudomonas aeruginosa* to antimicrobials reported on the susceptibility to topical antimicrobial agents. The testing of susceptibility to topical agents is however poorly standardized and the literature on that is very scant.[18] No study from a dedicated paediatric burns unit reported on the susceptibility of *Pseudomonas aeruginosa* to antimicrobials.

The % total burn surface area (TBSA) and the length of hospital stay have been shown to be important risk factors for developing nosocomial infections.[23,30,50] The incidence of different pathogens isolated from burn wounds also changes over time, with Gram positive organisms isolated more frequently early during admission and Gram negative organisms, in particularly *Pseudomonas aeruginosa,* more frequently isolated later during hospital admission. It is therefore critical that any study looking at the incidence of *Pseudomonas aeruginosa* infections in burns units also state the mean %TBSA and length of hospital stay, especially when reporting on the incidence with regards to the number of patients developing *Pseudomonas aeruginosa* infections. The length of hospital stay was not indicated in the study by Rodgers *et al.*[28]

There were no studies identified that specifically analyzed the incidence, resistance patterns, management and complications of *Pseudomonas aeruginosa* infections in an exclusively paediatric burns unit.

5. Conclusion

Pseudomonas aeruginosa is a common organism colonizing burn wounds in burns units around the globe. Its prevalence increases the longer patients stay in hospital. It has a remarkable capacity to develop resistance against antimicrobials and the incidence of resistance against anti-pseudomonal agents are common in burns units. Every effort must be made to prevent patient-to-patient transmission of *Pseudomonas aeruginosa* in burns units by diligent infection control practices. Judicious use of antibiotics according to susceptibility results should be employed to try and prevent the development of resistant strains in burns units.

Chapter 2

Retrospective audit of *Pseudomonas aeruginosa* burn wound infection at the Red Cross War Memorial Children's Hospital

1. Introduction

The Red Cross War Memorial Children's Hospital is a dedicated paediatric hospital situated in Cape Town, South Africa. It is the referral hospital for a large drainage area. The hospital has a dedicated burns unit that is the only paediatric burns unit, and referral center, for all of the Western Cape Province. Approximately 1100 patients are admitted to this unit annually.

As in most burn units across the globe, *Pseudomonas aeruginosa* burn wound infection is a major cause of morbidity in the burns unit of the Red Cross War Memorial Children's Hospital. However, the incidence of *Pseudomonas aeruginosa* infection in our burns unit is not known. The local resistance patterns of *Pseudomonas aeruginosa* in our unit have not recently been studied and the morbidity and cost implications have not been analyzed.

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The aim was to describe the incidence, current management and complications of pseudomonas burn wound infection at the Red Cross War Memorial Children's hospital. Resistance patterns to systemic and topical antimicrobial agents were also investigated.

2. Methods

All patients who were diagnosed and treated for Pseudomonas wound infection in the burns unit at the Red Cross War Memorial Children's Hospital in Cape Town between April 2007 and January 2010 were included in this study. A retrospective review of all the patient folders was undertaken and the following data extracted:

- Patient demographics.
- %Total Burn Surface Area (%TBSA).
- Length of hospital stay before diagnosis of *Pseudomonas aeruginosa* burn wound infection.

- Management data:
    - The site from where the positive culture was obtained i.e. wound swab, blood culture, CVP tip, urine, sputum, etc.
    - Systemic antibiotic usage.
    - Number of surgical debridements needed after the diagnosis of *Pseudomonas aeruginosa* infection until the wound was suitable for skin grafting.
    - Wound dressings and topical antimicrobial usage.
    - Time from positive culture until successful treatment of wound infection clinically, confirmed by negative cultures.
- Bacteriological data:
    - The incidence of *Pseudomonas aeruginosa* from samples taken from patients in the burns unit.
    - Resistance patterns of organisms on first positive cultures to topical and systemic antibiotics.
    - Development of resistance in hospital to topical or systemic antimicrobial agents during treatment.
- Complications of pseudomonas burn wound infection. The following were reviewed :
    - Biobrane or Allograft loss.
    - Skin Graft loss.
    - Additional theatre visits.
    - Delay in definitive skin grafting (in weeks).
    - Prolonged hospital stay (in weeks).

All patients were managed according to the standard management protocols of the unit. Admission criteria to this unit were all patients with greater than 10% TBSA partial thickness burns, all patients with full-thickness burns, circumferential limb involvement, facial burns, perineal burns and burns to hands or feet. Standard of care in our unit during the study period was to dress wounds infected with *Pseudomonas aeruginosa* (even if clinically suspected and not yet proven with positive swab) with daily sodium hypochlorite soaks and flamazine dressings. Flamazine was substituted with chlorhexidine, if an organism resistant to flamazine was isolated.

3. Results

3.1. Patient demographics

During the thirty six month study period, a total of 2632 patients were admitted in the burns unit at the Red Cross War Memorial Children's Hospital. Thirty four patients were recorded to have clinically significant pseudomonas burn wound infection in the thirty six month period. Therefore the incidence *Pseudomonas aeruginosa* burn infection per patient in our unit was 1.29%. Three patient records were unobtainable for review as the hospital records were lost. This represents less than 10% of the total number of patients. It is not possible to determine if this influenced the results/ in any way. Thirty one patients were therefore analyzed.

Clinically significant *Pseudomonas aeruginosa* wound infection was defined as a combination of clinical evidence of wound infection (excessive slough and erythaema, characteristic green colour change or characteristic smell) together with a positive isolate from a wound swab. Quantitative cultures are not routinely done at our laboratory.

Of the thirty one patients folders analyzed, 20 were male and 11 were female with a male:female ratio of approximately 2:1.The mean age of the patients who developed Pseudomonas infection was three years (range: 4 months to 10 years). The mean % total burn surface area (%TSBA) involved was 27% (range: 8-65%). The mean time in days between admission and diagnosis of pseudomonas infection was ten days (range: 1-34days). The mean time between diagnosis and negative cultures was thirteen days (range: 2-32days). The mean time between a positive culture and initiation of appropriate management was two days (0-9 days). Seven patients had positive blood cultures and one had positive urine cultures. All the patients who had positive blood or cultures also had positive burn wound swabs on the day of blood culture. The patient with the positive urine culture had positive wound swabs for *Pseudomonas* aeruginosa about one week prior to the positive urine culture and another positive wound swab more than a week after the positive urine culture, but not at the time of positive urine culture. The diagnosis of Pseudomonas wound infection was confirmed on eight tissue biopsies. Thirteen patients were treated with systemic antibiotics and eighteen without. All patients who had positive blood or urine cultures were treated with systemic antibiotics.

3.4. Bacteriology

During the study period from 1 April 2007 until 31 January 2010, a total of 2791 bacteriology samples were received from the burns unit at the Red Cross war Memorial Children's Hospital. Of these, 1885 samples had "No Growth" or "Mixed Growth". Samples labeled as "Mixed Growth" grew only skin commensals and no pathogens and were therefore regarded as not significant. Therefore a total of 906 samples had an organism isolated. Of these 906 samples, 406 samples were positive for *Pseudomonas aeruginosa* with an incidence of 50.3%.

Pseudomonas aeruginosa resistant to flamazine was isolated from six patients. In three of these six patients, the first positive swab for *Pseudomonas aeruginosa* was sensitive to flamazine, and only later on was an organism resistant to flamazine isolated. Therefore in three patients, the first positive swab was resistant to flamazine and only sensitive to chlorhexidine. All the patients that developed resistance to flamazine were treated with flamazine for a period after diagnosing the initial pseudomonal infection.

A multi-resistant *Pseudomonas aeruginosa*, defined as an organism resistant to three classes of anti-pseudomonal antimicrobials, was isolated in only one patient. It was isolated from a wound swab. This isolate was resistant to ciprofloxacin, ceftazidime, cefipime, gentamycin, pipperacillin- tazobactam and the carbapenems. It was also resistant to flamazine and betadine. This patient came from the general intensive care unit to the ward, had a %TBSA of 36% and did not receive any systemic antibiotics during his management for Pseudomonas wound infection in the burns ward.

In most patients (22), the first isolate was sensitive to all systemic anti-pseudomonal antibiotics and resistant to betadine. Of these twenty two patients, organisms with resistance to one or more systemic antibiotics were later isolated from seven patients. Of these seven patients, three were treated with systemic antibiotics. In two patients who were treated with pipiracillin-tazobactam an isolate with intermediate resistance to pipiracillin-tazobactam was cultured later. The other patient was treated with systemic ceftazadine, but an isolate with intermediate resistance to pipiracillin-tazobactam was cultured from a wound swab from that patient. Four of the seven patients were not exposed to systemic antibiotics.

Isolates with resistance to systemic antimicrobials were cultured from nine patients as the first positive wound swab. Four of these nine patients received systemic antibiotics according to the sensitivities of the cultured isolates.

In summary:

- The incidence of *Pseudomonas aeruginosa* of samples taken from patients in the burns unit during the study period is 50.3%.
- All but four of the isolates of *Pseudomonas aeruginosa* cultured from these patients were resistant to betadine; whereas all of the isolates were sensitive to chlorhexidine.
- *Pseudomonas aeruginosa* resistant to flamazine was cultured from six patients.
- One multi-resistant *Pseudomonas aeruginosa* was isolated.
- Most patients' (22 of the 31) first isolate was sensitive to all systemic antibiotics.

The resistance patterns of *Pseudomonas aeruginosa* from our unit to antimicrobials were investigated by analyzing records of isolates from the thirty one patients with clinical significant *Pseudomonas aeruginosa* burn wound infection. Sixty one isolates of *Pseudomonas aeruginosa* were tested against systemic antimicrobials and fifty three isolates were tested against topical antimicrobials. Of the sixty one isolates tested against systemic antimicrobials, forty nine (80.3%), were sensitive to ciprofloxacin and ceftazidine, fifty (82%) were sensitive to cefepime, fifty nine (96.7%) were sensitive to tobramycin, fifty six (91.8%) were sensitive to gentamycin and amikacin, thirty nine (63.9%) were sensitive to piperacillin-tazobactam, fifty five (90.2%) were sensitive to imipenem and fifty seven (93.4%) were sensitive to meropenem.

Table 4: Susceptibility results of isolates

	Sensitive Isolates	Percentage
Systemic	**N=61**	
Ciprofloxacin	49	80.3%
Ceftazadine	49	80.3%
Cefepime	50	82.0%
Tobramycin	59	96.7%
Gentamycin	56	91.8%
Amikacin	56	91.8%
Piperacillin-Tazobactam	39	63.9%
Imipenem	55	90.2%
Meropenem	57	93.4%
Topical	**N=53**	
Flamazine	46	86.7%
Betadine	4	7.5%
Chlorhexidine	53	100%

3.4. Patient management

In the thirty one patients with pseudomonas wound infection, a total of 371 dressing days were needed, until negative wound swabs were obtained with an average of twelve days per child (range: 2-30 days).

Twenty eight of the thirty one patients in our series were treated with daily sodium hypochlorite soaks and flamazine dressings for a total of 278 days with an average of fifteen days per patient (range: 2-30 days).

Six children were treated with daily sodium hypochlorite soaks and chlorhexidine dressings for a total of fifty three days, with an average of nine days per child (range: 5-16 days). Five children were initially treated with daily sodium hypochlorite and flamazine dressings for an average of nine days (range: 5-16 days) before the dressings were changed to daily sodium hypochlorite and chlorhexidine dressings, because an organism resistance to flamazine was isolated. Only one patient was treated exclusively with daily sodium hypochlorite and chlorhexidine dressings from the day the diagnosis of *Pseudomonas aeruginosa* wound infection was made for a total of six days.

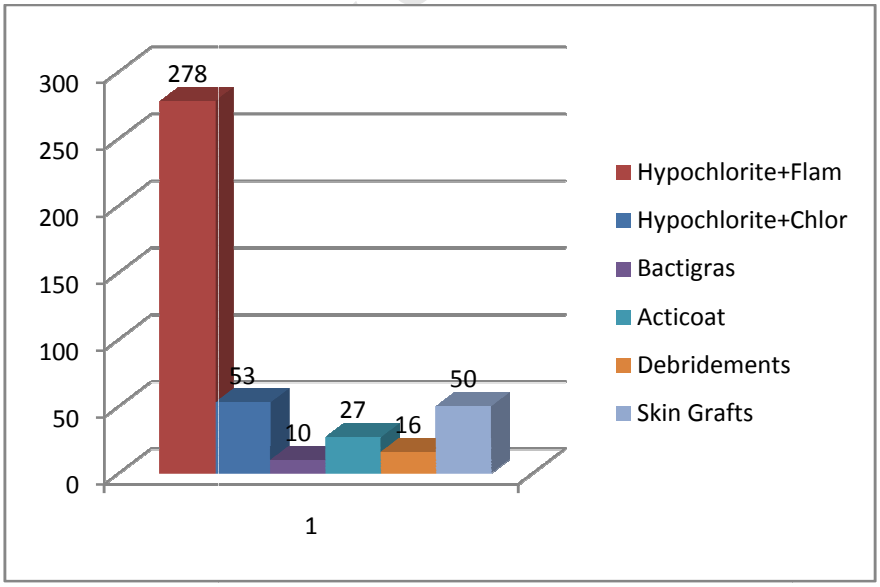
Three of the patients were treated with chlorhexidine (Bactigras) dressings after the diagnosis of flamazine resistant *Pseudomonas aeruginosa* was made for a total of ten days with an average of three days per patient (range: 3-4 days). All three of these patients were also managed with daily sodium hypochlorite and flamazine dressings during the course of *Pseudomonas aeruginosa* burn wound infection.

Four children were treated with Acticoat(Smith&Nephew) dressings for a total of twenty seven days at an average of seven days per patient (range: 5-8 days), after the wounds were debrided surgically.

Eleven children needed surgical debridement of wounds to help control Pseudomonas wound infection. Three of these patients needed additional debridements.

Fifty skin grafts were done on twenty six of the patients in this series with an average of two skin grafts per patient (range: 1-4).

There was no relapse of Pseudomonas infection after successful treatment of the wound infection.

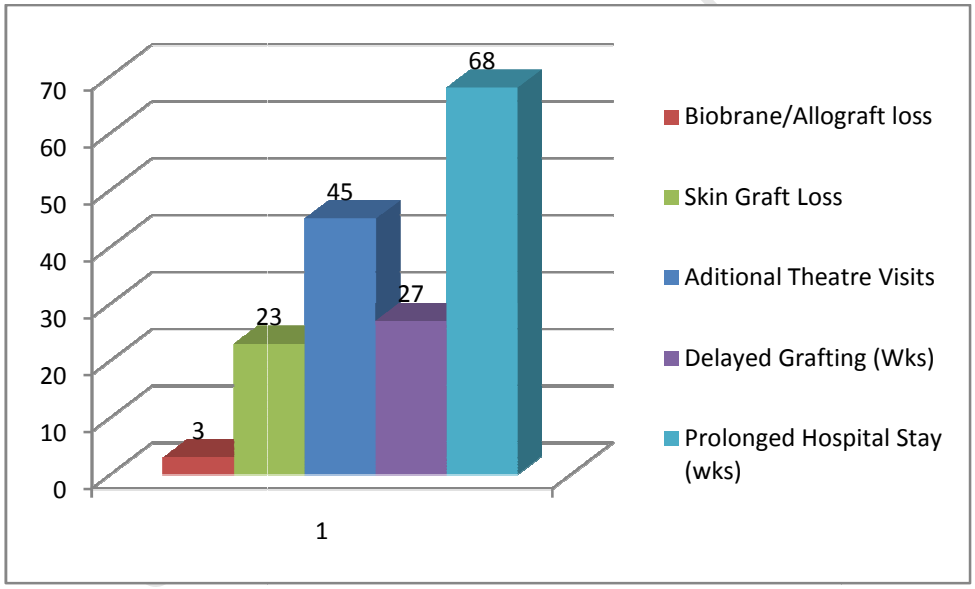


Graph 1: Number of Dressings Days, debridements and skin grafts

3.4. Complications

Three patients had loss of Biobrane or allografts. Allograft or Biobrane loss was defined as clinical evidence of infection necessitating removal, or failure of incorporation of graft secondary to Pseudomonas wound infection. A total of twenty three cases of skin graft loss occurred in eighteen patients (thirteen patients lost only one skin graft, while five patients lost two graft attempts). None of these patients had concomitant β-haemolytic streptococcal wound infection.

There were forty five additional theatre visits amongst eighteen of the thirty one patients (mean of 2.5 additional theatre visits per patient [range: 1-5]). A cumulative total of sixty eight additional weeks were spent in hospital for the management of the Pseudomonas wound infection at an average of two weeks per child (range: 1-4weeks).



Graph 2: Complications of pseudomonas wound infection

4. Discussion

The average %TBSA of burn wounds in the children with *Pseudomonas aeruginosa* infection in our study was 27% and the average time spent in hospital prior to diagnosis of *Pseudomonas aeruginosa* infection was ten days. This is in keeping with the work of other authors.[1,7,14,22,29]

The incidence of patients developing *Pseudomonas aeruginosa* infection in our unit during the 36 month period was 1.29%. This is lower than the incidence of 5.7% and 9% reported in the literature. [28,30] The one study looking at the incidence of *Pseudomonas aeruginosa* in a dedicated paediatric burns unit, that reported their incidence as the number of patients that developed *Pseudomonas aeruginosa* infection, had an incidence of 5,7%.[28] In that study, the average %TSBA was 15%, compared to the 27% TSBA in our review.

The incidence of samples positive for *Pseudomonas aeruginosa* sent from our unit is 50.3%. This is higher than the average incidence of 43%, from the reviewed literature (11.4-74%).

Compared to the reported susceptibility of *Pseudomonas aeruginosa* to antimicrobials in the literature reviewed, we have a low prevalence of resistance of *Pseudomonas aeruginosa* to systemic antibiotics.[27,31,32,49] This may be due to the relatively infrequent use of systemic antibiotics in our unit. The only isolate of a multi-resistant *Pseudomonas aeruginosa* in our thirty six month observational period was cultured from a patient that came from the general intensive care unit.

One of the most dreaded non-lethal complications for a burn patient is graft loss. *Pseudomonas aeruginosa* is a major cause of graft loss and occurred in twenty three of our thirty one patients. Nine patients lost more than one graft attempt. Skin grafts were only performed after negative wound cultures as well as satisfactory clinical appearance of the wound. None of the patients underwent skin grafting while still on systemic antibiotics. Negative wound cultures prior to skin grafting were therefore not influenced by the administration of systemic antibiotics.

Conclusion

The management of a patient with pseudomonas colonization or infection with daily sodium hypochlorite soaks and flamazine or chlorhexidine dressings seem to be effective. Surgical debridement of infected eschar or granulation tissue in combination with topical management with sodium hypochlorite and flamazine/chlorhexidine resulted in the fast return to negative culture wound swabs.

The cost in additional theatre visits and extended hospital stay and added wound dressing days, makes pseudomonas wound infection a serious and expensive condition.

5. Limitations and suggested future research

This study has the following limitations:

- This is a retrospective review from data collected from the burns unit of the Red Cross War Memorial Hospital. There is also no control group. Nor are there similar reviews published in the literature. The outcomes of management of *Pseudomonas aeruginosa* burn wound infection in an exclusively paediatric burn population with an un-buffered sodium hypochlorite solution was therefore not compared to a control group.

The following future research is suggested:

- A prospective, randomized control trial to compare the management of *Pseudomonas aeruginosa* burn wound infection using an un-buffered sodium hypochlorite solution with the standard of care in other burn units.

Chapter 3

The effects of un-buffered sodium hypochlorite solutions

1. Introduction

The bactericidal action of sodium hypochlorite has been known since the 1880's and it has been used in clinical practice for more than seventy years.[3] Its bactericidal action is directly related to its concentration and duration of exposure.[3,16]

The toxic effects of sodium hypochlorite on wound healing are confined to a restricted range of concentrations. But as the bactericidal activity of sodium hypochlorite is also related to its concentration and time of exposure, it is fundamental to use the optimum concentration that has both adequate bactericidal capabilities as well as minimal toxicity.[3,15,16] There have been no reports of the development of resistance to sodium hypochlorite by bacteria or yeasts, which make it a valuable topical agent to use in burn wound infection.[3,16]

In their landmark study in 1991, Heggers *et al* looked at the optimum concentration of sodium hypochlorite for adequate bactericidal capabilities with acceptable toxicity at 5, 10, 15 and 30minute intervals. They found that a sodium hypochlorite concentration of 0.025% was bactericidal within 30 minutes. The 0.025% sodium hypochlorite solution was buffered by adding 0.3 N sodium dihydrogen phosphate-disodium monohydrogen phosphate (NaH_2PO_4-Na_2HPO_4). This resulted in a solution with an osmolality of 354mOsm/L and a pH of 7.5. The buffered 0.025% sodium hypochlorite solution had no influence on fibroblast cytoarchitecture or viability.[16] The buffered 0.025% sodium hypochlorite solution had no influence on fibroblast cytoarchitecture and viability.[16] Fibroblast proliferation was used as a marker for wound healing.

A buffered sodium hypochlorite is currently not available for use at the burns unit of the Red Cross War Memorial Children's Hospital thus an un-buffered solution is being used (Appendix 1). An un-buffered sodium hypochlorite solution was investigated as follows:

- The effects of un-buffered sodium hypochlorite on fibroblast proliferation.

- The MBC (minimum bactericidal concentration) of un-buffered sodium hypochlorite dilutions was determined for three common pathogens in burns patients.
- The effectiveness and stability of un-buffered 0.025% sodium hypochlorite after 24-, 48-, 72- and 96-hours from manufacture.

The aim was to establish at what concentration of sodium hypochlorite fibroblast proliferation would be inhibited. A toxic concentration for wound healing could thereby be established. Together with the MBC concentration, the optimal concentration of an un-buffered sodium hypochlorite solution to be used in the management of patients with *Pseudomonas aeruginosa* burn wound infection would then be established. The shelf life of this un-buffered concentration would also be established

2. Methods

2.1. Fibroblast proliferation Assay (Appendix 2)

The effects of serial dilutions of the un-buffered Sodium Hypochlorite on fibroblast proliferation were analyzed. These effects were studied with fresh sodium hypochlorite solution as well as after 24-, 48-, 72- and 92-hours of shelf life at room temperature**.**

Human fibroblasts were cultured in DMEM containing 10% Foetal Calve Serum and Penicillin-Streptomycin at 37^{o}C in a 5% carbon dioxide incubator. Fibroblasts were used when the cultures reached 80% confluence.

$3x10^{7}$ cells were seeded per well in a ninety six well plate with 100µl of DMEM and allowed to grow for 24-hours. The cells were exposed to serial dilutions of sodium hypochlorite in sterile water for thirty minutes. The hypochlorite was then removed by pipetting it out of the swells and rinsing the wells with DMEM. The cells were then incubated in DMEM for twenty hours before assaying for cell viability using the XTT cell proliferation kit II (Roche).

2.2. Modified broth dilution to determine MBC (Appendix 3)

The effectiveness of dilutions of un-buffered sodium hypochlorite against *Pseudomonas aeruginosa* isolates as well as *Staphylococcus aureus* and β-haemolytic streptococcus (*Streptococcus pyogenes*) isolates was studied using a modified broth dilution method. The concentration at which this un-buffered sodium hypochlorite solution would be bactericidal was determined. The effectiveness of the un-buffered sodium hypochlorite against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and β-haemolytic streptococcus (*Streptococcus pyogenes* was tested after 24-, 48-, 72- and 96-hours of shelf life at room temperature, again using the modified broth dilution method.

The modified broth dilution method:

0.5 McFarland standard of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and β-haemolytic streptococcus (*Streptococcus pyogenes* (equivalent to approximately 1-2x 10^8cfu/ml) was prepared by using an overnight culture of organisms grown on blood agar. This was diluted 1:150 in nutrient broth and added to serial dilutions of sodium hypochlorite prepared to achieve final concentrations of 0.025%, 0.0125%, 0.006%, 0.003%, 0.0015%, 0.0008% and 0.0004%.

The test tubes were incubated at 35°C for eighteen to twenty four hours. Test tubes with *Streptococcus pyogenes* were incubated in CO_2 at 35°C for eighteen to twenty four hours.

Reading was done by checking the test tubes for turbidity. The lowest concentration of hypochlorite that produced no turbidity was read as the "MBC". An aliquot of the remaining organism suspension was then sub-cultured into blood agar, to check purity. This blood agar plates were incubated at 35°C for eighteen to twenty four hours. *Streptococcus pyogenes* was again incubated in CO_2 at 35°C for eighteen to twenty four hours. When reading these plates, results were recorded as "growth" or "no growth".

2.3. Biochemical properties

Biochemical properties and pH of the un-buffered 0.025% Sodium Hypochlorite solution were analyzed using the standard biochemistry methods with serial sodium hypochlorite

dilutions of freshly made sodium hypochlorite, as well as after 24-, 48-, 72- and 96-hours of shelf life at room temperature. The pH was measured using the ABL 520 Blood Gas Analyzer (GMI Medical), concentration of sodium and chloride was measured using the CX3 Chemical Analyzer (Beckmann) and the osmolality was measured using the OSMOMAT 030 osmolality analyzer (Gonontec).

3. Results

3.1. Effects of sodium hypochlorite on fibroblast proliferation

After the human fibroblast were exposed to a concentration of 0.025% un-buffered sodium hypochlorite for thirty minutes, only 24% of fibroblasts were viable. After thirty minutes exposure of 0.0125% sodium-hypochlorite, 86.2% of fibroblasts were viable. Thirty minutes exposure to a 0.006% solution left 88% fibroblasts viable and 98.9% of fibroblasts were viable after being exposed to a 0.003% solution of un-buffered sodium hypochlorite for thirty minutes.

These results remained constant for six consecutive days for which the un-buffered sodium hypochlorite was stored at room temperature. As the results of day five were not consistent with all the other results of day one to six, it was disregarded as a technical error most likely caused the inconsistency. The day 5 experiment could not be repeated as the inconsistency could only be detected on day 6 when the fibroblast proliferation assay was read. To repeat the experiment, a new batch of sodium hypochlorite would have had to been made and stored at room temperature for another five days. A new batch of fibroblast would have had to be prepared and a new proliferation assay kit would have had to been used. All these variables could have lead to inaccurate results. As the day 6 experiment results were consistent with the previous days, it was regarded that the sodium hypochlorite solution is equally -effective for the full six days at room temperature.

Table 5: Percentage fibroblasts surviving thirty minute exposure to dilutions of un-buffered sodium hypochlorite solutions in sterile water.

Day:	0.025%	0.0125%	0.006%	0.003%
1	24.0	86.2	88.0	98.9
2	16.7	81.7	100	100
3	27	74	82.7	87.2
4	27.4	63.4	83.5	100
5	15	39.4	64.6	64.4
6	30.1	95.4	100	100
Average	**25.0%**	**80.1%**	**90.8%**	**97.2%**

The same experiment was repeated with sodium hypochlorite solutions diluted in 0.9% saline, instead of sterile water. This yielded similar results.

Table6: Percentage fibroblasts surviving thirty minute exposure to dilutions of un-buffered sodium hypochlorite solutions in 0.9% saline.

Day	0.025%	0.0125%	0.006%	0.003%
1	23.38	42	85	93.1
6	21.6	29.6	71.9	100
Average	**22.5%**	**35.8%**	**82.3%**	**96.6%**

3.2. The effectiveness of sodium hypochlorite against isolates

The minimum bactericidal concentration (MBC) of the un-buffered sodium hypochlorite solution was tested using a modified broth-dilution method. A concentration of 0.006% of sodium hypochlorite was bactericidal to all the isolates of the deferens species tested.

The MBC for all the *Pseudomonas aeruginosa* isolates was 0.003%. The MBC for the *Staphylococcus aureus* isolates was 0.006% and the MBC for the *Streptococcus pyogenes* isolates was 0.0015%. The MBC remained unchanged for all the isolates that were tested with un-buffered sodium hypochlorite stored for ninety six hours at room temperature.

Table 7: MBC of sodium hypochlorite against different isolates

Organism	Number Isolates Tested	MBC 0.006%	MBC 0.003%	MBC 0.0015%	MBC 0.008%	MBC <0.004%	Overall MBC
P.aeruginosa	**31**		20	11			**0.003%**
P. putida	**2**			2			**0.0015%**
S. aureus	**12**	4	4	1		3	**0.006%**
S. pyogenes	**5**			2	1	2	**0.0015%**
Total:	**50**						

3.3. Biochemical properties and pH of the un-buffered 0.025% Sodium Hypochlorite solution

The un-buffered 0.025% sodium-hypochlorite solution has a pH of 10 and an osmolality of 168. The sodium concentration is 89mmol/dl and the concentration of Chloride 84mmol/dl. This remained stable with the 0.025% stored at room temperature over fourteen days.

Table 8: Biochemical properties of 0.025% sodium hypochlorite solution stored at room temperature

Time	Sodium	Chloride	pH	Osmol
Day 1	90.2	84.4	10	168
Day 2	87.8	81.7	10	167
Day 3	98.2	82	10	169
Day 4	89	85	10	169
Day 5	88	84	10	166
Day 6	88.9	83.3	10	167
Day 7	87.5	83.9	10	169
Day 9	88.7	82.4	10	167
Day 14	89	82.3	10	168

4. Discussion

The un-buffered sodium hypochlorite solution was bactericidal to all the organisms at a concentration of 0.006%. A concentration of 0.003% of un-buffered sodium hypochlorite was bactericidal to all the isolates of Pseudomonas tested in this study.

On average, 90% of fibroblasts were viable after thirty minutes exposure to 0.006% sodium hypochlorite solution whereas 97% of fibroblasts were viable after thirty minutes exposure to a 0.003% solution of un-buffered sodium hypochlorite. This difference, however, was not significant.

The optimum concentration of un-buffered sodium hypochlorite would therefore be a solution of 0.006%. This will bactericidal to all gram positive and gram negative organisms, and more than 90% of fibroblasts will be viable after thirty minutes of exposure to this solution.

The bactericidal effects as well as the effects on fibroblast proliferation of the un-buffered sodium hypochlorite solution were stable for a minimum of five days. The biochemical properties remained unchanged for fourteen days. It is therefore safe to use the un-buffered sodium hypochlorite for at least five days, when stored at room temperature.

5. Conclusion

The addition of daily soaks for thirty minutes in a buffered.025% Sodium Hypochlorite solution, in combination with other topical agents, for the management of *Pseudomonas aeruginosa* burn wound infection has been shown to be effective in previous studies.[16]

If an un-buffered solution is being used, a solution with a concentration of 0.006% seems to be optimal. It has a shelf life of at least five days at room temperature.

With the worrying development of resistance of *Pseudomonas aeruginosa* to betadine and flamazine in our unit, as well as the well documented ability of *Pseudomonas aeruginosa* to develop resistance to antimicrobials, the use of an effective topical agent to which no

development of resistance in the more than seventy years of use in clinical practice has been documented, is reassuring.

6. Limitations and suggested future research

This study has several limitations:

- The minimum bactericidal concentrations as well as fibroblast proliferation assays were all *in vitro* studies and no *in vivo* experiments were done.
- The systemic effects of sodium hypochlorite on the patient's electrolytes and pH were not assessed in this study.

The following future research is suggested:

- A study to evaluate the systemic effects on electrolytes and pH of the un-buffered sodium hypochlorite solution.
- A similar experiment to determine the effect of serial dilutions of sodium hypochlorite on keratinocytes should be done. Keratinocytes are more important in re-epithelialization of superficial partial thickness burn wounds.
- A study to compare effects sodium hypochlorite to those of acetic acid in the topical management of *Pseudomonas aeruginosa* burn wound infection.

Part 5

Discussion

Discussion of the findings of the research study

Pseudomonas aeruginosa burn wound infection is a serious condition that is difficult to manage.[1,4,5,17,511,4,13] Every effort must be taken to prevent Pseudomonas wound infection in burns patients. The use of topical antimicrobial agents is the single most important factor in preventing wound infection.[1,4,13] As soon as a patient is diagnosed or suspected to have Pseudomonas burn wound infection, the patient must be isolated from other patients.[1] Routine cultures must be obtained as surveillance for colonization of wounds.[1,4,5,17,51]

From the meta-analysis it is evident that there is very little data published on *Pseudomonas aeruginosa* burn wound infection or the management and complications thereof, in a dedicated paediatric burns unit. The retrospective audit done in this study, therefore adds valuable information to a small pool of published data.

None of the studies reviewed in the meta-analysis reported on the complications of *Pseudomonas* aeruginosa burn wound infection. No articles on the management, complications and cost implications of *Pseudomonas aeruginosa* burn wound infection in a dedicated paediatric burns unit was identified. The retrospective audit done at the Red Cross War Memorial Children's Hospital burns unit is therefore, to our knowledge, the only study reporting on this.

The incidence of patients developing *Pseudomonas aeruginosa* infection in our unit during the 36 month period was lower than the incidence reported in the literature.[28,30] The one study looking at the incidence of *Pseudomonas aeruginosa* in a dedicated paediatric burns unit, that reported their incidence as the number of patients that developed *Pseudomonas aeruginosa* infection, had an incidence of 5,7%[28] which is also higher than the 1.29% in our unit. In that study, the average %TSBA was 15%, compared to the 27% TSBA in our review.

The incidence of samples positive for *Pseudomonas aeruginosa* sent from our unit is higher than the average incidence in the reviewed literature. This was an unexpected finding, as our incidence of clinically significant *Pseudomonas aeruginosa* wound infection is lower than in other studies.

Compared to the reported susceptibility of *Pseudomonas aeruginosa* to antimicrobials in the literature reviewed, we have a low incidence of resistance of *Pseudomonas aeruginosa* to systemic antibiotics.[27,31,32,49] This may be due to the relatively infrequent use of systemic antibiotics in our unit. The only isolate of a multi-resistant *Pseudomonas aeruginosa* in our thirty six month observational period was cultured from a patient that came from the general intensive care unit. This patient received systemic antibiotics for nosocomial pneumonia in the intensive care unit, but this was stopped more than a week before the diagnosis of *Pseudomonas aeruginosa* burn wound infection was diagnosed and the patient did not receive systemic antibiotics for the treatment of the *Pseudomonas aeruginosa* burn wound infection.

A worrying factor is the resistance to betadine in all but four of the isolates of *Pseudomonas aeruginosa* in these patients. The routine standard of care in our unit was to treat all burn wounds without clinical evidence of wound infection with betadine impregnated swabs (Inadine, Johnson & Johnson), or Acticoat(Smith&Nephew). Our standard practice would therefore not prevent wound infection in all our patients to the strains of *Pseudomonas aeruginosa* colonizing our burns unit. This has prompted us to change the standard dressing to a chlorhexidine imprinted paraffin gauze (Bactigras, Smith&Nephew). None of the studies reviewed that reported on the susceptibility of *Pseudomonas aeruginosa* to antimicrobials, reported on the susceptibility to topical antimicrobial agents. The testing of topical antimicrobials is also very poorly standardized and there is not a lot of data that correlates laboratory results of topical susceptibility testing with clinical outcomes.[18] The average of thirteen days between diagnosis and negative cultures, reflect on the difficulty of managing pseudomonas burn wound infection.

Another problem that was identified with this study, is that results of routine wound swabs were not always adequately followed up as the time between a positive swab and initiation of appropriate management was nine days in one case. The average time between collection of swab and initiation of adequate treatment was two days. This problem has been adequately addressed. Doctors working in this unit now document all the recent microbiology results and current wound management in the patient's clinical notes on Mondays, Wednesdays and Fridays.

The morbidity associated with *Pseudomonas aeruginosa* burn wound infection included significantly longer hospital stay as long as additional theatre visits for debridement of

wounds and additional skin grafts. In addition to the physical pain and discomfort associated with additional procedures, *Pseudomonas aeruginosa* burn wound infection therefore is an expensive condition.

Sodium hypochlorite is an effective and inexpensive topical agent for the management of *Pseudomonas aeruginosa* burn wound infection. The un-buffered solution is easy to prepare and readily available. It is an ideal topical agent to use in a developing health care setting with limited resources, but effective and safe enough to use, regardless of resource limitations. The *in vitro* studies done are invaluable to determine a safe and effective sodium hypochlorite solution for the topical management of *Pseudomonas aeruginosa* burn wound infection.

The un-buffered sodium hypochlorite solution was bactericidal to all the organisms at a concentration of 0.006% and average, 90% of fibroblasts were viable after thirty minutes exposure to 0.006% sodium hypochlorite solution. The optimum concentration of un-buffered sodium hypochlorite would therefore be a solution of 0.006%. This will bactericidal to all gram positive and gram negative organisms, and more than 90% of fibroblasts will be viable after thirty minutes of exposure to this solution.

The bactericidal effects as well as the effects on fibroblast proliferation of the un-buffered sodium hypochlorite solution were stable for a minimum of five days. The biochemical properties remained unchanged for fourteen days. It is therefore safe to use the un-buffered sodium hypochlorite for at least five days, when stored at room temperature.

With the worrying development of resistance of *Pseudomonas aeruginosa* to betadine and flamazine in our unit, as well as the well documented ability of *Pseudomonas aeruginosa* to develop resistance to antimicrobials, the use of a safe and effective topical agent to which no development of resistance in the more than seventy years of use in clinical practice has been documented, is reassuring.

Part 6

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Part 7

Addendum

Appendix 1

Preparation of the un-buffered 0.025% sodium hypochlorite solution

The solution is prepared in the sterile pharmacy.

One liter of sterile water for irrigation (SABAX, Adcock Ingram) is used, and 25ml is withdrawn from the bottle.

Twenty five milliliters of Milton® (Cueta) is filtered through a 0.22µm filter (Millex GS 0.22µm, Millipore LTD) and add to the sterile water to have a concentration of 0.025% sodium hypochlorite.

Appendix 2

Fibroblast proliferation Assay

Human fibroblasts are cultured in DMEM containing 10% Foetal Calve Serum and Penicillin-Streptomycin at 37^{o}C in a 5% carbon dioxide incubator. Fibroblasts are used when the cultures reach 80% confluence.

3.0x10^{7} cells are seeded per well in a ninety six-well, flat bottom plate with 100µl of DMEM and allowed to grow for twenty four hours.

The cells are exposed to serial dilutions of sodium hypochlorite in sterile water for thirty minutes. The hypochlorite is then removed and cells incubated in DMEM for twenty hours before assaying for cell viability using the XTT cell proliferation kit II (Roche).

Preparation of human fibroblasts:

Human fibroblasts are cultured in a petri dish in DMEM containing 10% Fetal Calve Serum and Pen-Strep at 37^{o}C in a 5% carbon dioxide incubator, until cells reach 80% confluence.

Cells are lifted into suspension using trypsin and EDTA. The trypsin/EDTA is removed and DMEM added to neutralize the trypsin.

The cells in the suspension are then counted and 3.0x10^{7} cells in 100µl of DMEM are seeded into each well of a ninety six-well, flat bottom plate.

The cells are then incubated at 37^{o}C in a 5% carbon dioxide incubator for twenty four hours.

Preparation of hypochlorite dilutions:

Aliquot 2ml 0.05% hypochlorite solution into one test tube (labeled 1).
Aliquot 1ml of sterile water into 3 test tubes (labeled 2-4).
Transfer 1ml of hypochlorite into tube 2.
Transfer 1ml from tube 2 into tube 3, etc.

Discard 1ml from tube 4 – so all the tubes will have 1ml solution, with the following hypochlorite concentrations:

1. 0.05%
2. 0.025%
3. 0.0125%
4. 0.006%

Aliquot 100µl of dilutions of the sodium hypochlorite into the fibroblast cultures wells ($3.0x10^7$ cells per well). The final concentration of sodium hypochlorite in each well will then be:

1. 0.025%
2. 0.0125%
3. 0.006%
4. 0.003%

Incubate for thirty minutes.

After 30minutes the sodium hypochlorite solution and DMEM is removed and 100µl DMEM is added and the cells are incubated again at 37°C in a 5% carbon dioxide incubator for twenty hours.

Preparation of the XTT labeling mixture:

5ml of XTT labeling agent is mixed with 0.1ml of electron coupling reagent. Add 50µl of XTT labeling mixture to each well with cells.

Incubate for four hours at 37°C in a 5% carbon dioxide incubator.

Reading the cell viability assay:

The cell viability is read with the XTT machine.

Appendix 3

Modified Broth Dilution Method for Testing Hypochlorite

Preparation of hypochlorite dilutions:

Aliquot 2ml 0.05% hypochlorite solution into one test tube (labeled 1).
Aliquot 1ml of nutrient broth into six test tubes (labeled 2-7).
Transfer 1ml of hypochlorite into tube 2.
Transfer 1ml from tube 2 into tube 3, etc.
Discard 1ml from tube 7 – so all the tubes will have 1ml solution, with the following hypochlorite concentrations:

1. 0.05%
2. 0.025%
3. 0.0125%
4. 0.006%
5. 0.003%
6. 0.0015%
7. 0.0008%

Preparation of inoculums:

Prepare 0.5 McFarland standard of the organism (equivalent to approximately 1-2x 10^8cfu/ml) by using an overnight culture of organisms grown on blood agar. Use a wire to lift a colony of organisms approximately 1mm in diameter. Inoculate 0.9% normal saline with these organisms and mix the suspension well by using a Pasteur pipette. The suspension is then placed in a spectrophotometer. An absorbance of 0.15 at 640nm is equal to 0.5 McFarland standard.

Dilute this 1:150 in nutrient broth:

Dilute 100µl of suspension in 900µl broth (1:10).
Dilute 200µl of this in 2800µl broth (1:15).

Inoculation:

Inoculate 1ml organism suspension into tube 1-7.
Incubate tubes 1-7 at 35°C for eighteen to twenty four hours.
β-Haemolytic streptococcus spp. must be incubated in CO_2 at 35°C for eighteen to twenty four hours.

The final concentration of hypochlorite in tube is thus:

1. 0.025%
2. 0.0125%
3. 0.006%
4. 0.003%
5. 0.0015%
6. 0.0008%
7. 0.0004%

Subculture an aliquot of the remaining organism suspension into blood agar, to check purity
Incubate this blood agar plate at 35°C for eighteen to twenty four hours.
β-haemolytic streptococcus spp. should be incubated in CO_2 at 35°C for eighteen to twenty four hours.

Reading:

Check the tubes for visible turbidity. The lowest concentration of hypochlorite that produces no turbidity will read as the "MIC".

Subculture 10µl of the "MIC" tube into blood agar, to determine whether organisms have been killed. These plates should be incubated at 35°C for 18-24 hours. β-haemolytic streptococcus spp. should be incubated in CO_2 at 35°C for 18-24 hours.
When reading these plates, record results as "growth" or "no growth".

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19 May 2010

REC REF: 162/2010

Dr E Coetzee
Surgery

Dear Dr Coetzee

PROJECT TITLE: THE MANAGEMENT OF PSEUDOMONAS AERUGINOSA BURN WOUND INFECTION WITH SODIUM HYPOCHLORITE AND THE LOCAL EFFECTS AS WELL AS EFFECTIVENESS OF AN UN-BUFFERED SODIUM HYPOCHLORITE SOLUTION.

Thank you for submitting your study to the Research Ethics Committee.

It is a pleasure to inform you that the Ethics Committee has **formally approved** the above-mentioned study.

Approval is granted for one year till the 30th May 2011.

Please submit an annual progress report if the research continues beyond the expiry date. Please submit a brief summary of findings if you complete the study within the approval period so that we can close our file.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS
Federal Wide Assurance Number: FWA00001637.

S Thomas