BACTERIAL CAUSES AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF AEROBIC ISOLATES FROM BURN WOUND INFECTIONS IN DAR ES SALAAM, TANZANIA

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By

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A Dissertation Submitted in (partial) Fulfillment of the Requirement for the Degree of Master of Science (Microbiology and Immunology) of

Muhimbili University of Health and Allied Sciences
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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled: ***''Bacterial causes and antibiotic susceptibility patterns of aerobic isolates from burn wound infections in Dar es salaam, Tanzania''*** in (partial) fulfillment of the requirements for the degree of Science of Master in Medicine (Microbiology/Immunology) degree of Muhimbili University of Health and Allied Sciences.

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Dr. Agricola Joachim

(Supervisor)

_____

Date

DECLARATION AND COPYRIGHT

I, **Fatima Kabanangi**, declare that this **dissertation** is my own original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award.

Signature: …… **Date:** …….

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DEDICATION

This dissertation is dedicated to my best friend and the love of my life Seleman Rajabu Nyakusamaga and to our beloved children Rahma Tatu and Rajab Seleman Nyakusamaga

ABSTRACT

Background: Burn wound is one of the most common, devastating and painful trauma. Infection remains the most common cause of morbidity and mortality in burn patients. Information on local pathogens and sensitivity to antimicrobial agents is crucial for successful treatment of these infections.

Aim: To determine bacterial causes of BWI and antibiotic susceptibility patterns of aerobic isolates from burn patients at tertiary and regional hospitals in Dar es Salaam, Tanzania.

Methods: This was a cross-sectional study that was conducted between May and July 2017. Burn wound swabs were collected from patients with signs of wound infection. Swabs were cultured on blood agar and MacConkey agar and incubated aerobically at 37°C for 18–24 hours. In addition, a blood sample was collected from patients with a history of fever and/or temperature of ≥ 38º C. Bacterial identification was done using conventional method and API20E. Antimicrobial susceptibility was determined by Kirby-Bauer disk diffusion method.

Results: A total of the 70 patients with clinically diagnosed burn wound infections had their wound cultured. Sixty six (94%) had positive bacterial growth after overnight incubation. A total of 131 pathogenic bacteria were isolated, with gram negative organisms 91(69.5%) being more prevalent. The most frequently isolated bacteria were *Pseudomonas aeruginosa* (26%), followed by *Acinetobacter* spp (22%) and Coagulase negative staphylococci (CoNS) (18%), *Klebsiella* spp (11%) and *Staphylococcus aureus* (9.9%). CoNS were most common in BSI. Proportion of BSI among patients with BWI was 37.5%. Chloramphenicol was found to be most effective drugs towards gram positive bacteria and *Klebsiella*, while imipenem was effective against *Acinetobacter* spp. Up to 73% *Enterobacteriaceae* were extended spectrum beta-lactamase producers, while 61% of *Staphylococci* spp were resistant to methicillin. Proportion of MDR among all isolates was 73.5%. Two (2/70, 2.9%) paediatric patients died during the period of the study.

Conclusions and recommendations: *P. aeruginosa* was the most common isolate in BWI while CoNS were common in BSI. Most of the organisms isolated were resistant to commonly prescribed antibiotics. High proportion of infections were due to ESBL producers and methicillin resistance *staphylococci* spp. Routine culture and antimicrobial susceptibility testing should be performed before the start of therapy; including MRSA and ESBL screening

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LIST OF ABBREVIATIONS

ABA	American Burn Association
ATCC	American Type Culture Collection
API	Analytical Profile Index
BA	Blood agar
BMC	Bugando Medical Centre
BSI	Blood stream infection
BWI	Burn wound infection
CA	Chocolate agar
CoNS	Coagulase negative Staphylococci
CLSI	Clinical Laboratory Standard Institute
DDST`	Double disk synergy test
ESβLs	Extended spectrum beta lactamases
KCMC	Kilimanjaro Christian Medical Centre
KIA	Kligler iron agar
TBSA	Total body surface area
NBR	National Burn Repository
MBL	Metallo beta lactamase
MCA	MaConkey agar
MDR	Multi drug resistant
MHA	Mueller Hinton agar
MNH	Muhimbili National Hospital
MSA	Mannitol salt agar
MUHAS	Muhimbili University of Health and Allied Science
MRSA	Methicillin resistant *Staphylococcus aureus*
MRSE	Methicilllin resistant *Staphylococcus epidermidis*
MSSE	Methicillin sensitive *Staphylococcus epidermidis*
USA	United States of America

DEFINITION OF KEY TERMS

Burn wound infection – Presence of pus and/or foul smelling discharge from wound, and/or change in burn wound appearance or character, such as dark discolouration of the eschar, increased bleeding tendency, signs of inflammation in or around the wound and positive swab culture.

Colonization - The presence of bacteria on the surface of the wound in low numbers without invasive infection.

CHAPTER ONE

1.0. INTRODUCTION

1.1 Background

Severely burned patients are prone to several microbial infections such as wound infections, blood stream infections (BSIs), respiratory tract infections (pneumonia) and urinary tract infections (1–3). This is because thermal injury imparts generalized immunosuppression resulting from impaired function of both cellular and antibody mediated immune responses, loss of the skin which is the primary barrier to infection, and systemic inflammatory response syndrome (4). It is estimated that 75 % of deaths in severely burn victims are related to infections (5).

Burn wound infection (BWI) is one of the most common and severe complication in burn patients and is considered the number one cause of morbidity and death (6,7). The burn wound is a favourable and rich medium that nourishes and supports the growth of colonizing microorganisms due to the presence of large amounts of necrotic tissue and protein rich wound exudates. This can lead to subsequent invasive wound infections and septicaemia until the wound heals completely (8). Notably, burn patients are at an increased risk of blood stream infections (BSIs) especially those with central venous catheter compared with patients in other intensive can units (ICUs) (1,4).

A variety of microorganisms have been isolated from burn wound infections (6,8–11). Immediately following thermal injury, the burn wound is considered sterile (12,13). Initial wound colonization and infections is due to auto infection by gram positive bacteria from the patient's normal flora such as *streptococci* species from the throat, staphylococcal species that survive thermal injury by hiding in the pores of the skin and some *Enterococcus spp* (6,12). This is gradually taken over by mostly gram negative organism from the patient's gastrointestinal tract with *Pseudomonas aeruginosa*, *Escherichia Coli*, *Klebsiella pneumoniae* and *Acinetobacter* spp being the most common (6). It is this initial colonization that has the potential to cause serious wound infections and BSIs (6). In addition, the longer the patient

stays in the hospital, the higher the chances for the patient to acquire nosocomial infections in the wound (2,6,12,13). Isolates from burn wound infection have been shown to cause BSIs, making the wound the source of the infection, however, this is not always the case (14). Proliferation of bacteria in the wound results in high numbers that cause deep tissue infections. These bacteria can enter the lymphatic system or blood stream especially following invasive therapeutic procedures. This can result in sepsis with worse prognosis especially if caused by multi-resistant gram negative bacteria (14).

Bacterial isolates from burn patients have become increasingly resistant to most antibiotics (15). Changes in resistance pattern may be attributed to factors such as, cross infection, inappropriate use of antibiotics, selective pressure and even mutations in bacterial genome (16,17). Even with the use of topical and systemic antibiotics, colonization cannot be avoided. Topical agents prevent overgrowth of microbes but may fail to inhibit the growth of some invasive bacteria (18,19). Moreover, the efficacy of these topical agents changes as bacterial quickly develop resistance towards them (17). Currently, it is not recommended to give prophylactic systematic antibiotics to inpatients with burn wounds as this only adds to the emergence of resistant strains (11,13).

S. aureus belonging to methicillin resistant *S. aureus* (MRSA) group, is the most predominant gram positive causing infections, with methicillin resistant *S. epidermidis* (MRSE) following closely (10,14,16). MRSA in burn patients is favoured by prolonged hospital stay and extended use of antibiotics (20). These two factors have also led to a selection of mainly MRSA and MRSE in BSIs (10). Gram negative organisms have also emerged to cause serious infections in burn patients due to their virulence factors, and also have become increasingly resistant to commonly used antibiotics (21). *P. aeroginosa* and *Acinetobacter* have developed resistance to drugs that they were once sensitive to including ceftazidime, ciprofloxacin and carbapenems (20). Furthermore, most of these gram-negative bacilli have an ability to produce the extended spectrum beta lactamase (ESBL) enzymes that make them resistant to most third generation cephalosporins. These enzymes are able to hydrolyze broad-spectrum antibiotics such as cephalosporins, and are also capable of showing co-resistance to other classes of

antibiotics like quinolones and aminoglycosides. These capabilities limit the options of antibiotics available for therapeutic use (22). The present study is designed to update the profile of aerobic bacteria in burn wound infection, blood stream infection, and their antimicrobial sensitivity patterns at Muhimbili National hospital (MNH), Mwananyamala and Temeke regional hospitals in Dar es salaam, Tanzania.

1.2. Conceptual framework

Occurrence of infections in burn patients can be influences by several factors; patient factors such as age, length of hospital stay, total body surface area burnt (TBSA %), degree of burn, anatomical location of injury, previous use of broad-spectrum antibiotics and microbial factors. These patient factors predisposed burn patients to infections and microbial factors such as the number of organisms in the wound, and motility can result in invasive wound infections leading to BSI.

1.3. Problem statement

Burn wound infections are the most common and devastating forms of clinical infections in burn patients requiring immediate and appropriate treatment (14). It is estimated that 75 % of deaths in burn patients are related to infections. Reports have shown that burn wound infections prolong morbidity, hospital stay and increase the cost of hospitalization (5, 23). Burn patients are at an increased risk of colonization by drug resistance organisms especially MRSA (10). Compared to other patients, burn patients are at a higher risk of acquiring nosocomial infection from multi-drug resistant gram negative bacilli, thereby limiting the choice of empirical therapy (4,8,14,24). It is these infections by multi-drug resistant organisms that cause significant morbidity and mortality in these patients (10,14).

Studies from around the world and the region have shown different bacteria predominating as the cause of BWIs. These studies also document an increase in drug resistance of these isolates to commonly prescribed antibiotics, including an increase in multidrug resistance (23, 25–27). In Tanzania, very few studies have focused on BWIs (27), and therefore, it is not known whether the pattern of isolates and antibiotic resistance is similar to those reported in other countries and within hospitals in Dar es Salaam. Thus, there is very limited data regarding the organisms causing BWIs, their link to BSIs and also their drug susceptibility patterns and emergence of multidrug resistance in this group of patients. Lack of this information makes the choice of empirical therapy difficult for the clinician and may lead to inappropriate management of patient with burn infections especially in our settings where culture and sensitivity testing are not routinely and timely performed. This has negative implications in the treatment outcomes and development of antibiotic resistance.

1.4. Rationale

There are some similarities but also differences in the spectrum of bacteria implicated in BWI and hence BSI, locally and internationally (4). Therefore, a better understanding of the local spectrum of pathogens causing BWI, BSI, as well as their susceptibility pattern is important for prompt management of patients, as antimicrobial therapy significantly influences the outcome of the patients with BWI and BSI.

This study is therefore aiming to provide data on pattern of bacteria infecting burn wounds, their link to BSI, antimicrobial susceptibility patterns and factors predisposing burn patients to infections and their outcomes at MNH, Mwananyamala and Temeke regional hospitals. The information will help clinicians to provide appropriate antibiotics to patients with BWIs and BSIs as the empirical drugs will be 'tailor made' based on the predominating microorganism in burn unit and at that particular time, thus improving the overall infection-related morbidity and mortality. This will in turn allow burn centres to establish gains in infection control and control the emergence of multi-drug resistant organisms cause by the irrational and over use of broad spectrum antibiotic.

1.5. Research questions

1. What are the common bacterial pathogens causing BWI in Dar es Salaam?
2. What are the antibiotic susceptibility patterns of bacterial pathogens isolated from burn wound infections?
3. Are the bacterial pathogens isolated from BWI phenotypically similar to the ones causing BSI?
4. What factors predispose burn patients to infections by ESBL producing and methicillin resistant organisms
5. What are the outcome of patients with burn wound and blood stream infections during the study period

1.6. Objectives of the study

1.6.1. Broad objective

To determine the bacterial causes of BWI, BSI and their antimicrobial susceptibility patterns, and also factors predisposing these patients to infections and their outcome at tertiary and regional hospitals in Dar es Salaam, Tanzania.

1.6.2. Specific objectives

1. To determine the bacterial causes of burn wound infections at Muhimbili national hospital, Mwananyamala and Temeke regional hospital
2. To determine the proportion of burn patients with BWIs who will develop BSIs during the study period
3. To determine the antimicrobial susceptibility pattern of isolates from BWIs and BSIs in burn patients
4. To determine factors predisposing burn patients to infections due to ESBL producing and methicillin resistant organisms
5. To determine the outcome of patients with burn wound and blood stream infections during the study period

1.7. Literature Review

1.7.1 Common bacterial causes of burn wound infection

Different types of microorganisms have been shown to colonize and cause burn wound infection (6). In addition, the profile of organisms causing burn wound infections changes with time and geographical location, and from primarily gram positive to gram negative (11,15).

S. aureus and specifically MRSA, has been found to be the most common organism causing wound infection in several burn units worldwide (5,6,23,28–31). In a three-year prospective study of infections in burn patients in Sweden, 72 burn wound infections were registered. Of these, the most frequent organism causing BWIs was MSSA (85%), followed by *P. aeruginosa* (37.5%), B-haemolytic *streptococcus* spp (36%), Coagulase-negative Staphylococcus (CoNS) (24%), *Enterococcus* (18%) and *Enterobacter* (14%) (28). Similar patterns with *S. aureus* as the most prevalent organism causing BWIs have been seen in some developing and low income countries (5,32). In a one-year prospective study in Brazil in 2005, *S. aureus* (28.4%) was the most prevalent organism in their burn unit followed by *P. aeroginosa*. Also CoNS were high. It was also notices that *S. aureus* was the most prevalent organism in the first week, but was later surpassed by *P. aeruginosa* from the third week onwards (5). Similarly, in a South African study, the most common organism isolated form burn wounds was *S. aureus* (27.7%), followed by *K. pneumoniae* (13.4%), *Proteus mirabilis* (12.4%), Group D *streptococcus* (9.4%), *P. aeruginosa* (8.9%) and *E. coli* (6.2%) (6). In a study done in Blantyre, Malawi *S. aureus* (37.6%), *P. aeruginosa* (22.4%) and β-haemolytic streptococci (13.6%) were the most common organism isolated from burn wound. Other gram negatives isolated were *P. mirabilis*, *E. coli* and *K. pneumoniae* (32). In a similar study at the same hospital, *P. mirabilis* (22.7%) was found to be the predominating gram negative (29). In Ethiopia, a cross sectional, prospective study of 114 burn patients showed *S. aureus* to be highly prevalent at 69.5% (23). In a study done at Bugando Hospital in Mwanza, Tanzania on paediatric burn patients, *S. aureus* was more common in the first week of admission, with *P. aeruginosa* becoming more evident after 10th day. MRSA was detected in 19.2% of *S. aureus* (27).

In a study carried out on 51 burn patients in India, *Staphylococcus epidermidis* (56%) or CoNS was found to be the predominating organism followed by *P. aeruginosa* (18%) and *S. aureus* (13.4%) (33). CoNS was also the most prevalent isolate in 1204 paediatric patients in Pakistan at 14.5%, followed by *E. coli* (10.7%), *Enterobacter* species (9.9%) and *Pseudomonas* species (9.6%) (34). Similar finding were also reported in a study conducted in Ethiopia where CoNS was the most common infecting organism at 14.5% (35).

In other studies, gram negatives rods have been shown to cause serious nosocomial infections with *Pseudomonas* being predominant (11,18,36). In a recent quantitative survey of burn centres in the USA, 44% of them identified *P. aeruginosa* as the most prevalent gram-negative pathogen, followed by *Acinetobacter baumannii* and *Enterococcus* spp (20,37). In a study involving 95 burn patients in Pakistan, the most frequent isolate was *P. aeruginosa* (35%) followed by *K. pneumoniae* (20%), *S. aureaus* (19%), *Proteus* (9.8%), *E. coli* (6.9%), and *Acinetobacter* (6.9%). Among the *S. aureus* isolates, 68% were MRSA (36). Similar findings have also been reported across Africa (11,18,25,38). In a 5 year retrospective study involving 71 patients in Enugu, Nigeria, *Klebsiella pneumonia* (26.7%) was found to be the most prevalent gram negative causing BWIs, followed closely by *S. aureus* (25.6%) (39). In Tanzania, and particularly in Dar es Salaam, there is a paucity of information on the bacterial causes burn wound infection. It is also unknown if the organisms implicated in infection at the national hospital are similar to those at the regional level.

1.7.2. Blood stream infection in burn patients

Blood stream infections in burn patients may occur as a result of burn wound infection, use of devices such as central venous catheters and translocation of GIT flora (2,14,40). The etiologic agents of BWI have been shown to cause BSI resulting into life threatening septic episodes, with more invasive pathogens such as *S. aureus, P. aeruginosa, A. baumannii* , and *K. pneumoniae* being organisms most commonly associated with bacteremia in burn patients (8–10,40).

In a retrospective case control review of all patients in the National Burn Repository (NBR) in the USA, *S. aureus* was the most prevalent gram positive organism (32%) and *P. aeroginosa*

(35%) was the most common gram negative isolate causing BSIs (31). A study conducted in Poland on the type of microorganism isolated from blood and wound following treatment of sepsis in patients with burns reported that the commonly isolated microorganisms in cured patients were MRSE (19.8%) and MRSA (18.8%). *A. baumannii* (35.6%) and *P. aeruginosa* (22%) were isolated in the patients who were to die, showing just how serious infections caused by these multi-drug resistant (MDR) gram negative organisms can be (14).

In Brazil, the most common bacteria isolated from blood culture were *S. aureus*, CoNS, *A. baumannii, Enterobacter cloacae* and *K. pneumoniae.* The source of the septic episodes that occurred was the wound (24 %) and few cases resulted from the catheter (4.8%) (40). In Kuwait, studies have confirmed the wound as being the direct source of bacteria causing BSI as culture of tips from catheters did not yield positive results. Here, the most common isolates from BSI were MRSA and MRSE (9,10). In a South African study on bacteremia in burn patients, the three organisms most commonly cultured from blood were *A. baumannii, P. aeruginosa* and MRSA. However, due to limited resources, no analysis was done to related these isolates to those from wound swabs or catheter tips (4). In Tanzania, a retrospective analysis of blood cultures at MNH 7 years ago reported CoNS to be the most common, followed by *S. aureus, E. coli* and *Klebsiella* spp (41). *K. pneumoniae* was also the most frequently isolated organism in blood stream infection in Zanzibar followed by *E. coli, Acinetobacter* spp. and *S. aureus* (42). Regardless, the cause of the BSIs has not been well defined in burn patients.

1.7.3. Factors predisposing patients to infections due to ESBL and methicillin resistant organisms and outcomes

Various factors have been shown to increase the prevalence of BWIs leading to BSIs in patients and these can be grouped into two; patient and microbial factors. Patient factors include: size of the injury (%TBSA), degree of burn, anatomical location, duration of hospital stay, systemic prophylaxis, co-morbidities (i.e. obesity, diabetes, immunosuppression, malnutrition, HIV) and the extremes of age (25,43–45). For BSIs, the presence of intravascular catheters and their proximity to the wound has shown to be a factor (31).The

microbial factors are: virulence, numbers of organisms, motility, extra-cellular products such as proteinases, collagenases, hyaluronidase, exotoxins and antimicrobial resistance (45).

A prospective multicentre study in North America showed that children with TBSA over 60 % and adults with TBSA over 40% had increased risk of morbidity and mortality. The incidence of BWIs was higher among paediatrics but the incidence of nosocomial infections was comparable among all age groups (46). Another USA study concluded that mortality and other outcomes such as increased length of hospital stay and ICU days and cost of care are significantly associated with BSIs (31). In Malawi, Liwimbi *et al*, showed an increase in the ratio of bacteria grown per swab in relation to the increasing TBSA%. Also the location, i.e. being burned on the perineum area is a predisposing factor to infection by gram negatives due to its close proximity to the anus (29). In Tunisia, Higher mortality rate and increased length of hospital stay were recorded among children with larger wounds (10% to 20 % TBSA) (47). Also the length of hospital stay, previous use of broad-spectrum antibiotics, and previous use of meropenem/imipenem has been shown to predispose burn patients to infections by MDR organisms such as *P. aeroginosa* (48). Limited studies have been done in Tanzania to determine factors predisposing these patients to infections. This study sets out to describe these factors in hopes of curtailing infections in these patients and improving their survival.

1.7.4. Antibiotic susceptibility pattern and multidrug resistance of isolates from burn patients

The development of resistance to antibiotics by microorganisms is a challenge for clinicians caring for burn patients as it reduces the effectiveness and choice of treatment and threatens to be a future medical disaster (16,29). This challenge is even immense in resource limited countries in Africa where culture and antibiotic sensitivity are not routinely done and the choice of antibiotics is limited (29).

Antibiotic-resistant organisms such as MRSA, vancomycin-resistant Enterococci (VRE), and multiply-resistant gram-negative rods, including *P. aeruginosa*, *Acinetobacter* spp., and different members of the family *Enterobacteriaceae (K. pneumoniae, E. coli)*, have been implicated in infections of the burn wound, blood and other anatomic sites in patients with

major thermal injury, occasionally in the form of nosocomial outbreaks (2,49,50). Furthermore, infections with multi-drug resistant pathogens whether in hospitals or in the community increase morbidity, decrease treatment success, reduce hospital turn-over rate and increase cost of patient care (23).

In the 1920s and 30s, *S. pyogenes* was the most common gram-positive organism causing infections in burn wounds. This however, has been replaced predominantly by *S. aureus,* mostly MRSA (18). MRSA has proved to be a public health problem in both developed and developing countries (23,40,49). For example, in Canada, a single strain of MRSA was implicated in a multi-institutional outbreak in a study done in an adult plastic surgery/burn unit of a tertiary hospital. The breakout affected patient healing and increasing the cost of patient care (49). In Sweden, a 20 year study showed increasing resistance of staphylococci isolates to most of the broad-spectrum antibiotics such as ciprofloxacin and penicillinase-based penicilins but with no single resistance to vancomycin (51). Together with MRSA, MRSE has also been implicated in BSIs in ICU and burn centers as they are commonly found in the hospital environment (10). A Kuwait study reported staphylococcal septicaemia caused by MRSA and MRSE in 65% and 14% of the cases respectively (10). Continuing with the same trend, *S. aureus* isolates in a South African study showed increased resistance to penicillin (99%) and 58% of the isolates were MRSA. However, all the MRSA (100%) were sensitive to vancomycin (6). Also in an unrelated study in the same country, all MRSA isolates from BSIs were sensitive to vancomycin (4). In a Brazilian study on bacteriology of burn wounds, a high incidence of oxacillin resistance was observed among CoNS (44.6%) but was low among *S. aureus* (4.7%). However, as with other studies, all staphylococci were susceptible to vancomycin. *S. aureus* showed high susceptibility to a wide range of antibiotics while CoNS showed low to moderate susceptibility to amoxicillin/clavulanic acid, cephalothin, oxacillin, gentamicin, clindamycin, ciprofloxacin, ampicillin, and cotrimazole (5). In study conducted in Ethiopia, on BWIs all staphylococci isolates (100%) were MRSA. These isolates showed sensitivity to vancomycin, clindamaycin, Kanamycin and Erythromycin 94%, 91%, 86%, respectively and were resistant to penicillin, methicillin, polymyxin-B and chloramphenicol 96%, 77%, 68% and 52%, respectively (34). Also in Uganda, a high prevalence on MRSA

(46%) was reported in the burn unit, with 63% on the MRSA being MDR showing resistance to β-lactams, sulphamethoxazole-trimethoprim and tetracycline (52).

Gram negative organisms have emerged to cause serious and life threatening infections in burn patients and isolates that originate from the hospital environment and more multi-drug resistant than from the GIT (2,7,53). *P. aeruginosa* in particular carries many natural and acquired antimicrobial resistance traits that make infected burn wounds difficult to treat (2,50). Also the ability of this organism to form biofilms makes it less susceptible to antibiotics used to combat infections (54). Multi-drug resistant *P. aeruginosa* which is defined as being resistant to at least three antipseudomonal drugs has become a common feature of burn wound infections (55). Furthermore, the presence of ESBL producing *P. aeroginosa* in burn wounds has being noted (15,22,56). In Pakistan, *P. aeruginosa* isolates showed reduced sensitivity to many of the commonly used antibiotics; amikacin (8%), ceftazidime (11%), polymyxin B (36%), ciprofloxacin (44%) and imipenem (64%) (36). Still in Pakistan, a study prevalence of ESBL producing *P. aeruginosa* found 36% of the isolates to be ESBL producers and a significant proportion (29%) being MDR. The most potent antibiotics were meropenem and imipenem. The isolates showed reduced susceptibility to amikacin was 70%, gentamicin (25%), ciprofloxacin (49%), gatifloxacin (42%), and to co-trimoxazole (16%) respectively (22). In a study done in Nigeria on bacteriology of burn wounds, *P. aeruginosa* showed increasing and worsening resistance to gentamycin (33%) and colistin (67%) as compared to earlier reports where it was 84% and 100 % sensitive to gentamyicn and colistin (39).

Organism from the family *Enterobacteriaceae (E. coli*, *K. pneumoniae* and *Proteus spp.*) and *Acinetobacter* spp. that possess several types of beta-lactamases, including ESBL and metallo beta- lactamases (MBL) have been emerging as serious challenges in hospitalized patients (38). A study done in Bangladeshi on the bacteriology of burns wound and their antibiotic susceptibility showed increasing resistance to common antibiotics among the gram negative bacteria. All isolates were sensitive to imipenem. *E. coli* were highly resistant to cephradine (90%), amoxicillin (81%), co-trimoxazole (70%) and cefotaxime (64%). *Klebsiella* species were highly resistant to cephradine (88%) followed by amoxicillin (86%), co-trimoxazole

(81.4%) and ciprofloxacin (65%). *Proteus* species showed 93% resistance to cephradine, amoxicillin (86%), co-trimoxezole (79%) and gentamicin (76%). In addition, the study found 25% *E. coli*, 43% *K. pneumoniae* and 21% *Proteus* spp to be ESBL producers which were resistant to amoxicillin, cephradine, ceftriaxone, aztreonam, ceftazidime and cefotaxime (56).

In Tanzania, there is little to no data on isolates causing infections and their antimicrobial susceptibility patterns in burn patients. However, plenty of data exists on similar clinical isolates from other types of infections such as surgical site infections (SSI) (57,58). In a study at MNH on SSI, *S. aureus* isolates were highly resistant (83%) to penicillin based antibiotics and *P. aeruginosa* (92%) were sensitive to both gentamicin and ciprofloxacin with only one isolate being MDR, all *Enterobacteriaceae* showed high magnitude of resistance (69% to 100%) to multiple antibiotics tested but all were sensitive to meropenem. About 20-56% of the isolates were resistant to ciprofloxacin. *A. baumannii* isolates were also highly resistant (73% to 100%) to most antimicrobial agents, but 60% of them were moderately sensitive to imipenem (57). In BSIs, an MRSA prevalence of 23% has been documented at MNH, which is considerable higher that that reported in previous studies at the same hospital among the isolates (41). ESBL and MDR gram negative organism have also proven to be a problem in clinical isolates from Tanzania (57,59). A local study reported that most of the *Enterobacteriaceae* showed increased resistance (62.5%) to third-generation cephalosporins as most of the isolates were probably ESBL producers (41). ESBL producing *E. coli, K. pneumoniae* have also being reported in BSIs in Zanzibar (42). At Bugando Medical Centre in Mwanza, 29.2% of all the gram negative rods were ESBL producers, with more isolations from in patients (35.3%) than out patients (10.6%) (59). It is unknown if similar patterns of antimicrobial susceptibility patterns occur in isolates specifically from burn wounds. Also the emergence of multi drug resistance among the isolates needs to be addressed. This study sets out to provide local data on drug susceptibility pattern of isolates causing wound and blood stream infections burn patients.

CHAPTER TWO

2.0. MATERIALS AND METHODS

2.1. Study design and Setting

This was a hospital based cross-sectional study that was conducted at MNH and two regional hospitals located in Dar es Salaam city, Tanzania namely Mwananyamala and Temeke hospitals from May to July 2017. MNH is the largest tertiary health care facility in Tanzania with 1,500-bed capacity, which serves as a teaching and referral hospital to the population of Dar es Salaam and the whole country. The hospital attends to 1000 to 1,500 outpatients per day, and admitting 1,000 to 1,200 inpatients per week with nearly 3000 employees. Mwananyamala regional referral hospital is located in Kinondoni municipality with 240-bed capacity attending to an average of 1500 outpatients per day. Temeke regional referral hospital is located in Temeke municipality attending to 1000 to 1500 patients per day.

2.2. Study population

During this study, all children and adults with burn wounds admitted at MNH, Mwananyamala and Temeke regional hospitals were enrolled.

2.3. Inclusion and Exclusion criteria

2.3.1. Inclusion criteria

1. Children and adult patients with burn wound infections, treated at the three selected hospitals.
2. Patient accepting to give informed consent/assent. For children below 18 years of age, only those whose parents/guardians will sign informed consent will be included.

2.3.2. Exclusion criteria

1. Refusal to give consent to participate in the study

2.4. Case definition

In this study, burn wound infections were defined using any of the criteria below as stated by Appelgren *et al*, 2002 (28,60) and CDC (61).

1. Pus and/or foul smelling discharge from skin, wound, blister, abscess, drain or vascular insertion site above fascia.
2. Change in burn wound appearance or character, such as dark discolouration of the eschar, increased bleeding tendency, signs of inflammation in or around the wound or vascular insertion site and positive swab culture
3. Skin graft detached more than two days after grafting and positive swab culture
4. Pus and/or foul smelling discharge from wound, abscess, drain or fistula beneath fascia.
5. Positive swab culture from burn wound and at least two signs below
    i. Temperature >38.5 ◦C during at least 12 h or ongoing antipyretic therapy
    ii. Leukocyte count <4 or $>12\times10^9$/l in blood.

2.5. Sample Size

The sample size was calculated in terms of the number of isolates using the following formula.

$$\underline{n = z^2\, p(1-p)}$$
$$e^2$$

Where

n = Sample size

z = at 95% confidence interval z value = 1.96

p = Proportion of *S. aureus* isolated from BWIs at Yekatit 12 hospital burn unit in Addis Ababa, Ethiopia 69.5% (0.695)

e = Margin of error at (8%) (0.08)

Thus:

$$n = \underline{(1.96)^2 x\ 0\ .695(1\text{-}0.695) =}\ 127.24$$
$$(0.08)^2$$

The minimum required samples size was 127 isolates.

2.6. Sampling Procedure

Convenient sampling was used to recruit the available patients for this study. Everyday new patients who met the inclusion criteria were enrolled until the required sample size was attained.

2.7. Variables of the study

2.7.1. Independent variables: Age, Sex**,** degree of burn, hospitalization, antibiotic use, duration of hospital stay

2.7.2. Dependant variables: Burn wound infection, blood stream infection, antimicrobial resistance pattern, infection by ESBL producing *Enterobacteriaceae* and MR *Staphylococci*

2.8. Data Collection

Structured standard questionnaire was used to collect socio-demographic data such as age, sex, physical address and education. Clinical information including type and degree of burn, history of antibiotic use, time and date of admission, mechanism of injury, total body surface area (TBSA) burned, co-morbidities, date and type of operative procedures, length of hospital stay, and survival were also collected from patients after signing the informed consent.

2.9. Specimen collection and Laboratory procedures

2.9.1. Specimen collection

Two surface swabs were collected per patient. These taken from the depth of the wound showing signs of infection including discharge, pain, swelling, foul smelling and from chronic wound. Surface swabs were collected from burn wounds after removing any dressings and topical antimicrobial agents and cleansing of the wound surface with sterile normal saline (2). Specimen were places in the Stuarts transport media and transported to the microbiology laboratory for processing as soon as possible (62). Blood samples for culture were collected from patients with burn wounds showing signs of infection and a history of fever and/or have

a fever of ≥ 38 ºC. Blood collection was done using aseptic technique. Three to five (3-5) ml of blood was drawn from children (63).

2.9.2. Laboratory Procedures

2.9.2.1. Isolation and Identification of bacterial pathogens

Two pus swabs were collected per patient. One swab specimen was Gram stained and examined microscopically for presence of bacteria and pus cells, then quantified as number of cells/high power field. The other swab specimen was inoculated onto Blood agar (BA) and MacConkey agar (MCA) and incubated aerobically at 37 ºC for 24 to 48 hours (63). Preliminary identification of bacterial isolates was done based on colonial morphology and characteristics such as pigmentation, haemolysis pattern on blood agar, etc) and Gram staining characteristics. Inoculated blood culture bottles were incubated in BD BACTEC FX 40 for 24 hours to 5 days until the machine detect positive growth. Gram stain was performed on blood culture positive vials and then subcultured on either MCA or BA, for detection of microorganism causing fever. MCA was incubated aerobically at 37 ºC for 24h while BA was incubated in 5-10% Co_2 for 24 hours (63).

2.9.2.2. Biochemical test

Isolates were confirmed by conventional biochemical tests, and API 20E for a few isolates, which were performed on colonies subcultured on nutrient agar (NA) (63). Gram-negative organisms were tested for; carbohydrate utilization tests, indole production, urease test, citrate utilization, oxidase test, and H_2S production. Isolates exhibiting ambiguous taxonomic classification were confirmed by API 20E (BioMerieux, France) following the manufacturer's instructions. Isolates were also checked for motility. Gram-positive cocci were identified based on catalase test, coagulase test, mannitol fermentation and DNase test result (63).

2.9.2.3. Antibiotic susceptibility test

Antimicrobial susceptibility testing were performed using the Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) (64). Briefly, from a pure culture, 3- 5 selected colonies of bacteria were picked and transferred to a tube containing

5 ml sterile normal saline and mixed gently until a homogenous suspension was formed. Turbidity of the culture suspension was equilibrated to match 0.5 McFarland standards. A sterile cotton swab was used and the excess suspension removed by gentle pressing and rotation of the swab against the inside wall surface of the tube. The swab was then used to distribute the bacteria evenly over the entire surface of Mueller-Hinton agar (MHA). For *Streptococci spp,* MHA with 5% sheep's blood was used. The plates were incubated at 37 ºC for 18-24 hours and the diameters of the zone of inhibition around the disk were measured using vernier callipers in millimeters and interpreted according to CLS1 2015 criteria; sensitive, intermediate, and resistant. *P. aeroginosa* American Type Culture Collection (ATCC) 35218, *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were used as control organism.

The following antibiotic disks (Oxoid, UK) were tested for Gram-positive bacteria; ampicillin (10 µg), penicillin G (10 units), ceftriaxone (30 µg), chloramphenicol (30µg), vancomycin (30µg), erythromycin (10 µg), gentamicin (10 µg), ciprofloxacin (5µg), tetracycline (30 µg), trimethoprim-sulphamethoxazole (1.25/23.75 µg). Gram-negative *Enterobacteriaceae* isolates were tested against amoxicillin clavulanic acid (20 µg /10 µg), ampicillin (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), ciprofloxacin (5µg**)**, chloramphenicol (30 µg), and gentamicin (10 µg). For *Psuedomonas*, aztreonam (30 µg), ceftazidime (30 µg), ciprofloxacin (5µg), imipenem (10 µg), and gentamicin (10 µg) while *Acinetobacter spp* were tested against aztreonam (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), ciprofloxacin (5µg), imipenem (10 µg), gentamicin (10 µg) and tetracycline (30 µg). Antibiotic disks were applied on the surface of the plates at least 15mm apart from the edges of the plates to prevent overlapping of inhibition zones.

The *Staphylococcus* isolates were tested for methicillin resistance using cefoxitin as outlined by CLSI 2015 (64). Cefoxitin disks (30 µg) (Oxoid UK) were used following the standard disk diffusion method. Incubation of plates was at 33 to 35°C; ambient air for 16 to 18 hours. For interpretation, zone of inhibition $\leq$ 21 mm was reported as MRSA, and $\geq$ 22mm was considered as Methicillin susceptible *S.aureus* (MSSA).

All strains which showed a diameter < 22mm for ceftazidime and < 25 for ceftriaxone, were selected for detection of extended spectrum β-lactamases (ESBL) production (64). ESBL production was tested by Modified Double Disk Synergy Test (MDDST) using a amoxicillin-clavulanate (20/10 μg) disk along with two third generation cephalosporins; cefotaxime and ceftazidime. A lawn culture of the organisms was made on a Mueller-Hinton agar plate, as recommended by CLSI. Amoxicillin-clavulanate (20/10 μg) was placed in the centre of the plate. Ceftazidime and cefotaxim disks were placed 20 mm apart, centre to centre to that of the amoxicillin-clavulanate disk. Any distortion or increase in the zone towards the disk of amoxicillin-clavulanate was considered as positive for ESBL production. *Klebsiella pneumoniae* 700603 was used as a control strain for a positive ESBL production and *Escherichia coli* 25922 for negative control for the ESBL production (65).

Multidrug resistant (MDR) was defined as resistance to at least one antibiotic agent in three or more antimicrobial classes (66). Antimicrobial classes tested were Penicillin class (ampicllin, penicillin); Cephalosporin class (ceftazidime, ceftriaxone); Aminoglycosides class (gentamicin); Tetracycline class (Tetracycline); fluorquinolones class (ciprofloxacin); folate pathway inhibitors class (sulfamethmoxazole-trimethoprim); phenicols class (chloramphenicol); macrolides class (erythromycin)

2.9.2.4. Quality control

Burn wound swabs were aseptically obtained using sterile cotton swabs from wound sites after the wound was cleaned by sterile normal saline. Care was taken not to swab health skin surfaces. Following collection from patients, specimens were transported in Stuarts transport media to the microbiology laboratory for processing.

Blood samples: Nurses assisting in blood collection were trained and orientated in the use of BD blood culture vials and butterfly safety lock blood collection kits. Aseptic techniques were insured to avoid contamination of the sample. Gloves were worn and the venupucture site disinfected with 70% ethanol before drawing blood and culture bottles were cleaned with an alcohol swab before dispensing blood.

All stains and reagents were clearly labeled, dated, and stored correctly. The operating temperature of the refrigerator and incubator were monitored and documented. All culture

media were prepared according to the manufacturer's instructions and were tested for performance and sterility. To standardize the inoculums density of bacterial suspension for the susceptibility test, a 0.5 McFarland standard was used and standard reference strain *S. aureus* ATCC-25923, *S. aureus* ATCC 29213 for MRSA, *.E. coli* (ATCC-25922), *K. pneumoniae* ATCC -700603 for ESBL and *P. aeruginosa* (ATCC-27853) were used as control strains.

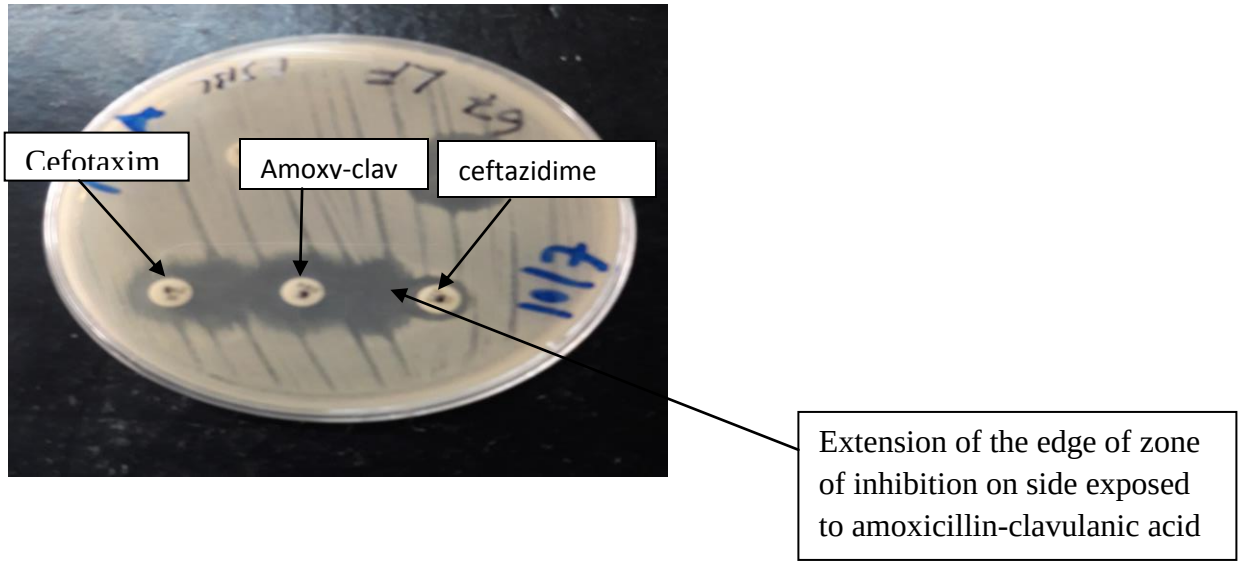


Figure 1 Double disk synergy test (DDST) positive for ESBL production in *Enterobacteriaceae* isolates

2.11. Data Management and Analysis

2.11.1. Data management

Socio-demographic, clinical information and laboratory results of participants were crosschecked and coded before being entered into computer software. Data was edited, cleaned, entered and analyzed using statistical package for social science (SPSS) version 20.0

2.11.2. Statistical analysis

Descriptive analyses such as frequencies and proportions have been used. Binary logistic regression (BLR) was employed to determine the association between independent and dependent variables and odds ratio was used for interpretation. P-value of < 0.05 was considered as statistically significant. BLR was performed on all associated factors with $P \leq 0.2$ to determine independent risk factors on multivariate analysis. The results have been presented in tables and figures.

2.12. Ethical considerations

Ethical clearance was obtained from the Senate Research and Publications Committee of Muhimbili University of Health and Allied Sciences (MUHAS). Letters of permission to conduct the study were also obtained from MNH, Mwananyamala, and Temeke regional hospital administrations. Written informed consent was obtained from each participant or patient's legal guardian prior to enrolment in the study. Confidentiality of the study participants was ensured by using codes instead of patient's names. Participant results were communicated to the attending clinician for appropriate antibiotic treatment to the patient**.** Refusing to participate in this study did not in any way affect the services provided to the patients, especially on management at respective hospital, as participation was voluntary.

2.13. Study limitations

1. Use of antibiotics prior to specimen collection may have affected the rate of the isolation
2. Due to financial and time constraints it was not possible to do the following:
    a. Recruit more patients in order to get a more representative sample size
    b. To do blood culture for all patients who met criteria for BSI
    c. Collect more than one blood specimen for culture in order to distinguish infection from mere contamination
    d. Isolation of anaerobic bacteria
    e. Genotypic tests for confirmation of ESBL and MRSA isolates

2.14. Dissemination of study findings

Following data analysis, a research report was written and submitted to the Department of Microbiology and Immunology, MUHAS which will be submitted to the directorate of postgraduate, MUHAS. Copies of the report will also be submitted to the director of MNH and district medical officers of Temeke and Mwananyamala regional hospitals. The research findings will be been submitted for publication in a respected peer reviewed journal.

CHAPTER THREE

3.0 RESULTS

3.1. Demographic characteristics of patients with burn wound infections

A total of 70 patients with clinical signs of burn wound infections were enrolled in the study from May to July 2017. Social demographic and clinical characteristics of the patients are summarized in Table 1. More than half 39/70 (56%) of the study participants were females with 94 % being paediatric patients. Majority 51/70 (73%) of the patients were from MNH paediatric burn unit. A high proportion 55/70 (79%) of the patients had a percent total body surface area (%TBSA) between 1-20% and was admitted to the respective hospital on the day of thermal injury. From the patients case files, most patients 52/70, (74%) had documentation of antimicrobial exposure prior to specimen collection, and the majority 53/70, (76%) of the patients had their specimen taken within one week of hospitalization.

Table 1 Demographic and clinical characteristics of patients with burn wound infections

Variables	Number	Percentages
Age (yrs)		
≤ 5	61	87
6 - 17	5	7
Adult	4	6
Gender		
Male	31	44
Female	39	56
Admitting hospital/ward		
MNH paediatric burn unit	51	73
MNH- adult ward	3	4
Temeke paediatric ward	6	9
Temeke adult ward	1	1
Mwananyamala paediatric ward	9	13
Time to admission		
Day of injury	53	76
One day after injury	15	21
Few days after (3-5 days)	2	3
Extent of burn (TBSA %)		
1-10%	28	40
11-20%	27	39
21-60%	15	21
Antibiotic use prior to specimen collection		
Yes	52	74
No	18	26
Length of hospital stay prior to specimen collection		
1 week	53	76
2 weeks	10	14
≥ 3 weeks	7	10

3.2. Number of aerobic bacteria isolates from culture

Figure 2 shows the number of bacterial isolates obtained from wound swab culture. Of the wound swabs cultured from 70 patients with burn wound infections, 66/70, (94%) had significant bacterial growth after overnight incubation. Of this, 62% was mixed growth with two or more isolates while 38% had monoisolate.

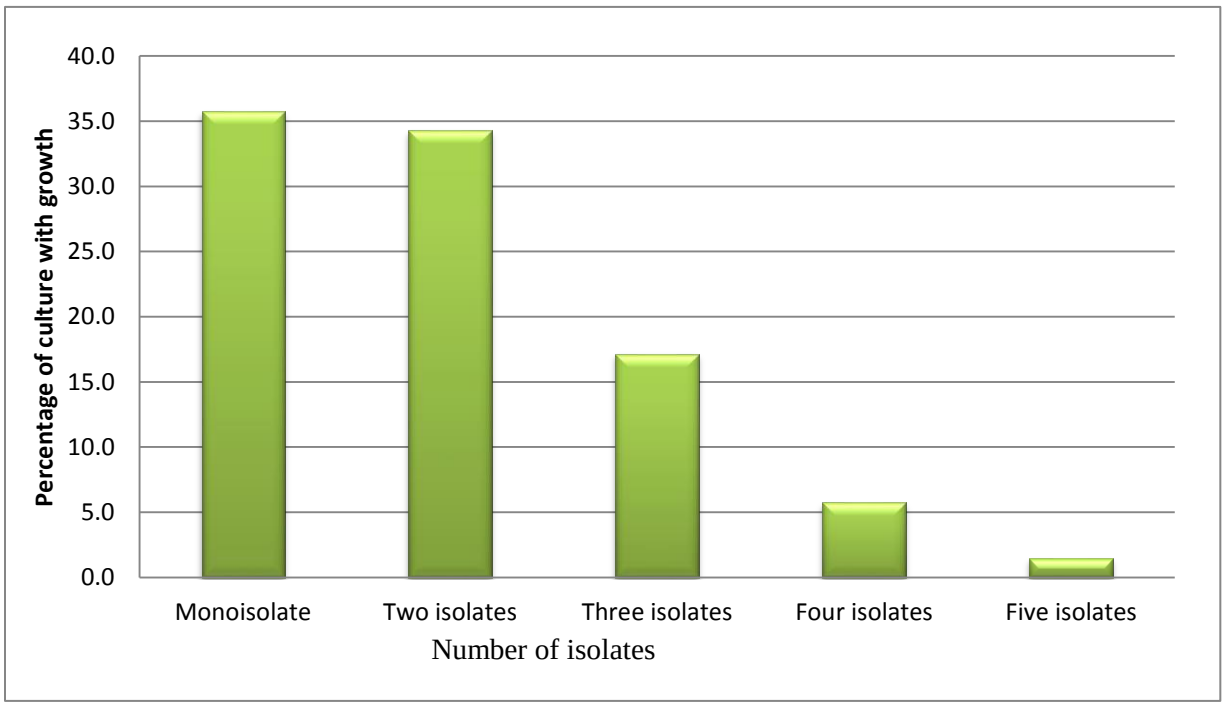


Figure 2: Number of bacteria isolates from culture

3.3. Frequency of isolates from burn wound infections

A total of 131 different aerobic bacteria were isolated from the 66 patients' samples. Gram negative bacteria were more prevalent (67%) compared to gram positive bacteria (30%). The most commonly isolated organism was *P. aeruginosa* 34/131 (26%) followed by *Acinetobacter spp*., 29/131 (22%), CoNS 23/131 (18%), *Klebsiella spp.* 14/131 (11%), and *S. aureus* 13/131, (9.9%) (Figure 2). Although in only a few isolates 4/131 (3%), we were able to isolates *Rahnella aquatilis* with the help of API20E.

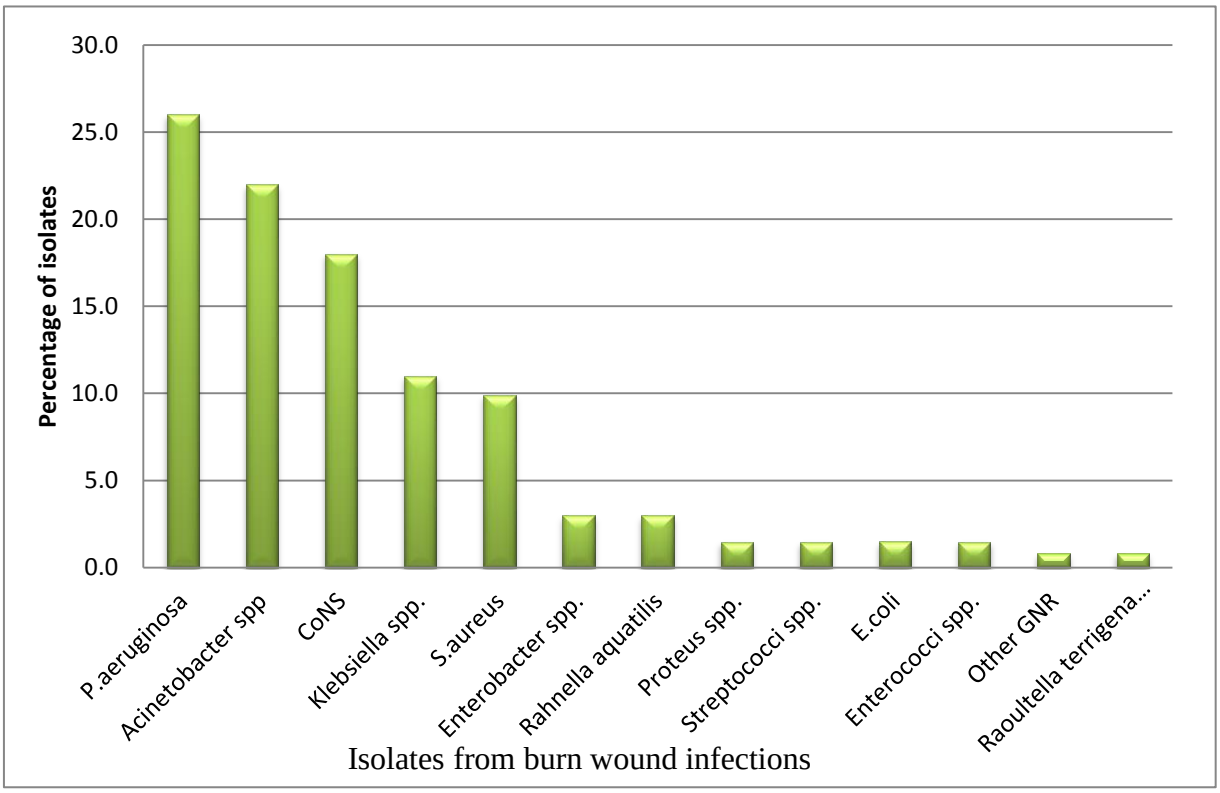


Figure 3: Frequency distribution of aerobic bacteria isolates from burn wound infections

3.5. Distribution of aerobic bacteria isolates in relation to admitting hospital and ward

Table 2 summarizes the frequency of bacterial isolates from the different hospitals**.** Seventy two percent (71%) of all isolates were from MNH paediatric burn unit and 18%, 7.6% and 3.8% were from Mwananyamala, Temeke and MNH adult ward respectively. Common isolates differed between hospitals. Of the isolates from MNH paediatric burn unit, *P. aeruginosa* 27/93 (29%) was the most common isolates. *Acinetobacter spp*. 4/10 (29%) were the most common isolate at Temeke hospital while CoNS 5/23 (22%) were the most common at Mwananyamala general paediatric ward.

Table 2: Distribution of pathogenic bacteria isolates in relation to hospital and ward

Admitting hospital	*Acinetobacter* spp. n (%)	*Klebsiella* spp. n(%)	*P.aeroginosa* n (%)	GNR n (%)	*S.aureus* n (%)	CoNS n (%)	GPC n (%)	Total isolates N	%
MNH paediatric burn unit	21(23)	9(9.7)	27(29)	8(8.6)	9(9.7)	17(18)	2(2.2)	93	71
Temeke hospital	4(40)	1(10)	1(10)	2(20)	1(10)	1(10)	0	10	7.6
Mwananyamala paediatric ward	4(17)	3(13)	4 (17)	2(8.7)	3 (13)	5(22)	2(8.7)	23	18
MNH Adult ward	0	1(20)	2(40)	2(40)	0	0	0	5	3.8
Total isolates	29	14	34	14	13	23	4	131	100

GNR = *Proteus spp., Enterobacter spp., E. coli, R. aquatilis. Raoultella spp.*

GPC = *Streptococci spp., Enterococci spp*.

3.6. Proportion of BSI and frequency of aerobic bacteria isolate from the bloodstream

Of the 70 patients enrolled in the study, blood culture was performed on 40 patients who met criteria. Out of the 40 blood cultures performed 15/40 (37.5%) were positive, resulting in 16 isolates obtained. The most frequently isolate organisms from the blood were CoNS 9/16 (56%) followed by *Acinetobacter spp*. 5/16 (31%) and 2/16 (13%) *Streptococci spp*. Only one isolate each of *S. aureus* and *P. aeroginosa* were obtained (Figure 4). Of the patients with BSI, two who had *Acinetobacter* spp, two with CoNS, and one with *P. aeruginosa*, had phenotypically similar organisms isolated from the burn wounds, resulting in a concordance of 33% (5/15).

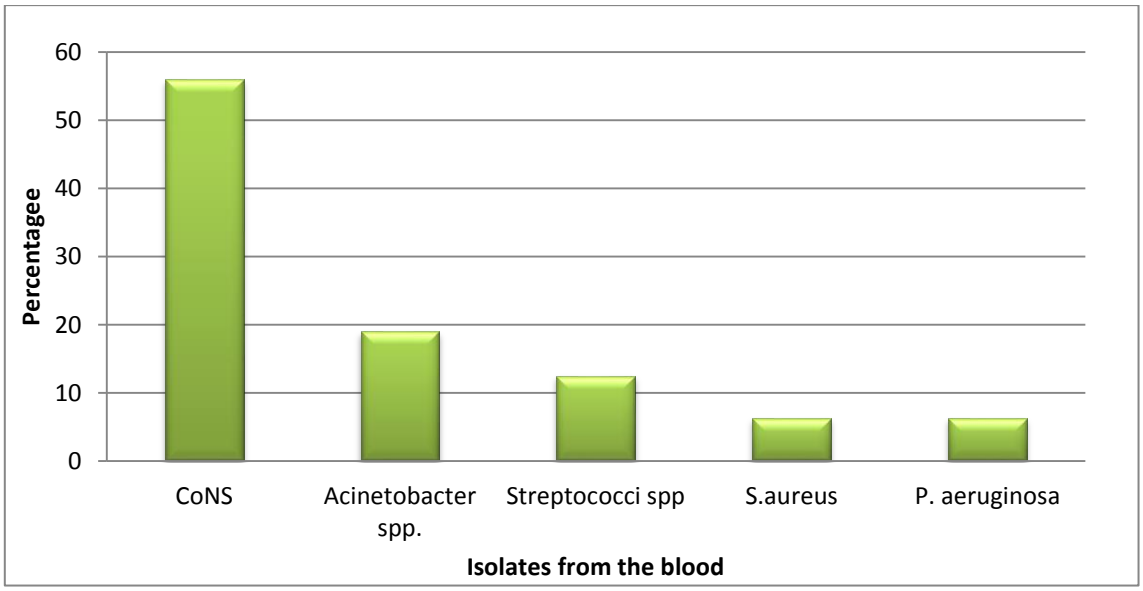
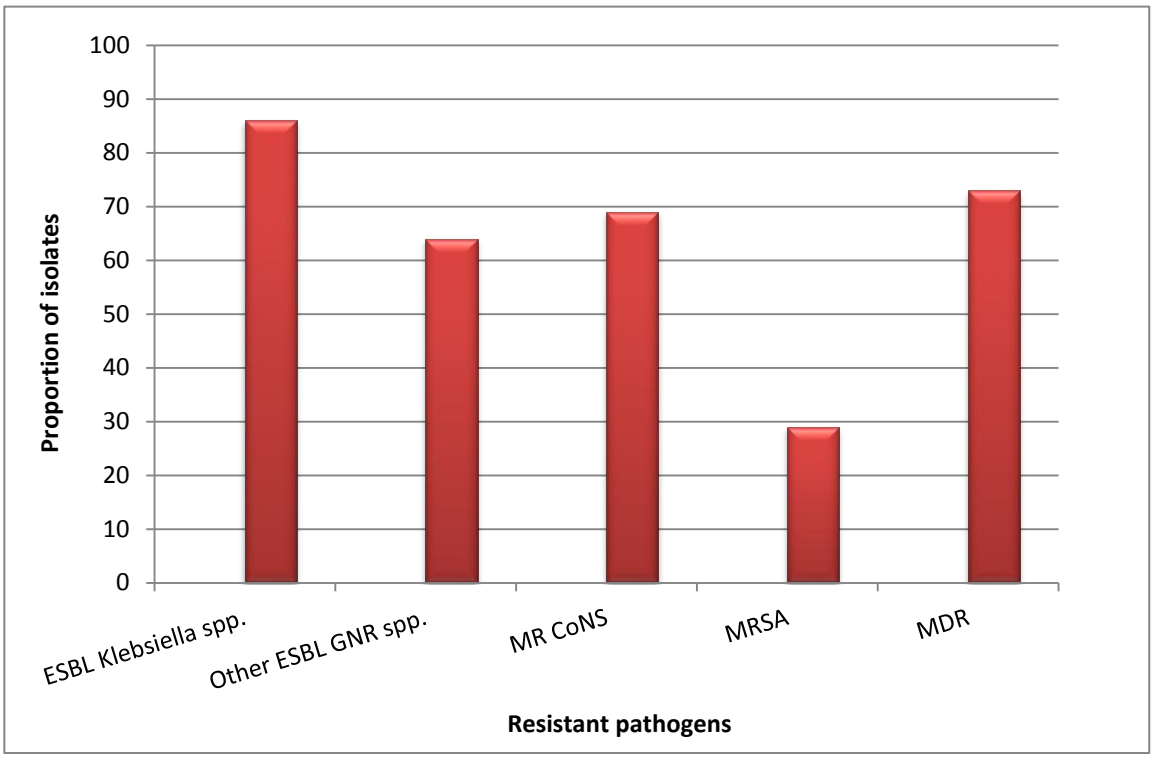


Figure 4: Frequency distribution of aerobic bacteria isolates from BSIs

3.7. Distribution of resistant pathogens among bacteria isolates

Out of the 14 *Klebsiella spp*. isolated from the three hospitals, 12 (86%) were ESBL producers. ESBL producers among other GNR accounted for 9/14 (64%) of the isolates tested. Among the *S. aureus* isolates, 4/14 (29%) were MRSA (one from blood), whereas 22/32 (69%) of CoNS were methicillin resistant including six (6) isolate from blood. Overall Multidrug resistant organism i.e. isolates resistant to at least three classes of antibiotics, made up 107/147 (73.5%) of all isolates from blood and burn wounds (Figure 3).



* Other ESBL GNR spp***;*** *E.coli, Enterobacter spp., Proteus spp., Rahnella aquatili,Roultella spp* *MDR – Multidrug resistant

Figure 5:Distribution of resistant pathogens among burn wound and bloodstream isolate

3.8. Antimicrobial resistance pattern of gram positive bacteria isolates from BWIs

Table 3 summarizes the antibiotic resistance pattern of gram positive isolates. All *S. aureus* isolates tested were sensitive to ciprofloxacin and chloramphenicol while resistance was low towards gentamicin (10%), sulfamethoxazole-trimethoprim (25%) and tetracycline (20%). High rates of resistance (70%) were observed with penicillin and erythromycin. All MRSA isolates were resistant to erythromycin whereas resistance to ciprofloxacin, gentamicin, sulfamethoxazole-trimethoprim and tetracycline was 33% to each antibiotic. All MRSA isolates were sensitive to chloramphenicol but resistant to erythromycin and gentamicin. Among the CoNS, resistance to chloramphenicol and tetracycline was low (20%) while high resistance was observed with cefoxitin (70%), penicillin ans sulfamethoxazole-trimethoprim

(74%). Other gram positive isolates (*Streptococci spp*. and *Enterococci spp*.) were highly resistant to erythromycin and tetracycline (67%), low resistance was seen with ampicillin and ceftriaxone (33%) and all isolates were sensitive to chloramphenicol, penicillin and vancomycin.

Table 3:Antibiotic resistance pattern of gram positive bacteria isolates from burn wound

ANTIBIOTICS	*S. aureus* n = 10 (%)	MRSA n = 3 (%)	CoNS n= 23(%)	Other GPC n= 3(%)
Ampicillin	-	-	-	1 (33)
Ceftriaxone	-	-	-	1 (33)
Cefoxitin	-	-	16 (70)	-
Ciprofloxacin	0 *	1 (33)	4* (31)	-
Chloramphenicol	0	0	5 (22)	0
Erythromycin	7 (70)	3 (100)	14 (61)	2 (67)
Gentamicin	1 (10)	3 (100)	14 (61)	-
Penicillin	7 (70)	-	17 (74)	0
Sulfamethoxazole trimethoprim	2 (25) *	1(33)	17 (74)	-
Tetracycline	2 (20)	1 (33)	5 (22)	2 (67)
Vancomycin	-	-	-	0

* Not all isolates tested with antibiotic

3.9. Resistance pattern of gram negative bacteria isolates

Table 4 shows antibiotic resistance pattern of gram negative isolates. All *Enterobacteriaceae* isolates showed high resistance towards most of the antibiotics tested. Overall, among the *Klebsiella* isolates, we observed a high resistance rates ranging from 57% for ciprofloxacin to as high as 100% for ampicillin and ceftazidime. Seventy nine percent (79%) of the isolates were sensitive to chloramphenicol. Other Gram negative isolates, (*E.coli, Enterobacter spp*., *Proteus spp*., *R. aquatilis*), also showed high rates of resistance (57 – 86%) to the tested

antimicrobials and only half (50%) were sensitive to chloramphenicol. In this study, *P. aeroginosa* isolates showed high resistance rate (74%) to aztreonam, ciprofloxacin and imipenem. Sixty two percent (62%) were resistant to ceftazidime and gentamicin. *Acinetobacter spp*. exhibited high resistance rates (55% – 86%) to most of the antibiotics tested, but were highly sensitive to imipenem (90%).

Table 4: Antibiotic resistance pattern of gram negative bacteria isolates from BWIs

ANTIBIOTIC	*Acinetobacter spp.* n = 29 (%)	*P. aeroginosa* n = 34 (%)	*Klebsiella spp.* n = 14 (%)	*Other GNR* n = 14 (%)
Amoxicillin-clavulanate	-	-	13 (93)	11 (76)
Ampicillin	-	-	14 (100)	12 (86)
Aztreonam	25 (86)	25 (74)	-	-
Chloramphenicol	-	-	3 (21)	7 (50)
Ceftazidime	20 (69)	21 (62)	14 (100)	8 (57)
Ciprofloxacin	17 (59)	25 (74)	8 (57)	9 (64)
Ceftriaxone	20 (69)	-	13 (93)	10 (71)
Imipenem	3 (10)	25 (74)	-	-
Gentamicin	16 (55)	21 (62)	12 (86)	10 (71)
Sulfamethoxazole-trimethoprim	21 (72)	-	12 (86)	9 (64)
Tetracycline	19 (66)	-	-	-

Other GNR - *E.coli, Enterobacter spp., Proteus spp.. Rahnella aquatilis, Raoultella spp.*

3.10. Antibiotic resistance pattern of ESBL producing *Enterobacteriaceae*

Antibiotic resistance pattern of ESBL producing *Enterobacteriaceae* isolates are summarized in table 5. Majority of ESBL producers were highly resistant (67-92%) to all tested antimicrobials. Higher percentages (75 %) of ESBL producing Klebsiella spp were sensitive to chloramphenicol compared to only 33 % of other ESBL GNR.

Table 5 Antibiotic resistance pattern of ESBL producing *Enterobacteriaceae*

ANTIBIOTIC	ESBL *Klebsiella spp*. n =12 (%)	Other ESBL GNR n = 9 (%)
Amoxicillin-clavulanate	92	89
Chloramphenicol	25	67
Ciprofloxacin	67	89
Gentamicin	92	89
Sulphamethoxazole-trimethoprim	92	89

3.11. Resistance pattern of bacteria isolates from the bloodstream

Table 6 summarizes the resistance pattern of bacterial isolates from blood stream. *Acinetobacter* spp were highly resistant to all antibiotics tested. The only *P. aeroginosa* isolate was resistant to all the tested antibiotics and only sensitive to imipenem. Among the CoNS, resistance to penicillin was high (89%) and all were resistant to sulfamethoxazole-trimethoprim. Seventy eight percent (78%) were sensitive to tetracycline. The only *S. aureus* isolated turned out to be MRSA and was sensitive to chloramphenicol and tetracycline. The two *Streptococci spp*. were resistant to ampicillin. Erythromycin, tetracycline and sulfamethoxazole-trimethoprim and only one isolate was resistant to the other tested antibiotics.

Table 6. Antibiotic resistance pattern of organisms isolated from the blood stream

ANTIBIOTIC	*Acinetobacter* spp. n = 3 (%)	*P. aeruginosa* n = 1 (%)	*S. aureus* n=1(%)	CoNS n = 9 (%)	*Streptococci* spp. n = 2 (%)
Ampicillin	-	-	-	-	100
Erythromycin	-	-	100	44	100
Cefoxitin	-	-	-	56	-
Ceftazidime	67	100	-	-	-
Ciprofloxacin	67	100	-	-	-
Chloramphenicol	-	-	0	60*	50
Gentamicin	67	100	100	44	50
Imipenem	100	0	-	-	-
Penicillin	-	-	100	89	-
Sulfamethoxazole trimethoprim	-	-	100	100*	100
Tetracycline	67	-	0	22	100
Vancomycin	-	-	-	-	50

* Not all isolates were tested

3.12. Factors associated with harbouring ESBL and MR organisms burn wound infections

Table 7 shows patients' characteristics associated with harbouring ESBL producing gram negative *Enterobacteriaceae* isolates. With regards to age, children ≤ 5 yrs old were 24 times more likely to harbour ESBL producing organisms compared to adults (OR 24 & 95% CI: 0.87-684.47). Males had nearly twice the risk (OR 1.7 & 95% CI: 0.32 - 8.74) to have infection due to ESBL organism when compared to females. Patients whose specimens were collected in week two of hospital stay, were three times as likely to harbour ESBL organisms as those in week 3. Patients, who had larger burn surface area, also had higher chances of

getting infection due to ESBL producing gram-negative organisms. Those who used antibiotics were at increased risk of infections due to ESBL organisms than those who did not, despite more ESBL producers being isolated from those with antibiotics use. None of the factors yielded statistically significant P values.

Table 7: Univariate and multivariate analysis of patient factors associated with harbouring ESBL producing *Enterobacteriaceae* isolates

Patient characteristics	N	ESBL *Enterobacteriaceae* positive patients			
		Univariate		Multivariate	
		P-value	OR (95% CI)	P-value	OR (95% CI)
Age (yrs)					
≤ 5	21	0.137	7 (0.54-91.11)	0.061	24 (0.87-684.47)
Adults	1	1	1	1	1
Gender					
Male	11	0.546	1.7 (0.32-8.74)		
Female	11	1	1		
Length of hospital stay at isolation					
Week 1	13	0.592	1.1 (0.08-14.41)		
Week 2	7	0.441	3.5 (0.14 – 84.69)		
> Week 3	2	0.595	1		
Extent of burn (%TBSA)					
1-10%	9	0.557	1		
11-20%	6	0.322	3.3 (0.31 - 36.11)		
21-60%	7	0.496	1.9 (0.29 – 13.19)		
Antibiotic use					
Yes	19	0.935	0.9 (0.08-10.21)		
No	3		1		

Table 8 summarizes patients factors associated with isolation of methicillin resistant *Staphylococci* species from burn wounds and blood stream. Children below five yrs of age were at higher risk of infection by methicillin resistant (MR) organisms (MRSA and MR CoNS) than their older counterpart, and no adult patient had *Staphylococci* spp isolated from their wounds or blood stream. Males were more likely to be infected by MR *Staphylococci* spp as compared to females. Length of hospital stay at isolation had no significant association with MR *Staphylococci* spp while those with larger burn surface area had higher chances of being inhabited by MR *Staphylococci*. Those who used antibiotics had more than twice higher chances of having infections due to MR *Staphylococci* spp than those without history of antibiotic use. However, all the factors assessed had p- values > 0.05.

Table 8: Univariate and multivariate analysis of patient factors associated with harbouring methicillin resistant organisms

Patient characteristics	n	Methicillin resistance positive patients			
		Univariate		Multivariate	
		P value	OR (95%CI)	P value	OR(95%CI)
Age (yrs)					
≤ 5	23	0.691	1.6 (0.14 – 19.65)		
> 5	2	1	1		
Gender					
Male	13	0.382	1.7 (0.51-5.79)		
Female	12		1		
Length of hospital stay at isolation					
Week 1	20	0.683	1		
Week 2	3	0.382	0.35 (0.03 – 3.67)		
≥Week 3	2	0.999	0.00 (0.00)		
Extent of burn (%TBSA)					
1-10%	10	0.272	0.3 (0.05 – 2.37)		
11-20%	12	0.775	1.3 (0.22 – 7.87)		
21-60%	3	0.158	1		
Antibiotic use					
Yes	10	0.157	2.7(0.69-10.36)	0.322	2.1 (0.47-9.83)
No	14		1		1

3.13. Outcome of patients with burn wound and blood stream infections

At the end of the study period, majority (70%) of the patients had survived and been discharged from the hospitals and 27 % were still receiving care. Patients who were still admitted by the time study ended were all from MNH paediatric burn unit. Two paediatric patients died, one from MNH and the other from Temeke hospital. Cause of the death was registered as septicaemia.

CHAPTER FOUR

4.0 DISCUSSION

In this study, 131 bacteria isolates from burn wound infection were investigated to determine their identity and antimicrobial susceptibility pattern. Overall, we demonstrate the predominance of gram-negative organism in burn wound infections with *P. aeruginosa* as the most common bacteria isolated. This finding is similar to other studies done in Egypt and other countries (18,67,68) that reported *P. aeruginosa* as the commonest isolate in BWI. *Acinetobacter spp* was the second most frequent organism followed by Coagulase negative *staphylococci* spp., *Klebsiella spp*., *S. aureus* and other gram-negative rods. These bacteria have been reported in most studies in the spectrum of nosocomial infections in burns, albeit with a difference in their order of frequency (4,27,62,69,70). However, *S. aureus* was the most common isolate from similar studies done in Mwanza, Tanzania, where surface swabs were collected on admission and day 10, and in Egypt where surface swabs were collected on admission and the 5^{th} day (18,27). In the current study, swabs sample was collected only once from burn wounds that showed signs of infection (foul smell, discharge, itch etc). The difference in the timing of specimen collection could explain the variation in the most common isolate reported. Additionally, *S. aureus* was also the most common isolate in a similar study in Ethiopia, which had similarities in surface swab collection as our study(35). The variation between our study and the one done in Ethiopia could be explained by the difference in the age of the study population which ranged from 7 to 50 yrs where as in this study, the population was predominantly under 5 yrs. Children under 5 years of age are very mobile and prone to hospital acquired infections due to increased contact with hospital surfaces which might be contaminated with pathogens such as *P. aeruginosa*. We also report the isolation of *R. aquatilis* among our isolates. Although low in frequency, this is an import organism to report as it is rarely isolated in human specimens and is usually isolated from immune compromised patients(71–73). Therefore, it is plausible that it has been isolated from burn patients who are considered to have generalized immune suppression due to thermal injury (4).

Our study has shown that there are some similarities but also some differences between the main causes of infection in our unit and other local wards. *P. aeruginosa* was the most prevalent isolate from burn wounds in the specialized paediatric burn unit at MNH. Similar findings have been reported in a specialized paediatric burn unit in Turkey (70). Silver sulfadiazine, which is bactericidal against GNR and *P. aeruginosa* (2), is used as the topical agent for burn wound dressing in this unit. Therefore, it is of concern that despite its use, *P. aeruginosa* is still predominant. Although only a small number of samples were obtained from Temeke general paediatric and adult wards, *Acinetobacter spp* were most common isolates while CoNS were common at Mwananyamala general paediatric ward. The difference between the three hospitals reported here is similar to findings in a study done in China that compared frequency of isolates from specialized burn unit to a general ward (24).

With regard to BSI, the proportion of patients with BWI who has positive blood culture results was 37.5%. This proportion is lower than 62.5% and 56% reported by a similar study in South Africa and Turkey respectively (4,74) which included both paediatric and adult patients. The lower proportion could due to the small sample size in our study. The most common organisms isolated from blood culture positive specimens were CoNS, which is in line with finding from previous study done at the same tertiary hospital and from other countries (75). Although CoNS should be considered as an important pathogen for sepsis in burns due to it being ubiquitous in hospital environments, and burn wounds being the ideal medium for its multiplication (40), it is difficult to say whether the CoNS were true cause of BSI or contaminants, since only one blood specimen was collected from each patient. Other isolates included *Acinetobacter, P. aeroginosa, S aureus* (MRSA), which have been shown to cause septicaemia in burn patients (4,8,10,67). It is important to note that there was 33% concordance in patients with *Acinetobacter* and *P. aeroginosa*, and CoNS in BSI since phenotypically similar organisms were also isolated from the burn wound. This finding concurs with studies done in Brazil by De Macedo *et al*, where burn wounds were found to be the source of organisms isolated from the blood (40,76). The low level of concordance in our study can be explained by the small sample size studied.

In this study, *S. aureus* isolates from BWI showed high resistance to penicillin, results similar to those reported by others (15,23). However, these studies reported relatively low resistance to erythromycin as compared to our findings. The increase in resistance could be due to widespread use of erythromycin in staphylococcal infections, resulting in high occurrence of mechanisms responsible for resistance to this drug. Relatively low resistance was observed with sulfamethoxazole-trimethoprim, gentamicin and ciprofloxacin similar to finding from other studies on *S. aureus* isolates at the same hospital (41,57). Chloramphenicol was the most effective antibiotic tested against *S. aureus*.

In our investigation, 30% of *S. aureus* from BWI were MRSA. This finding was slightly lower than that reported in South Africa (6). This could be due to the small number of isolates we had and also short hospital stay of the patients before isolation. All MRSA isolates were sensitive to chloramphenicol, which could be an alternative treatment option. Of importance is the high resistance rate of CoNS to cefoxitin. This could imply an increase in nosocomial infections due to methicillin resistant CoNS, as CoNS are very common and highly pathogenic in burns due to immune suppression following thermal injury. Similar results have been reported in Switzerland (51).

Gram-negative isolates from BWI also showed high resistance towards commonly prescribed antibiotics such as ampicillin, amoxicillin-clavulanate, gentamicin and sulfamethoxazole-trimethoprim, signifying MDR nature of these isolates, although this requires confirmation. These finding are in line with those by Manyahi *et al* and Mawalla *et al* and others researchers on similar isolates (57,58,77). Additionally, 73% of the gram negative organisms were MDR, which was slightly higher than the 61% reported by other studies done at MNH on similar isolates (57). The high rate of resistance observed in this study could be attributed to the fact that, these antibiotics are easy to administer, relatively inexpensive and widely prescribed in empirical treatment of various bacterial infections in our settings. The majority of the *Enterobacteriaceae* isolates also showed very high to resistance to third generation cephalosporins, findings similar to those by Moyo *et al* at MNH (41) . This could be explained by the presence of ESBL enzymes in the majority of these isolates.

ESBL production was higher in *Klebsiella* isolates (86%) than in other enteric gram negative rods (64%). In general, ESBL producing strains displayed high rates of resistance to most antibiotics tested in this study, findings that concur with those by Mshana *et al* on isolates from different clinical specimens in Mwanza, Tanzania (59). Additionally, 73% of all the isolates from both burn wound and blood stream infection were MDR, a finding similar to studies done at MNH on similar isolates (57).The high resistance can be attributed to the widespread and indiscriminate use of these broad-spectrum antibiotics. This poses a challenge for clinicians in management of these infections as they may require more expensive drugs such as carbapenems (59), which are not readily available in most hospitals in our settings.

Similarly, *P. aeruginosa* isolates showed very high resistance towards most of the tested antibiotics including third generation cephalosporins, ciprofloxacin and aztreonam. Of note is the relatively high resistance to the carbapenem (imipenem) (74%), findings similar to other countries (69). This could indicate the carbapenemase producing *Pseudomonas* isolates causing these infections although this needs to be confirmed. These findings are in contrast to those reported at MNH where imipenem was the antibiotic of choice for treatment of pseudomonal infections (57). The increasing use of imipenem in the treatment of infections by MDR-GNR can be the cause of increasing development of resistance among *P. aeruginosa* isolates. All *Acinetobacter* isolates from BWI were highly resistant to most of antimicrobial agents tested but sensitive to imipenem (90%), a finding that concur with previous data from the study done at the same hospital (57). With the extensive use and/or abuse of antimicrobial drugs, *Acinetobacter* has become one of the major bacteria causing hospital-acquired infections (24).

With regard to antibiotic resistance among isolates from the bloodstream, the only *S. aureus* isolate was MRSA and was resistant to all drugs tested except chloramphenicol and tetracycline. CoNS were highly resistant to cefoxitin and penicillin and all *Streptococci* isolates were resistant to ampicillin. Acinetobacter isolates showed high resistance to the drugs tested and all were resistant to imipenem, findings that concur with those reported by a similar study in South Africa, where Acinetobacter isolates were found to be highly resistant to most

of the drug tested including carbapenems (4). The only pseudomonas isolates was sensitive to imipenem but resistant to all other antibiotics tested.

We also investigated associated factors such as age, gender, %TBSA, hospital stay and broad spectrum antibiotic usage in relation to acquisition of resistant organisms (ESBL producers and MR *Staphylococi* spp) in the burn wounds and blood stream. However, none of these factors were found to be associated with isolation of ESBL and MRSA as they all yielded P values > 0.05. The majority of the patients enrolled in this study survived and were discharged from the hospital, with only a small proportion (27%) remaining in hospital by the time the study ended. Two of the paediatric patients died due to septicaemia. However, no pathogens where detected from these two patients at the time of blood culture. Moreover, these two patients had larger %TBSA of 20% and 45% indicating that they were at high risk of acquiring nosocomial infection by MDR gram negative organisms and even fungi or anaerobic infection (48,69). Both patients had *P. aeruginosa*, and *Klebsiella spp* isolated from their burn wounds, and therefore, it is possible that blood stream infection occurred later on in their hospital stay as is very common with gram negative septicaemia and candidaemia (69).

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

Gram negative organisms predominated, with *P. aeruginosa* being the most common isolates in BWIs while CoNS were common in BSIs. We found most of the isolates had multiple resistant to commonly prescribed antimicrobial agents including Ampicillin, Amoxicillin-clavulanate, ceftriaxone, ciprofloxacin, sulfamethoxazole-trimethorpim, penicillin and erythromycin. Furthermore, we found high number of BWI due to ESBLs producing *Klebsiella spp* and other enteric gram negative rods, methicillin resistant *Staphylococci spp* as well as MDR organisms. In vitro, chloramphenicol was found to be the most effective antibiotic towards gram positive organisms and *Klebsiella*, while imipenem was effective against *Acinetobacter*.

5.2 Recommendations

From our study findings, we recommended the following:

- Culture and antimicrobial susceptibility testing should be performed routinely, including MRSA and ESBL screening, whenever burn wound and blood stream infections are suspected. Antimicrobial sensitivity test results should be used to guide choice of antibiotics.
- To limit the use of beta lactam antibiotics and third generation cephalosporins in treatment of BWI
- Chloramphenicol should be considered as a suitable treatment option
- To conduct larger study which will also investigate the role of anaerobes in causing infections in patients with thermal injury
- To conduct molecular studies on the isolates to determine their resistance genes and mode of spread
- To establish regular surveillance of bacteria isolates from the burn unit in order to monitor their antibiotic susceptibility pattern.

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APPENDICES

Appendix I: Informed consent form

Title: Bacterial causes and antibiotic susceptibility patterns of aerobic isolates from burn wound infections in Dar es Salaam, Tanzania.

Identification Number_____

Greetings! My name is .....I am a postgraduate student at MUHAS, doing a research on bacterial causes of burn wound infections, blood stream infections and antimicrobial susceptibility patterns of aerobic isolates from burn patients at Muhimbili national hospital, Mwananyamala and Temeke regional hospitals. This research is being done to acquire knowledge of bacteria that causes infections in burn patients and how to treat them.

Purpose of the study: To determine the bacterial causes of BWIs and antimicrobial susceptibility patterns of aerobic isolates at tertiary and regional hospitals in Dar es Salaam, Tanzania. In addition, I will also determine factors that predispose burn patients to infections and their outcomes.

Participants and methods

If you agree to join the study, you will be interviewed using questionnaire, detailed information on social demographic characteristics, past and present medical history will be requested. A pus swab will be taken from the site of the burn wound and blood will be drawn to detect the presence or absence of infection.

Confidentiality

Identification number will be used on all collected information instead of names. The principle investigator, research assistance, supervisor and MUHAS at large are obliged to maintain confidentiality of all data or information collected from you. No unauthorized persons will have access to the data collected.

Benefits and Potential Risks

If you agree to take part in this study, the benefits may be direct or indirect. You will benefit by knowing the result of culture and sensitivity pattern of a collected specimen, and whenever there are culture positive results, appropriate medications will be given according to hospital guidelines. This information will also be helpful to others as the hospital will also know what kind of bacteria and common in their wards, and what type of antibiotics they are susceptible to, allowing them to use the effective drugs empirically. No harm is expected to happen to you because of your participation in this study. Sometimes, minimal pain may occur when taking swabs and drawing blood.

Rights to Withdraw and Alternatives

Participation in this study is completely optional. You can decide to participate or not and you are allowed to stop participating in this study at any stage, even if you have already given your consent without any penalty or loss of any benefits entitled to you.

Who to Contact

For any inquiries on this study, please contact;

The Principal Investigator: Fatima Kabanangi,

Muhimbili University of Health and Allied Sciences, MUHAS, P. O. Box 65001,

Mobile: + 255 764 559 999 Dar es Salaam, OR

The Chairperson of the Senate Research and Publications Committee, Muhimbili University of Health and Allied Sciences, MUHAS P. O. Box 65001,

Telephone: +255 22 2152489, Dar es Salaam.

Do you agree? : Participant agrees ……..Signature…… Date……

Participant does NOT agree ……...

Kiambatanishi No. 1

Fomu ya ridhaa

Namba ya utambulisho:.....

Ridhaa ya kushiriki katika utafiti juu ya maambukizi ya vimelea vya vidonda vya kuungua katika hospitali ya taifa ya Muhimbili na hospitali za rufaa za Mwanayamala na Temeke.

Salamu! Jina langu ni ..... mwanafunzi wa uzamili katika Chuo kikuu cha Afya na Sayansi Shirikishi Muhimbili (MUHAS). Nafanya utafiti juu ya maambukizi ya vimelea vya vidonda vya kuungua katika hospitali ya taifa ya Muhimbili na hospitali za rufaa za Mwananyamala na Temeke. Utafiti huu ni muhimu kwa kuwa tutafahamu wadudu wanaosababisha ugonjwa kwa wagonjwa wenye vidonda vya kuungua na jinsi yakutibu

Madhumuni ya utafiti

Kujua aina ya bakteria wanaosababisha maambukizi katika vidonda vya kuungua na maambukizi yake katika damu. Pia kufahamu aina ya dawa inayoweza kutibu.

Jinsi ya kushiriki

Ikiwa utakubali kushiriki, nitakuuliza maswali machache kuhusu matatizo haya na nitaomba kutoa sampuli ya usaha na damu kwa ajili ya uchunguzi zaidi.

Usiri

Itatumika namba ya utambulisho tu badala ya jina katika kukusanya taarifa zinazotakiwa. Mtafiti mkuu, msaidizi , msimamizii na MUHAS kiujumla,itawalazimu kutunza na kuhifadhi taarifa zote zilizochukuliwa/kukusanywa toka kwa mgonjwa. Hairuhusiwi kwa mtu wa aina yoyote kupata taarifa zilizokusanywa.

Faida na athari

Ukikubali kushiriki sehemu ya utafiti huu, faida zake zinaweza onekana kwako au kwa mwingine. Ushiriki wako utakufaya ufahamu aina ya wadudu wanaosababisha madhara na matibabu yake.Taarifa hizi zitawanufaisha na wengine hasa hospitali zenye wodi ya wagonjwa

walioungua. Watajifunza aina ya wadudu wanaopatikana sana katika wodi, watafahamuaina ya dawa inayotibu. Hii itaruhusu kutumia madawa yaliyobora na yenye viwango. Hakuna madhara au athari inayoweza kutokea kwako kwa sababu ya ushiriki kwako katika utafiti huu. Unaweza pata maumivu kidogo wakati wakuchukua sampuli ya damu na usaha.

Uhuru wa kushiriki

Kushiriki katika utafiti huu ni hiari yako. Kama utachagua kutokushiriki katika utafiti utaendelea kupokea huduma zote kama kawaida kutoka hospitali hii. Unaweza kuacha kushiriki katika utafiti huu wakati wowote, hata kama baada ya kutoa idhini yako.

Taarifa/Mawasiliano

Endapo utahitaji kupata maelezo zaidi au taarifa yeyote kuhusu utafiti huu,wasiliana na Fatima Kabanangi, simu namba 0764 559 999, Chuo Kikuu cha Afya na Sayansi (MUHAS) S.L.P 65001, Dar es Salaam. Kama utakuwa na maswali kuhusu haki yako kama mshiriki, unaweza wasiliana na Mwenyekiti wa Utafiti, S.L.P 65,001, Dar es Salaam. Tel: +255 22 2152489 Dar es Salaam.

Uthibitisho

Je, unakubali kushiriki kwenye utafiti? Ndiyo..... Hapana…….

Mimi _____ nimesoma na nimeelewa yaliyomo katika fomu hii. Maswali yangu yamejibwa na nimekubali kushiriki kwa ridhaa yangu.

Sahihi ya mshiriki/mlezi _____

Sahihi ya ushahidi (kama mama / mlezi hajui kusoma) _____

Sahihi ya mtafiti _____

Tarehe ya ridhaa _____

Appendix II: Questionnaire (English version)

Title: Bacterial causes and antibiotic susceptibility patterns of aerobic isolates from burn wound infections in Dar es Salaam, Tanzania.

Name of the facility----------Participant Identification number--------

Participant study number----------

Name of interviewer----------

A. Participants Socio-Demographic information

1. Name of the participant----------
2. Current physical address…….
3. Mobile number…….
4. Age in years----------
5. Sex: 1. Female 2. Male
6. Educational level (circle one) a) non formal b) Primary c) Secondary d) College and University

B. Participants Clinical information

1. Date of admission ……
2. Date of thermal injury …….
3. Cause of burn trauma (circle one); a. Scald b. Flame c. electricity d. chemical agents e. others (specify) ……..
4. Place of injury (circle one): a. home b. factory c. others (specify)
5. Anatomic location of the burn (circle as many): a. extremities b. trunk c. head & neck d. perineum
6. Depth of the burn wound (circle one): a. full thickness b. partial thickness
7. Extent or size of burn (% of total burn surface area)

8. Duration of time before getting medical attention after burn injury: Immediately after injury? Yes /No If No, how long after injury? ……..
9. History of fever a) Yes b) No Temperature ..... ^{0}C (At the time of blood collection/ measured the same day)
10. Signs of infection in the burn wound a) Yes b) No
11. Antibiotic use prior to or after admission Yes/No If yes, what type? .....
12. Any known immunodeficiency disease? E.g. HIV, diabetes Yes/No If yes, Which disease?.....
13. Date and type of operative procedures ……
14. Length of hospital stay …….
15. Survival Yes/No

Kiambatanisho No. 2

Dodoso/Maudhui

Utafiti juu ya maambukizi ya vimelea vya vidonda vya kuungua katika hospitali ya taifa ya Muhimbili na hospitali za rufaa za Mwanayamala, Temeke na Amana.

Jina la kituo.....namba ya utambulisho ya mshiriki.....

Jina la msaili.....

A. **Taarifa ya washiriki**
    a. Jina la mshiriki.....
    b. Anuani kamili.....
    c. Namba ya Simu.....
    d. Umri.....
    e. Jinsia 1. Mke. 2. Mme.....
    f. Elimu (zungushia moja) a) shule ya msingi b) secondary c) elimu ya juu d) hajawai kupata elimu

B. **Taarifa ya mgonjwa/mshiriki**
    a. Tarehe ya kulazwa.....
    b. Tarehe yakuungua ……..
    c. Chanzo cha kuungua,(zungushia moja); (a) moto (b) umeme (c) chemikali (d) mengineyo eleza.....
    d. Eneo la ajali (zungushia moja) (a) nyumbani (b) Kiwandani, (c) mengine eleza.....
    e. Sehemu gani ya mwili imeungua (zungushia moja) a) mikono/miguu b) mwilini c) shingo na kichua d) makalio
    f. Ukubwa wa kidonda kilichoungua (% jumla ya eneo lililoungua)
    g. Ilichukua mda gani kupata huduma ya matibabu? Baada ya kuingia jeraha, muda tu baada ya ajali?
        i. Ndio/hapana. Kama hapana ilichukua mda gani baada ya kuumia?

h. Dawa ya antibiotiki alipewa kabla au baada ya kulazwa?

    i. Ndio/hapana. Kama ndiyo ni aina gani ya dawa alipewa

i. .Historia ya homa a) ndiyo b) hapana Joto la mwili (temprecha) …….. ºC (muda wa kuchukuwa damu / ipimwe siku hiyo hiyo)

j. Dalili za maambukizi (infection) kwenye kidonda (kama muwasho, usaha, maumivu, uvimbe, kidonda kutoa harufu, n.k)

k. Ugonjwa wowote unao athiri kinga ya mwili kwamfano (kisukari au Ukimwi) Ndio/Hapana. Kama ndio taja ugonjwa ……

l. Tarehe na aina ya upasuaji uliofanyika

m. Mda aliokaa hospitalini

n. Manusura: ndio/hapana

C. LABORATORY RESULTS

1. Organisms isolated

(1) ……..

2) ……..

3) ……

2. Sensitivity pattern of isolated organisms

Antibiotic					
Diameter					
Interpretation					