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Risk Factors and Antimicrobial Resistance of Pathogens Isolated from Burn Units at Local Hospitals in Gaza Strip, Palestine

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BY

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Dedication

This thesis is dedicated to my father, my mother, my wife, and my sons (Ahmed, Mohammad and Osama) who supported me all the way of my study to pursue my ambitions and my goals.

Finally, this thesis is dedicated to all those who believe in the richness of learning.

Declaration

“I herby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another nor material which to a substantial extent has been accepted for the award of any other higher degree or diploma of the university or other institute of higher learning, except where due acknowledgement is made in the text"

Name	**Signature**	**Date**
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Abstract

Background: Nosocomial infection is a recognized public health problem world-wide with an important cause of burn mortality. It has been estimated that 75% of all deaths following burns are related to infection. The emergence of resistance to antimicrobial agents is a major clinical and public health problem particularly in pathogens causing nosocomial infections.

Aim: The present cross sectional study was undertaken to determine burn infection bacterial etiological agents and their antimicrobial resistance pattern, the influence of environmental conditions, and risk factors associated with burn infections.

Method: Wound swabs were collected from 118 burn patients from two burn units (Al-Shifa and Nasser hospitals) from October 2010 to March 2011. Different environmental samples, health care workers (HCWs) samples (nasal, throat, fingers) and air samples for culture were investigated to determine possible infection source(s). Isolated bacteria from the samples were identified by conventional biochemical methods and API 20E system. Moreover, antimicrobial susceptibility testing was performed. Statistical analysis was performed using SPSS version 13.0 program.

Results and Conclusions: The overall percentage of positive cultures from burn patients samples in both hospitals was 45.8%, *Pseudomonas aeruginosa* was the most common pathogen isolated (50%) followed by *Enterobacter cloacae* (27.8%), Coagulase-negative staphylococci (CoNS) (9.3%), and *Escherichia coli* (5.6%). Meanwhile, fingers and nasal samples that collected from HCWs showed 78.6% and 32.3% positive cultures respectively, where *P. aeruginosa* was the highest pathogen isolated (32.3%), followed by CoNS (29%). Environmental and air samples also showed higher isolation rate of *Pseudomonas* and CoNS. *P. aeruginosa* was resistant to most antimicrobial agents tested in this study. All *Enterobacter* spp. isolates were resistant to ampicillin and cefazoline but most of them were sensitive to imipenem. Most of *Staphylococcus* spp. were resistant to oxacilllin and cefuroxime but sensitive to linezolid and imipenem. This study showed that

the most common route of transmission of pathogens was cross-infection. Similarities of antimicrobial resistance profiles of the isolated pathogen from patients and environment suggests that hospital environment may play a critical role as a source of nosocomial infections. Moreover, HCWs hands may play a considerable role in transmission of infection in these burn units. Moreover, the hospital and surgical procedures could be risk factors for transmission of nosocomial infections.

Finally, the main recommendations were: application of infectious diseases control program, training of HCWs on nosocomial infections control programs, using alcohol-based hand rubs, prevent crowding in burn units, creating isolation care unit and making a database for nosocomial infections.

Keywords: Antimicrobial resistance, burn units, nosocomial infection, Gaza strip.

مستخلص

عوامل الخطورة والميكروبات المقاومة للمضادات الحيوية المعزولة من وحدات الحروق في مستشفيات قطاع غزة، فلسطين

تمهيد: عدوى المستشفيات مشكلة صحية عامة في جميع أنحاء العالم وأحد أهم أسباب الوفيات في حالات الحروق. وقد قدر أن 75 ٪ من جميع وفيات الحروق ترتبط بهذه العدوى.إن ظهور المقاومة لمضادات الجراثيم هو من أهم المشكلات الصحية السريرية والعامة ولاسيما في الميكروبات التي تسبب عدوى المستشفيات.

الهدف: أجريت الدراسة الحالية لتحديد المسببات البكتيرية لالتهاب الحروق، ونمط مقاومة هذه البكتيريا للمضادات الجرثومية ، وتأثير الظروف البيئية وعوامل الخطر المرتبط بعدوى الحروق.

طريقة البحث: تم أخذ 118 مسحة من مرضى وحدتي الحروق (مستشفيي الشفاء وناصر) بين أكتوبر 2010 وحتى مارس2011. وقد تم أخذ العينات البيئية المختلفة، وعينات (الأنف والحنجرة ، وأصابع اليد) من العاملين في مجال الرعاية الصحية وعينات من الهواء في وحدة الحروق لتحديد مصدر العدوى المحتمل. وتم تحديد البكتيريا المعزولة من العينات بالطرق التقليدية والبيوكيميائية ونظام API 20E. علاوة على ذلك ، تم إنجاز فحوصات حساسية البكتيريا للمضادات الحيوية. وأجري التحليل الإحصائي باستخدام برنامج SPSS الإصدار 13.0.

النتائج والاستنتاجات : كانت النسبة الإجمالية للمزارع الايجابية من عينات مرضى الحروق في كلا المستشفيين 45.8 ٪ ، حيث أن *P. aeruginosa* كان الممرض المعزول الأكثر شيوعا (50 ٪)، يليه *Enterobacter cloacae* (27.8 ٪) ثم Coagulase-negative staphylococci (CoNS) (9.3 ٪) ، و*Escherichia coli* (5.6 ٪). وفي الوقت نفسه ، أظهرت المزارع الإيجابية لعينات الأيدي 78.6% وعينات الأنف 32.3 ٪ والتي تم جمعها من العاملين في مجال الرعاية الصحية ، حيث *P. aeruginosa* كان أعلى الممرضات المعزولة (32.3 ٪) ، تليها CoNS (29%). وأظهرت العينات البيئية وعينات الهواء ارتفاع معدل عزل *P. aeruginosa* وCoNS

كانت *P. aeruginosa* مقاومة لمعظم المضادات الجرثومية في هذه الدراسة. جميع *Enterobacter* المعزولة مقاومة لل ampicillin وcefazoline لكن معظمها كانت حساسة للimipenem. و معظم البكتيريا العنقودية كانت مقاومة للcefuroxime و oxacillin لكنها حساسة لل linezolid وimipenem.

وأظهرت هذه الدراسة أن معظم مسار انتقال البكتيريا المتعددة المقاومة للمضادات الجرثومية كان عبر العدوى المتبادلة. ومن خلال التشابه في فحوصات المقاومة للمضادات في الميكروبات الممرضة المعزولة من المرضى

والبيئة ، فإن البيئة ممكن أن تلعب دورا كمصدر لعدوى المستشفيات. وعلاوة على ذلك فإن أيدي العاملين في الحقل الطبي ممكن أن تلعب دورا كبيرا في انتقال العدوى في وحدات الحروق. علاوة على ذلك فإن المستشفى والعمليات الجراحية قد تكون عوامل خطر لانتقال عدوى المستشفيات.

أخيرا كانت أهم التوصيات: تطبيق برنامج مكافحة الأمراض المعدية، تدريب العاملين في الحقل الطبي على برنامج مكافحة العدوى المكتسبة، تدليك أيدي العاملين بالكحول، منع الازدحام في وحدات الحروق، خلق وحدة عزل وعمل قاعدة بيانات لعدوى المستشفيات.

الكلمات الرئيسية : مقاومة المضادات الجرثومية - وحدات الحروق - عدوى المستشفيات - قطاع غزة.

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List of abbreviations

AACN	American Association of Critical-Care Nurses
APIC	Association for Professionals in Infection Control and Epidemiology
API 20E	Analytical Profile Index 20 Enterobacteriaceae
BCUs	Burn Care Units
BSI	Bloodstream Infection
BU	Burns Unit
BWI	Burn Wound Infection
CDC	Centers for Disease Control and Prevention
CFU/50L	Colony Forming Unit / 50 Liter
CLSI	The Clinical and Laboratory Standards Institute
CoNS	Coagulase-negative staphylococci
EMB	Eosin Methyelene Blue
EPIIC	European Prevalence of Infection in Intensive Care
GI	Gastrointestinal
HCWs	Health Care workers
HICPAC	Healthcare Infection Control Practices Advisory Committee
MDR	multidrug-resistant
MMWR	Morbidity and Mortality Weekly Report
MRGN	Multi-Resistant Gram-Negative Bacilli
MRPA	Multi-resistant *Pseudomonas aeruginosa*
MRSA	Methicillin Resistant *Staphylococcus aureus*
NI	Nosocomial Infection
NNIS	National Nosocomial Infections Surveillance
PCR	Polymerase Chain Reaction
PHIC	Palestinian Health Information Center
RICPRAC	Rural Infection Control Practice Group
PPE	Personal Protective Equipment
RR	Recommendations and Reports
SARS	Severe Acute Respiratory Syndrome
SHEA	Society of Healthcare Epidemiology of America
SPSS	Statistical Package for Social Sciences

TBSA	Total Body Surface Area
TSB	Tryptic Soy Broth
USA	United States of America
UTI	Urinary Tract Infection
UV	Ultraviolet
VRE	Vancomycin-Resistant Enterococci
WHO	World Health Organization

Chapter (1)
Introduction

1.1 Overview

Nosocomial infections (NI) are those infections that develop during hospitalization and are neither present nor incubating at the time of patient's admission **(Samuel *et al.,* 2010)**. It represents a major problem in health care facilities, resulting in prolonged hospital stays, substantial morbidity and mortality, and excessive costs **(Stone *et al.,* 2002)**.

Nosocomial infections typically affect patients who are immunocompromised because of age, underlying diseases, or medical or surgical treatments. Aging of the population and increasingly aggressive medical and therapeutic interventions, including implanted foreign bodies, organ transplantations, and xenotransplantations, have created a cohort of particularly vulnerable persons **(Rebecca *et al.,* 2001)**.

Burn patients are at a high risk for infection as a result of the nature of the burn injury itself, the immunocompromizing effects of burns, prolonged hospital stays, and intensive diagnostic and therapeutic procedures **(Lari & Alaghehbandan, 2000)**.

In addition, the control and prevention of infectious diseases among burned patients present a greater and more specialized problem, because the skin barriers are disrupted, the environment in burn units (BUs) can become contaminated with resistant organisms, and these organisms can be transmitted easily from one patient to another. Thus, burn care units (BCUs) can be the site of explosive and prolonged outbreaks caused by resistant organisms **(Oncul *et al.,* 2009)**.

The skin forms a protective barrier against invasion by bacteria, fungi and viruses and any breach in this barrier provides easy access for microbial invasion. The burn wounds initially sterile; however, Gram-positive bacteria

from hair follicles and sweat glands, which may survive thermal injury, colonize the wound within 48 hours of injury. Following the initial period of shock, sepsis is the major complication in burns and it has been estimated that about 75% of the mortality associated with burn injuries is related to sepsis especially in developing countries. In addition, overcrowding in burn units is an important cause of cross infection which necessitates a regular monitoring of bacterial species and their antimicrobial susceptibilities because significant shifts in these data may be correlated with changes in clinical management with respect to drug choice for therapy **(Liwimbi *&* Komolafe*,* 2007)**.

There are two forms of acquiring infections; Endogenous infection, (self-infection, or auto-infection), in which the causative agent of the infection is present in the patient at the time of admission to hospital but there are no signs of infection. The infection develops during the stay in hospital as a result of the patients altered resistance. The other form is cross-contamination followed by cross-infection. During the stay in hospital, the patient comes into contact with new infective agents, becomes contaminated, and subsequently develops an infection. While there is no clinically significant difference between the endogenous self-infection and the exogenous cross-infection, the distinction is important from the standpoint of epidemiology and prevention **(WHO, 2002)**.

Microorganisms are probably still transmitted to the burn wound surfaces of recently admitted patients by the hands of personnel, by fomites, and perhaps, to some extent, by hydrotherapy. The gastrointestinal (GI) tract continues to be a potential reservoir for microorganisms that colonize the burn wound surface. It is likely that endogenous microorganisms continue to be transmitted to burn wound surfaces by feces. The infection sources of patients with burns include the hands of health care workers (HCWs), the environment, and food **(Mayhall, 2003)**.

Burn injury is a major problem in many areas of the world and it has been estimated that 75% of all deaths following burns are related to infection

(Sharma *et al.,* 2005). Although exposed burned tissue is susceptible to contamination by microorganisms from the GI and upper respiratory tract, many studies have reported the presence of aerobes and facultative anaerobes such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp., *Enterococcus* spp., and *Candida* spp.,. In other studies involving more stringent microbiological techniques, anaerobic bacteria have been shown to represent between 11 and 31% of the total number of microbial isolates from burn wounds **(Pisanelli *et al.,* 2008)**.

According to data from various medical records in different countries, the epidemiology of the pathogens of burn wounds is represented by: *P. aeruginosa* (25– 74%), *E. coli* (5–35%), *S. aureus* (9–17%), *Enterococcus spp.* (9–14%), coagulase-negative staphylococci (CoNS) (2–21%), and *Acinetobacter baumannii* (1– 24%) **(Song *et al.,* 2001)**.

The emergence of resistance to antimicrobial agents is a global public health problem particularly in pathogens causing nosocomial infections. Antimicrobial resistance results in increased illness, deaths and health care costs **(Savas *et al.,* 2006)**. Treatment of these infections is frequently complicated by antimicrobial resistance, a problem that has been increasing over time. The emergence of multi-drug resistant (MDR) strains in burn units (BUs), particularly in economically underdeveloped and developing countries is an increasing infection control problem **(Rastegar *et al.,* 2005)**.

Antimicrobial resistance has become a major clinical and public health problem within the lifetime of most people living today. Confronted by increasing amounts of antimicrobial agents over the past 60 years, bacteria have responded to the deluge with the propagation of progeny no longer susceptible to them. While it is clear that antimicrobial agents are pivotal in the selection of bacterial resistance, the spread of resistance genes and of resistant bacteria also contributes to the problem **(Stuart, 2002)**.

In Gaza strip, there are only two burn units in governmental hospitals (Al-Shifa and Nasser hospitals).

1.2 Objectives

The overall objective is to study the risk factors associated with nosocomial infection, to identify the bacterial etiological agents and their antimicrobial resistance pattern in burn units at Al-Shifa and Nasser hospitals in Gaza strip, Palestine.

The specific objectives of this study are:

1. Identifying type of bacterial pathogens causing nosocomial infections in these burn units.
2. Assess the environmental conditions and risk factors associated with burn infections.
3. Determination of antimicrobial resistance pattern of both clinical and environmental isolated bacteria.

1.3 Significance of the study

Burn patients are among the patients at highest risk for hospital-acquired infections. These patients have lost a portion of their integument that would ordinarily be a strong barrier to invasion by microorganisms. The presence of serum proteins provides a rich culture medium for microorganisms. Thermal injuries adversely affect both local and systemic immunity**,** data submitted from burn intensive care units to the National Nosocomial Infections Surveillance (NNIS) system at the Centers for Disease Control and Prevention (CDC) indicate that the cumulative incidence for burn wound infections is 4.5% and the incidence rate is 6.8 cases per 1,000 patient-days. Infections are the most common cause of death in burn patients. Bacteria probably cause the majority of infections in most burn care centers. The environmental and work conditions of the burn unit may be the most important contributor to infection **(Mayhall, 2004)**.

Al-Shifa (The largest hospital in Gaza strip) burn unit has reported 205 positive microbial cultures in 2009, of which 165 were caused by *P. aeruginosa* (80%), and 20% were caused by other bacterial pathogens. Nosocomial infections endanger the life of burn patients and prolong hospitalization and as a consequence elevate the cost of treatment with antimicrobial agents. In 2009, the units served 3469 patients with 507 admissions and a total of 26 deaths. While Nasser burn unit serve an average of 30 patients monthly and no death records because complicated cases are usually referred to other hospitals

The results of this study will provide original data on the etiological agents of burn infection and the associated risk factors in Gaza strip, Palestine. This would be important for local health authorities in planning intervention actions to reduce such infections.

Chapter (2)
Literature Review

2.1 Introduction

Burn injuries by fire, hot liquids, and contact with hot surfaces have been recognized as a significant and major public health problem in economically developing countries. Large open wound areas containing necrotic tissue make burn patients more susceptible to infection. In addition, a general state of immunosuppression is caused by the impaired functioning of neutrophils and the cellular and humoral immune system. In these conditions, microorganisms can easily multiply and colonize wounds to high densities. Immunologically compromised patients are also obliged to stay in high-risk intensive care units for prolonged periods of time, during which they may be submitted to endotracheal intubation and/or catheterization of the blood vessels and bladder; also, in these units, both the air and environmental surfaces are heavily contaminated. That is why burn patients are high-risk groups for infection **(Rastegar *et al.,* 2005)**.

Nosocomial infections, otherwise known as hospital-acquired infections are those infections acquired in hospital or healthcare service unit, that first appear 48 hours or more after hospital admission or within 30 days after discharge following in patient care. They are unrelated to the original illness that brings patients to the hospital and neither present nor incubating as at the time of admission. There are several reasons why nosocomial infections are even more alarming in the 21st century. These include hospitals housing large number of people who are sick and whose immune system are often in a weakened state, increased use of outpatient treatment meaning that people who are in hospital are sicker on average, many medical procedures that bypass the body's natural protective barriers, HCWs move from patient to patient thus providing a way for pathogens to spread, inadequate sanitation protocols regarding uniforms, equipment sterilization, washing and other preventive measures that may either be unheeded by hospital personnel or too lax to sufficiently isolate patients from infectious agents and lastly the

routine use of antimicrobial agents in hospitals creates selection pressure for the emergence of the resistant strains of microorganisms **(Samuel *et al.,* 2010)**.

2.2 Epidemiology of burn infection

All over the world, nosocomial infection is a recognized public health problem. Surveillance programmes estimate the rate of infection at 5-10% of hospital admissions. Nosocomial infections are responsible for about 90,000 deaths in the United States of America (USA) per year and approximately 10% of American hospital patients (about 2 million every year) acquired a clinically significant nosocomial infections **(Samuel *et al.,* 2010)**. Estimates of the annual cost range from $4.5-11 billion **(Klevens *et al.,* 2007)**.

In France, the prevalence of nosocomial infections is 6.9% to 7.5%. A rate of 5 to 19% hospitalized patients is infected and up to 30% are in ICUs. In Italy in 2000s, about 6.7% of hospitalized patients were infected; that means, between 450,000 and 700,000 patients had nosocomial infections out of which between 4,500 and 7000 died. In Switzerland, extrapolations assume about 70,000 hospitalized patients affected by nosocomial infections (between 2 and 14% of hospitalized patients **(Samuel *et al.,* 2010)**. In Nigeria, nosocomial infection rate of 2.7 % was reported from Ife **(Onipede *et al.,* 2004)** while 3.8 % from Lagos and 4.2 % from Ilorin **(Odimayo *et al.,* 2008)**.

The cause of nosocomial infections in burn patients might be endogenous or exogenous. Endogenous infections are caused by organism present as part of the normal flora of the patient, while exogenous infections are acquired through exposure to the hospital environment, hospital personnel or medical devices. Nosocomial infection rates vary substantially by body site, by type of hospital and by the infection control capabilities of the institution. The proportion of infections at each site is also considerably different in each of the major hospital services and by level of patient risk **(Samuel *et al.,* 2010)**.

The most important reservoirs for microorganisms that colonized the burn wounds of new patients were the collective burn wound surfaces and the (GI) tracts of patients. Microorganisms were transmitted by the hands of HCWs, by fomites and hydrotherapy water, and according to some reports, by the air **(Mayhall, 2003)**.

In Turkey, the 169 burn cases admitted to BCU during one year, 127(75%) acquired nosocomial infection **(Oncul *et al.,* 2009)**.

The development of infection depends on the presence of three conditions, a source of organisms; a mode of transmission; and the susceptibility of the patient **(Sharma *et al.,* 2005)**. These conditions will be discussed in details.

2.2.1 Sources of organisms

Sources of organisms are found in the patient's own endogenous (normal) flora, from exogenous sources in the environment, and from HCWs. Exogenous organisms from the hospital environment are generally more resistant to antimicrobial agents than endogenous organisms. Organisms associated with infection in burn patients include gram-positive, gram-negative, and yeast/fungal organisms. The typical burn wound is initially colonized predominantly with gram-positive organisms, which are fairly quickly replaced by antimicrobial-susceptible gram-negative organisms, usually within a week of the burn injury. If wound closure is delayed and the patient becomes infected, requiring treatment with broad-spectrum antimicrobial agents, this flora may be replaced by yeasts, fungi, and antimicrobial-resistant bacteria **(Sharma, 2007)**.

2.2.2 Mode of transmission

Among patients and HCWs, microorganisms are spread to others through four common routes of transmission: contact (direct and indirect), respiratory droplets, airborne spread, and common vehicle.

2.2.2.1 Contact transmission

This is the most important and frequent mode of transmission in the health care setting. Organisms are transferred through direct contact between an infected or colonized patient and a susceptible HCW or another person. Patient organisms can be transiently transferred to the intact skin of a HCW (not causing infection) and then transferred to a susceptible patient who develops an infection from that organism. This demonstrates an indirect contact route of transmission from one patient to another. An infected patient touching and contaminating a doorknob, which is subsequently touched by a HCW and carried to another patient, is another example of indirect contact **(Collins, 2008)**.

2.2.2.2 Droplet transmission

A person's coughing, sneezing and talking generate droplets. Procedures such as suctioning and bronchoscopy are also a source of droplets. Transmission occurs when an infected or colonized person generates droplets containing microorganisms which are propelled a short distance through the air and deposited on the conjunctivae, nasal mucosa or mouth of a host. Droplets do not remain suspended in the air; so special air handling and ventilation are required to prevent droplet transmission **(RICPRAC, 2005)**.

2.2.2.3 Airborne transmission

Airborne transmission of infectious agents involves droplets that are expelled by sneezing or coughing or are otherwise distributed into the air. Although the liquid/ vapor around the infectious agent evaporates, the residue (or droplet nuclei) may remain in the air for long periods, depending on such factors as particle size, velocity, force of expulsion, particle density, infectivity (i.e., viability of the microorganism when exposed to the environment and its ability to cause infection when a susceptible host is subsequently exposed), humidity, and rate of air flow **(Memarzadeh *et al.,* 2010)**.

2.2.2.4 Common vehicle

Common vehicle (common source) transmission applies when multiple people are exposed to and become ill from a common inanimate vehicle of contaminated food, water, medications, solutions, devices, or equipment. Bacteria can multiply in a common vehicle. Examples include improperly processed food items that become contaminated with bacteria, waterborne shigellosis, bacteremia resulting from use of intravenous fluids contaminated with a gram-negative organism, contaminated multi-dose medication vials, or contaminated bronchoscopes **(Collins, 2008)**.

2.2.3 Patient susceptibility

The patient has three principal defenses against infection: physical defenses, nonspecific immune responses, and specific immune responses. Changes in these defenses determine the patient's susceptibility to infection. Invasive devices, such as endotracheal tubes, intravascular catheters and urinary catheters, bypass the body's normal defense mechanisms. Infection from intravascular catheters is of particular concern in burn patients, as often these lines must be placed directly through or near burn injured tissue. Catheter associated bloodstream infection (BSI) is caused by organisms which migrate along the catheter from the insertion site and colonize the catheter tip, Catheter tips are also susceptible to colonization from hematogenous seeding of organisms from the colonized burn wound **(Sharma *et al.,* 2005)**.

2.3 Surveillance systems for hospital-acquired infections

Surveillance is the ongoing, systematic collection, analysis, and interpretation of health data essential to the planning, implementation, and evaluation of public health practice, closely integrated with the timely dissemination of these data to those who need to know **(Gaynes *et al.,* 2001)**.

Surveillance of infection has been shown to diminish the rate of nosocomial infection as well as reduce cost. Surveillance of infection in burn patients should be done to monitor incidence and rates which have been appropriately risk adjusted by size of burn injury and invasive device use. At a minimum, surveillance should include collection of data on pneumonia, burn wound

infection (BWI), urinary tract infection (UTI), and BSI. Systematic collection of data allows the burn unit to monitor changes in infection rates over time, identify trends, and evaluate current treatment methods **(Sharma *et al.,* 2005)**.

2.4 Sites of infection

Specific sites of infection that are particularly important for burn patients include pneumonia, BSI, BWI, and UTI. Fever, a highly specific indicator of infection for many patient populations, often does not correlate well with the presence of infection in patients with burn injuries, particularly large injuries. In burn injuries, the skin and core temperatures increase, and there is an increase in heat production, which is associated with the onset of a hypermetabolic response. The core temperature is commonly “reset” to a higher level (38° to 39°C)**,** because of this response, fever alone, without other signs and symptoms, is not indicative of infection **(Rieg, 1993 and Rafla & Tredget, 2011)**.

A study in Turkey included 169 burn patients showed that 127 of them acquired nosocomial infection with 56% BWI, 19.9% BSI, 15.7% pneumonia, and 8.4% UTI **(Oncul *et al.,* 2009)**.

2.5 Outbreaks in burn units

The exact cause for many of these outbreaks could not be determined, however certain patterns are clear. In almost all cases the colonized patient is thought to be a major reservoir for the epidemic strain. Other important sources include contaminated hydrotherapy equipment, common treatment areas, and contaminated equipment such as mattresses, which appear to pose unique risks of cross contamination in the burn environment. Risks associated with care of the burn wound, such as hydrotherapy and common treatment rooms, are related to the use of water sources that are frequently contaminated by gram-negative organisms intrinsically, and may also be contaminated by organisms from other patients **(Rafla & Tredget, 2011)**.

This aquatic environment is difficult to decontaminate because of continuous reinoculation of organisms from the patients' wound flora, adequate decontamination of this equipment (e.g., tanks, shower tables, straps) is difficult to achieve between patients using this equipment on a daily basis and monitoring techniques are insufficient to provide timely detection of contamination. In addition, the patient's own flora may be spread through the water and by caregivers to colonize other sites on the patient that are at increased risk of infection. For example, organisms from the wound may migrate to a central venous catheter site or bowel flora may be transferred to the burn wound. The risks associated with a "common treatment room" involve the contamination of the surrounding environment and the difficulty in assuring that the room is appropriately cleaned between successive patients. This is difficult to assure given the number of procedures which are performed each day and the necessity of stocking the room with dressing supplies for multiple patients **(Sharma, 2007)**.

The other principal modes of transmission in burn units are via the hands of the personnel and contact with inadequately decontaminated equipment or surfaces. The two areas most likely to become contaminated when caring for the burn patient are the hands and apron area of the person, as the surfaces (e.g., beds, side rails, tables, equipment) are often heavily contaminated with organisms from the patient. Likewise all equipment used on the patient (e.g., blood pressure cuffs, thermometers, wheelchairs, IV pumps) are also heavily contaminated and may be transmitted to other patients if strict barriers are not maintained and appropriate decontamination carried out. In fact, a single cause is uncommon in a burn unit outbreak; in almost all instances, multiple factors contribute to its occurrence and perpetuation **(Sharma *et al.,* 2005)**.

Several surveillance studies attempting to document the number of burn infections and the resulting deaths are found in the literature. Table 2.1 show the data collected from South West of Iran **(Panjeshahin *et al.,* 2001)**, while table 2.2 documented burn infections in Korea in the period 1996-1998 **(Song *et al.,* 2001)**. Admission of children due to burns in Tehran is summarized in table 2.3 **(Alaghehbandan *et al.,* 2001)**.

Concerning the situation in Gaza strip, there are two governmental hospitals that have burn units: Al-Shifa in Gaza and Nasser in Khan Younis. Al-Shifa the oldest and the largest in Gaza strip has 503 beds, while Nasser has 277 beds.

Table (2.1): Incidence of admissions due to burns in (1994–1998) in the South West of Iran **(Panjeshahin *et al.,* 2001)**

Year	All patients	All admissions		Number of deaths	
		No.	Percent (%)	No.	Percent (%)
1994–1995	9974	542	(5.4)	181	(33.3)
1995–1996	11 067	478	(4.3)	196	(41)
1996–1997	8961	505	(5.6)	156	(30.8)
1997–1998	11 666	518	(4.4)	171	(33)
Total	41 668	2043	(4.9)	704	(34.4)

Table (2.2): Incidence of organism's number isolated from the burn patients in Korea over 3 years (1996–1998) **(Song *et al.,* 2001)**

No. of isolates (%)									
Blood		Catheter		Respiratory tract		Wound or pus		Total	
No.	Percent (%)	No.	Percent (%)	No.	Percent (%)	No.	Percent (%)	No.	Percent (%)
476	(7.3)	654	(10.0)	853	(13.0)	4567	(69.7)	6550	(100)

In 2010, the admitted patients in Al-Shifa were 47416 and the major operations 12128 and the admitted patients in Nasser in the 2005 were 23984 and the major operations 3994 **(PHIC, 2010)**.

The beds in Al- Shifa burn unit are 10 and 3 beds in the BCU. In Nasser burn unit are 5 beds and does not have a BCU **(PHIC, 2010)**. Al- Shifa burn unit serves an average of 289 patients monthly, while Nasser burn unit serves an

average of 30 patients monthly. Data collected from Al-Shifa burn unit in 2009 is shown in table 2.4.

Table (2.3): Incidence of admissions due to childhood burns in Tehran, Iran (1994-1998) **(Alaghehbandan *et al.*, 2001)**

Year	All admissions	Pediatric admissions		Pediatric deaths	
		No.	Percent (%)	No.	Percent (%)
1995–1996	1020	512	(50.1)	72	(14.1)
1996–1997	1105	528	(47.7)	83	(15.7)
1997–1998	1216	414	(34.0)	79	(19.1)
Total	3341	1454	(43.5)	234	(16.1)

Table (2.4): Incidence of admissions due to burns in month in 2009 in Al-Shifa burn unit (personal contact with Al-Shifa burn unit 2009)

Month	All patients	All admissions	Number of deaths
Jan – Feb	728	193	10
Mar – Apr	719	71	6
May – Jun	453	71	1
Jul – Aug	378	59	2
Sep – Oct	676	58	6
Nov – Dec	515	55	1
Total	3469	507	26

2.6 Common pathogens isolated from burn unit

Burn injury is a major public health problem in many countries of the world. Infection is the most common cause of death and serious problems following thermal injury. According to data from various medical records in different countries, the epidemiology of the pathogens of burn wounds is represented by: *P. aeruginosa* (25–74%), *E. coli* (5–35%), *Enterococcus* spp. (9–14%),

S. aureus (9–17%), CoNS (2–21%), *A. baumannii* (1–24%). It is generally known that the spectrum of infective agents varies from time to time and from place to place. It is, therefore, desirable to carry out periodic reviews of the bacterial flora of burn wounds in order that preventive strategies might be modified as necessary **(Song *et al.,* 2001)**.

The isolated bacteria from children in an Indian burn unit; *Klebsiella* species was the predominant organism (33.91%), followed by *Pseudomonas* species (31.84%), *S. aureus* (18.2%), *E. coli* (12.9%), *Proteus* species (1.6), *Streptococcus* species (1.2), *S. epidermidis* (0.1%), *Acinetobacter* species (0.1%), and Yeast (0.1%) **(Srinivasan *et al.,* 2009)**.

A study in a BCU from Turkey showed the isolated bacteria were: *P. aeruginosa* (57%), *A. baumannii* (21%) and *S. aureus* (14%) **(Oncul *et al.,* 2009)**. While in India, *Pseudomonas* species was the commonest pathogen isolated (51.5%) followed by *Acinetobacter* species (14.28%), *S. aureus* (11.15%), *Klebsiella* species (9.23%) and *Proteus* species (2.3%) **(Mehta *et al.,* 2007)**.

An American study during the study period of six years showed that *A. baumannii* (22%) was the most prevalent organism, followed by *P. aeruginosa* (20%), *K. pneumoniae* (20%), and *S. aureus* (13%) **(Keen *et al.,* 2010)**.

However a study in burn unit in Egypt for 70 burned patients revealed that the most frequent isolate was *P. aeruginosa* (21.6%), followed by *K. pneumoniae* (15.2%), then *E. coli* (13.6%), *S*. *aureus* (13.2%), CoNS (11.6%), *Streptococcus pyogenes* (8.3%), *Enterobacter* species (6.6%), and lastly *Enterococcus faecalis* and *Candida albicans* (5.9 and 3.6%, respectively) **(Nasser *et al.,* 2003)**.

2.7 Isolation guidelines

The open burn wound increases the contamination of environment present around the patient, which is the major difference in burn versus non-burn patients. The degree or amount of contamination is roughly proportional to the

size of the open wound and amount of colonization present whereas it is inversely proportional to the distance from the patient. For this reason, appropriate barrier garb is recommended for any patient contact. Patients with greater than 30% total body surface area burn injuries are more immunocompromised due to the size of their injury. This, in combination with their loss of physical defenses and need for invasive devices, significantly increases their risk to infection. These patients also represent a significant risk for contamination of their surrounding environment with organisms (including multiply resistant organisms when broad spectrum antimicrobial treatment has been required) that may then be spread to other patients in the unit. For these reasons, it is recommended that patients with larger burn injuries be isolated in private rooms or other enclosed bed space to ensure physical separation from other patients in the unit **(Rafla & Tredget, 2011)**.

Special attention is also required for patients with smaller burn injuries who are colonized or infected with multiply resistant organisms, especially those with wound drainage that cannot be adequately contained in dry, occlusive wrapped outer dressings, or pediatric patients who cannot comply with hand washing or other precautions. Patients colonized with multiply resistant organisms must frequently have their need for isolation balanced against their need for rehabilitation and the rehabilitation needs should preferably be met in the private room **(Sharma, 2007)**.

2.8 Environmental issues

The principles of cleaning and disinfecting environmental surfaces take into account the intended use of the surface or item in patient care. CDC retains the Spaulding classification for medical and surgical instruments, which outlines three categories based on the potential for the instrument to transmit infection if the instrument is microbiologically contaminated before use. These categories are “critical,” “semi critical,” and “no critical.” In 1991, CDC proposed an additional category designated “environmental surfaces” to Spaulding’s original classification to represent surfaces that generally do not come into direct contact with patients during care. Environmental surfaces carry the least risk of disease transmission and can be safely decontaminated

using less rigorous methods than those used on medical instruments and devices. Environmental surfaces can be further divided into medical equipment surfaces (e.g., knobs or handles on hemdialysis machines, x-ray machines, instrument carts, and dental units) and housekeeping surfaces e.g., floors, walls, and tabletops **(CDC & HICPAC, 2003)**.

Plants and flowers should not be allowed in units with burn patients because they harbor gram-negative organisms, such as *Pseudomonas* species, other enteric gram-negative organisms, and fungi. Many of these organisms are intrinsically resistant to multiple antimicrobial agents, which may serve as reservoirs to colonize the burn wound **(Kates *et al.,* 1991 and Sharma *&* Taneja, 2007)**.

Pediatric burn patients should also have policies restricting the presence of non-washable toys such as stuffed animals and cloth objects. These can harbor large numbers of bacteria and are difficult to disinfect. Toys should be nonporous and washable, designated for individual patient use, and thoroughly disinfected after use and before being given to another child to use. Paper items, such as storybooks and coloring books, should always be designated for single patient use and should be disposed of if they become grossly contaminated or when the child is discharged **(Sharma *et al.,* 2005)**.

The health care environment surrounding a patient contains a diverse population of pathogenic microorganisms that arise from a patient's normal, intact skin or from infected wounds. Approximately 10 flat, keratinized, dead squamous epithelium cells containing microorganisms are shed daily from normal skin, and patient gowns, bed linens, and bedside furniture can easily become contaminated with patient flora **(Collins, 2008)**.

Surfaces in the patient care setting can also be contaminated with pathogenic organisms (e.g., from a patient colonized or infected with Methicillin Resistant *S. aureus* (MRSA), Vancomycin-Resistant Enterococci (VRE), or *Clostridium difficile*) and can harbor viable organisms for several days. Contaminated surfaces, such as blood pressure cuffs, nursing uniforms, faucets, and

computer keyboards, **(Boyce *et al.,* 1997 and Bures *et al.,* 2000)** can serve as reservoirs of health care pathogens and vectors for cross-contamination to patients. Studies have demonstrated that health care workers acquire microorganisms on gloved hands without performing direct patient contact and when touching surfaces near a colonized patient **(Boyce *et al.,* 1997 and Ray *et al.,* 2002)**.

Another study concluded that a HCWs hand became contaminated after entering a regular patient's room (one who was not on contact precautions) and only touching common surfaces close to the patient (bed rails, bedside table), without direct patient contact. The same hand contact was done by other personnel in unoccupied rooms that had been terminally cleaned after patient discharge. Ungloved hands became contaminated with low levels of pathogenic microorganisms more than 50 percent of the time, even from surfaces in rooms that had been terminally cleaned after patient discharge **(Bhalla *et al.,* 2004)**.

It is important to consider this likelihood of hand contamination could occur (contamination would also apply to the external surface of gloves, if worn) and to perform routine hand hygiene to bare hands or ungloved hands to reduce hand contamination before touching clean, general-use surfaces (e.g., computer keyboard, telephone, med cart, medical record, cleaning supplies, etc.). Proper disinfection of common surfaces and proper hand hygiene procedures (after direct contact to surfaces or contact with glove usage) is also critically important to reduce direct or indirect routes of transmission. Persistence of environmental contamination after room disinfection can occur and has been recently demonstrated to increase the risk of transmission to the next susceptible room occupants **(Zafar *et al.,* 1998 and Huang *et al.,* 2006)**.

Thus, patients with known colonization or diseases with MDR organisms as *C. difficile* require contact precautions in addition to the standard precautions to reduce the risk of transmission from the patient and the contaminated environment to others. Nurses can ensure clean medical equipment is used

between patients and can work with environmental services personnel to maximize clean conditions in and around patient rooms. It is necessary to consistently perform hand hygiene after routine patient care or contact with environmental surfaces in the immediate vicinity of the patient **(Sehulster *&* Chinn, 2003)**.

2.9 Health Care Workers (HCWs)

Health-care-associated infections are an important cause of morbidity and mortality among hospitalized patients worldwide. Such infections affect nearly 2 million individuals annually in the United States and are responsible for approximately 80,000 deaths each year. Transmission of health-care-associated pathogens most often occurs via the contaminated hands of HCWs. Accordingly, hand hygiene (i.e., hand washing with soap and water or use of a waterless, alcohol-based hand rub) has long been considered one of the most important infection control measures for preventing health-care-associated infections. However, compliance by HCWs with recommended hand hygiene procedures has remained unacceptable, with compliance rates generally below 50% of hand hygiene opportunities **(CDC, APIC, & SHEA, 2006)**.

2.9.1 Health care workers hands

The hands of HCWs are the primary mode of transmission of MDR pathogens and proper hand hygiene is the single most important, simplest and least expensive means of preventing nosocomial infections. Normal human skin harbors bacteria, usually between 10^2 and 10^6 CFU/cm^2. During daily activity, HCWs progressively accumulate microorganisms on their hands from direct patient contact or contact with contaminated environmental surfaces and devices **(Kavathekar *et al.,* 2004)**.

Traditionally, microorganisms residing on the hands are divided into resident and transient flora. Resident flora colonizes deeper skin layers and is more resistant to mechanical removal than transient flora. This group consists mainly of CoNS and Corynebacteria. These bacteria multiply in hair follicles and remain relatively stable over time. Resident flora generally has lower

pathogenic potential than transient flora and is considered important for colonization resistance, preventing colonization with other, potentially more pathogenic microorganisms. Transient flora colonizes the superficial skin layers for short periods and is usually acquired by contact with a patient or contaminated environment. These microorganisms are easily removed by mechanical means such as hand washing. Transient flora (e.g., *S. aureus*, gram-negative bacilli, or *Candida* species) is responsible for most health care–associated infections and the spread of antimicrobial resistance **(Trampuz & Widmer, 2004)**.

Washed hands can become recontaminated from faucets or by splashes from traps or sinks. *P. aeruginosa* is commonly found in tap water. In addition, plain soaps may become contaminated during use, and waterborne bacteria from the plumbing system may be present in the tap water. In contrast, alcohol hand rubs eliminate the risk of hand contamination or microbial dispersal into the environment because alcohol kills rather than removes microorganisms. Contamination of alcohol-based solutions with vegetative bacterial forms has not been reported. Alcohol dispensers can be reused as long as they are not visibly soiled **(Mondal & Kolhapure, 2004)**.

Scientific evidence and ease of use support the use of alcohol-based hand rubs for hand hygiene during patient care. The alcohol hand-rub technique is microbiologically more effective, more accessible, and less likely to cause skin problems and saves time and human resources. As a consequence, alcohol hand rubs are associated with substantially better adherence to hand hygiene than hand washing **(Trampuz & Widmer, 2004)**.

In a randomized controlled clinical trial study in India that involved 16 HCWs without any signs of skin abrasions or infections, *S. aureus* was the commonest isolate, isolated from all HCWs. Other isolates were CoNS, *B. subtilis* beside few fungal colonies. Gram negative bacilli isolates (*Klebsiella* spp. and *E. coli*) were non-significant. Not a single isolate of *P. aeruginosa* or *E. faecals* was found **(Kavathekar *et al.*, 2004)**.

2.9.2 Proper use of personal protective equipment

Infection control practices to reduce nosocomial infection include the use of protective barriers (e.g., gloves, gowns, face mask, protective eyewear, face shield) to reduce occupational transmission of organisms from the patient to the HCW and from the HCW to the patient. Personal protective equipment (PPE) is used by HCWs to protect their skin and mucous membranes of the eyes, nose, and mouth from exposure to blood or other potentially infectious body fluids or materials and to avoid parenteral contact. The Occupational Safety and Health Administration's Bloodborne Pathogens Standard states that HCWs should receive education on the use of protective barriers to prevent occupational exposures are able to identify work-related infection risks, and have access to PPE and vaccinations **(Collins, 2008)**.

Proper usage, wear, and removal of PPE are important to provide maximum protection to the HCWs. However, PPE may not be 100 % protective, individual work practices may lead to exposure (e.g., needlestick injury), breaches in PPE might occur, and some breaches may go unrecognized. All PPE should be removed when leaving the patient care area. Gloves prevent gross contamination of the hands when touching body fluids, reduce the likelihood that microorganisms present on the hands of personnel will be transmitted to patients during invasive or other patient care procedures, and reduce the likelihood that hands of personnel contaminated with microorganisms from a patient or a fomite can transmit these microorganisms to another patient. Gloves may have small, unapparent defects or may be torn during use, and hands can become contaminated during removal of gloves, thus hand hygiene is essential before donning another pair of gloves **(Olsen *et al.,* 1993 and Larson, 1995)**.

Various types of masks, goggles, and face shields are worn alone or in combination to provide barrier protection. A surgical mask protects a patient against microorganisms from the wearer and protects the HCW from large-particle droplet spatter that may be created from a splash-generating procedure. When a mask becomes wet from exhaled moist air, the resistance

to airflow through the mask increases. This causes more airflow to pass around edges of the mask. The mask should be changed between patients, and if at any time the mask becomes wet, it should be changed as soon as possible **(Collins, 2008)**.

Gowns are worn to prevent contamination of clothing and to protect the skin of HCWs from blood and body fluid exposures. Gowns specially treated to make them impermeable to liquids, leg coverings, boots, or shoe covers provide greater protection to the skin when splashes or large quantities of potentially infective material are present or anticipated. Gowns are also worn during the care of patients infected with epidemiologically important microorganisms to reduce the opportunity for transmission of pathogens from patients or items in their environment to other patients or environments. When gowns are worn, they must be removed before leaving the patient care area and hand hygiene must be performed **(Garner, 1996)**.

Improper use and removal of PPE can have adverse health consequences to the HCWs. During the 2003 severe acute respiratory syndrome (SARS) outbreak in Canada, 44 % of the probable SARS cases were in HCWs. After institutional implementation of SARS-specific infection control precautions, 17 workers developed disease. Fifteen were interviewed to determine their knowledge and work practices that could have contributed to their infection. Only 9 (60 %) reported they had received formal infection control training; 13 (87 %) were unsure of the proper order in which to don and remove PPE; 6 (40 %) reused items (e.g., stethoscopes, goggles, and cleaning equipment) elsewhere on the ward after initial use in the room of a SARS patient; and 8 (54 %) were personally aware of a breach in infection control precautions. Fatigue and multiple consecutive shifts may have contributed to the transmission **(Ofner-Agostini *et al.,* 2006)**.

From the experiences observed during the SARS outbreak, CDC developed training materials to increase the safety of the health care worker environment through improved use of PPE by health care worker **(Collins, 2008)**.

2.10 Antimicrobials and burns

Systemic antimicrobial treatment must be thoughtfully considered in the care of the burn patient to prevent the emergence of resistant organisms. The burn wound will always be colonized with organisms until wound closure is achieved and administration of systemic antimicrobials will not eliminate this colonization but rather promote emergence of resistant organisms. If antimicrobial therapy is indicated to treat a specific infection, it should be tailored to the specific susceptibility patterns of the organisms, as soon as this information is available. Also, if antimicrobial treatment is necessary, awareness should be heightened for the possibility of super infection with resistant organisms, yeasts, or fungi. Systemic antimicrobials are indicated to treat documented infections, such as pneumonia, bacteremia, BWI, and UTI **(Sharma, 2007)**.

2.10.1 Antimicrobial resistance

Although burn wound surfaces are sterile immediately following thermal injury, these wounds eventually become colonized with microorganisms. Wound colonization by yeasts and fungi usually occurs later due to the use of broadspectrum antimicrobial therapy. Microorganisms transmitted from the hospital environment tend to be more resistant to antimicrobial agents than those originating from the patient's normal flora **(Church *et al.,* 2006)**.

The emergence worldwide of antimicrobial resistance among a wide variety of human bacterial and fungal burn wound pathogens, particularly nosocomial isolates, limits the available therapeutic options for effective treatment of burn wound infections **(Taneja *et al.,* 2004)**.

Resistance to antimicrobial agents is an increasing clinical problem and is a recognized public health threat. *P. aeruginosa* has a particular propensity for the development of resistance. It is naturally resistant to many antimicrobial agents because of its relatively impermeable outer membrane and it can also easily acquire resistance, creating challenging therapeutic scenarios **(Parsnjothi *&* Dheepa*,* 2010)**.

2.10.2 Multi-Drug Resistance (MDR)

Multi-Drug Resistance (MDR) in the Gram negative isolates was defined as resistance to three or more first line classes (beta lactams, aminoglycoside, and fluroquinolone) of antimicrobials or resistant to carbapenem. Multi-resistant *P. aeruginosa* (MRPA) are usually defined as resistance to ≥3 antipseudomonal agents/groups of agents (third/fourth generation cephalosporins, piperacillin/tazobactam, imipenem, meropenem, fluoroquinolones, aminoglycosides). Surveillance of MDR *P. aeruginosa* in USA and Europe has reported resistance rates of 5-10%, whereas Japan has reported 2.8%. Data from South America are relatively scarce, but indicate around 8% MRPA (Burden of Resistance to Multi-Resistant Gram-Negative Bacilli (MRGN) **(Siegel *et al.,* 2006)**. Recent surveillance data shows an increasing incidence of MRGN worldwide **(Giske *et al.,* 2008)**.

Multi-resistance also occurs in Gram-negative bacilli like *Enterobacter* spp., *Serratia* spp. and *Citrobacter freundii*. The resistance is usually conferred by several mechanisms such as decreased permeability, efflux (active extrusion), and production of chromosomal ß-lactamase. Some transferable ß-lactamases (carbapenemases) can by themselves confer a multi-resistant phenotype, and these ß-lactamases are called metallo-ß-lactamases because their activity is dependent on zinc **(Talbot *et al.,* 2006)**.

A study of 9 years surveillance, MDR, gram negative bacilli increased from 1% to 16% for MDR *P. aeruginosa*, 4% to 13% for MDR *Enterobacter* species, 0.5% to 17% for MDR *Klebsiella* species, 0% to 9% for MDR *Proteus* species, and 0.2% to 4% for MDR *E. coli*. The most common pattern of MDR was co-resistance to quinolones, third-generation cephalosporins, and aminoglycosides **(Agata, 2004)**.

In a study conducted by **Mehta *et al.* (2007)**, *Pseudomonas* species were resistant to antimicrobials like amikacin (85.18%), gentamicin (89.22%), ciprofloxacin (78.81%), carbenicillin (88.26%), tobramycin (87.52%) and ceftazidmine (79.09%). And *S. aureus* were highly resistant to amoxycillin

(69.04%), erythromycin (75.27%), and netilmicin (77.75%); and 24% of isolated *S. aureus* were MRSA.

In a study by **Keen *et al.,* (2010)**, the percents of isolates were; *A. baumannii* (22%) was the most prevalent organism recovered, followed by *P. aeruginosa* (20%), *K. pneumoniae* (20%), and *S. aureus* (13%) and MDR prevalence rates among these isolates were *A. baumannii* 53%, *K. pneumoniae* 17% and *P. aeruginosa* 15%.

MDR strain of *P. aeruginosa* was isolated from four patients admitted to the Australian Burn Unit (BU) within one year. It was the cause of recurrent episodes of bacteraemia in two **(Douglas *et al.,* 2001)**.

2.10.3 Methicillin-resistant *S. aureus* (MRSA)

Resistance to methicillin was first described for *S. aureus* in 1960, shortly after the introduction of the drug into clinical practice **(Cunha, 2005)**. *S. aureus* is a common pathogen associated with serious community and hospital acquired diseases and has for long been considered as a major problem of public health **(Pesavento *et al.,* 2007)**. Most of the nosocomial *S. aureus* infections are caused by MRSA strains and have become a widely recognized cause of morbidity and mortality throughout the world **(Ardic *et al.,* 2006, Pesavento *et al.,* 2007, and Ho *et al.,* 2008)**. In addition, MRSA strains resistant to quinolones or multiresistant to other antimicrobial agents have been emerging, leaving a limited choice for their control **(Mee-Marquet *et al.,* 2004, Nejma *et al.,* 2006, and Pesavento *et al.,* 2007)**. Traditionally, methicillin or oxacillin has been tested and the results are representative of resistance to all β-lactam agents **(Brown *et al.,* 2005)**.

A study by **Keen *et al.* (2010)** reported that MRSA was 34% but in another study by **Mehta *et al.,* (2007),** only 24% of isolated *S. aureus* were MRSA.

In another study in Egypt **(Nasser *et al.,* 2003),** antibiograms revealed no incidence of MRSA. However, in other series, there was increasing evidence that MRSA has become a significant problem in Canada and Libya **(Husain *et al.,* 1989 and Taylor *et al.,* 1992)**.

2.10.4 Prevention of antimicrobial-resistant organisms

Authors of evidence-based guidelines on the increasing occurrence of MDR organisms propose these interventions: stewardship of antimicrobial use, an active system of surveillance for patients with antimicrobial-resistant organisms, and an efficient infection control program to minimize secondary spread of resistance **(Muto *et al.,* 2003)**.

Antimicrobial stewardship includes not only limiting the use of inappropriate agents, but also selecting the appropriate antimicrobial, dosage, and duration of therapy to achieve optimal efficacy in managing infections. A prospective study on hospital mortality due to inadequate antimicrobial treatment demonstrated that the infection-related mortality rate for patients receiving inadequate antimicrobial treatment (42%) was significantly greater than the infection-related mortality rate of patients receiving adequate antimicrobial treatment (17.7%) in a medical or surgical ICU setting **(Kollef *et al.,* 1999)**.

Interventions were aimed at varying outcomes (e.g., increase/decrease treatment, regimen, and timing of dosing, restrictive or persuasive methods to reduce unnecessary antimicrobial use). Studies showed that about half of the time, hospital physicians were not prescribing antimicrobial agents properly. Nonetheless, most interventions demonstrated some improvement in antimicrobial agents prescribing to reduce antimicrobial resistance or hospital-acquired infections. Hospital campaigns to prevent antimicrobial resistance include steps to (1) employ programs to prevent infections, (2) use strategies to diagnose and treat infections effectively, (3) operate and evaluate antimicrobial use guidelines (stop orders, restrictions, and criteria-based clinical practice guidelines), and (4) ensure infection control practices to reduce the likelihood of transmission **(CDC, 2008)**.

Chapter (3)

Material and methods

3.1 Study design

This study is a cross sectional study of all burn patients admitted to two burn units in governmental hospitals (Al-Shifa and Nasser hospitals), between October 2010 to March 2011. All burn admitted patients during this period were included in the study. Patient charts were stored in a computerized database for statistical evaluation.

3.2 Study setting

There are only two burn units in governmental hospitals (Al-Shifa and Nasser hospitals). Al-Shifa burn unit in Gaza city has 10 beds in burn department, 3 beds in BCU, and 2 dressing beds. It also has an operation room and Physiotherapy room. Nasser burn unit in Khanuons city has 5 beds and 1 dressing bed.

3.3 Types of sample

Different samples were collected from the burn units from Al- Shifa and Nasser Hospitals. These samples were taken from the patients admitted to the units, environmental elements, and HCWs as follows:

1. Sterile swabs were used to culture burn infection samples from each infected patient in the unit during the study period.
2. Sterile swabs were used to collect environmental samples (burn unit) from floors, detergents, fomite, gloves and other sources as needed.
3. Sterile swabs were used to collect samples from the burn unit HCWs (nasal, throat, hand fingers).

3.4 Study population & sampling

Samples were collected over a period of six months from October 2010 to March 2011. The number of burn wound swabs depended on the number of admitted patients in the burn units during this period. The total number of burn wound swabs was 118 (Al-Shifa 94 and Nasser 24).

The environmental samples were collected over the same period; Al-Shifa 72 and Nasser 25 and a similar sampling strategy was performed for 28 HCWs. The following table (3.1) summarizes the number of samples.

Table (3.1): Swab samples distributed according to source

Sample	Al-Shifa	Nasser	Total
Burn infection	94	24	118
HCWs	25	3	28
Environment	72	25	97
Total	191	52	243

3.5 Questionnaire

To achieve the objectives of this investigative work, a questionnaire was used to collect data from the HCWs and patients. The following data were obtained from all burn cases admitted to the burn units; registration data: age, sex, occupation, and past history. Clinical assessment of the wound: cause of burn, site affected, Total Body Surface Area (TBSA), degree, and complications. Chronological data: dates of admission and discharge.

The following data were obtained from HCWs: hygiene practices, hand washing related issues, personal protective equipment and others (See annexes 1 and 2).

A personal interview was held for filling in the questionnaire. All interviews were conducted face to face by the investigator himself. The questionnaire was based on the review of literature related to nosocomical infection in burn unit with some modifications.

3.6 Materials

3.6.1 Apparatus

Apparatus	**Manufacturer (Country)**
Air sampler	AES CHEMUNEX (France)
Autoclave	Sturdy (Germany)
Incubator	Heraeus (Germany)

Optical microscope	Olympus (Japan)
Refrigerator	Sanyo (Italy))

3.6.2 Reagents and stains

API- 20E (BioMérieux- France)

Barium chloride $BaCl_2$

Hydrogen peroxide (3% $H_2O_{2)}$

Glycerol

Gram stain kit (HiMedia- India)

Human serum

Normal saline

Oxidase discs (HiMedia- India)

3.6.3 Culture media (Manufacturer- Country)

Blood agar (HiMedia- India)

Brain heart infusion broth (BHIB) (HiMedia- India)

MacConkey agar (HiMedia- India)

Muller Hinton agar (HiMedia- India)

Nutrient agar (HiMedia- India)

3.6.4 Antimicrobial discs

Antimicrobial discs	Potency	Abbreviation	Manufacturer- Country
Penicillin G	10 units	P	HiMedia- India
Ampicillin	10 µg	Am	HiMedia- India
Piperacillin	100 µg	PIP	HiMedia- India
Cefazolin	30 µg	Cz	HiMedia- India
Ceftriaxone	30 µg	FR	Span Diagnostics- Span
Gentamicin	10 µg	GM	HiMedia- India
Amikacin	30µg	Ak	HiMedia- India
Imipinem	15 µg	IMIPM	Rosco- Denmark
Vancomycin	30 µg	Vm	Span Diagnostics- Span
Ciprofloxacin	5 µg	CI	Span Diagnostics- Span
Chloramphenicol	30 µg	C	HiMedia- India

Tetracycline	30 µg	Te	HiMedia- India
Erythromycin	15 µg	ER	Span Diagnostics- Span
Piperacillin+Tazobactam	100+10 µg	PI+TZ	Rosco- Denmark
Oxacillin	1 µg	OXA. 1	Rosco- Denmark
Linezolid	30 µg	LINEZ	Rosco- Denmark
Cefapime	30 µg	FEP30	Rosco- Denmark
Cefuroxime	30 µg	Cu	HiMedia- India
Co-Trimoxazole	30 µg	CT	Span Diagnostics- Span
Ceftazime	30 µg	CZ	Span Diagnostics- Span
Aztreonam	30 µg	AO	HiMedia- India
Norfloxacin	10 µg	Nf	HiMedia- India

3.7 Laboratory setting

Patient's swabs, HCWs and environmental samples were cultured at the Balsam hospital laboratory. Isolates from positive cultures were identified according to standard methods and were tested for antimicrobial susceptibility.

3.8 Sample collection

3.8.1 Clinical samples

The clinical samples were collected through surface swabs. Multiple samples from several areas of the burn were collected in order to obtain the most accurate assessment. Surface swabs were collected from burn wounds after the removal of dressings and topical antimicrobial agents and cleansing of the wound surface with 70% alcohol **(Church *et al.,* 2006)**.

Silver sulfadiazine or any topical antimicrobial (if present) were first removed with sterile, saline-soaked gauze. An area of about 4 cm^2 was swabbed using two sterile cotton swabs. Swab samples were taken from the wound area where the degree of burn is highest. If there are wounds with distinct color change, this was preferred to other wounds due to higher chance of infection. For a dry wound, the swab was moistened with sterile saline before swabbing.

Once collected, it was homogenized in 4-ml sterile saline **(Salehifar *et al.,* 2009)**.

Nosocomial infections were defined as infections diagnosed > 72 hr after the start of hospitalization **(Eldere, 2003)**, so only patients who are admitted for more than 3 days were included in this study.

3.8.2 Environmental samples

A sterile cotton swabs moistened in sterile normal saline were used to collect environmental samples from the floors, doors, sinks, incubators, and other instruments in the unit. The area of the swab was approximately 10 sq. cm **(Ness, 1994)**.

Ninety seven samples were taken, 72 from Al-Shifa and 25 from Nasser from patient`s rooms, dressing rooms, halls, toilets, ICU, operation room, and physiotherapy room, different places such as walls, beds, wheelchairs , trolley, halls, floors, doors, and patients instruments.

3.8.3 HCWs samples

The fingers of HCWs were rubbed for 1 minute in a Petri plate containing 10 ml of Tryptic Soy Broth (TSB)**.** After 24 hours incubation at 35 ^{o}C, the turbid plates were subcultured on Blood agar and MacConkey agar **(Kampf *&* Ostermeyer, 2005)**.

Throat and nasal swabs were also collected from HCWs and subsequently cultured on Blood agar and MacConkey agar. Any isolates found were subcultured and formally identified by standard biochemical profile.

3.8.4 Indoor air samples

Indoor air samples from different places in the two burn units such as patient's rooms, dressing rooms, BCU, operation rooms, and halls were collected using (Air Sample Suction) by suction of 50 liters of air from these places. Blood agar plates were used as enrichment media. The plates were transported to the laboratory and incubated for 48 hours at 37^{o}C. Any growth was counted in

term of CFU, and few colonies were picked for further identification and for antimicrobial susceptibility testing.

3.9 Sample transport

Although there are no published standards for transport of burn wound specimens, superficial swabs were sent to the laboratory as soon as possible after collection to ensure optimal recovery of all types of microorganisms **(Church *et al.,* 2006)**.

3.10 Microbiological investigation

The swabs were dipped in Stuart's transport medium, and then plated on blood agar and MacConkey. The isolates were identified using conventional identification techniques after incubation for 18–48 hours at 37°C **(Salehifar *et al.,* 2009).**

Positive cultures were subcultured on blood agar and MacConkey agar, as per routine bacteriologic guidelines. An oxidase test was used to differentiate *P. aeruginosa* from Enterobacteriaceae. API 20E system was used to identity the isolated gram negative bacteria. While gram stain, catalase, hemolysis on blood agar, coagulase and other tests were used to identify gram positive bacteria **(Pechorsky *et al.,* 2009)**.

3.11 Antimicrobial susceptibility test (The Kirby-Bauer method)

Small filter paper disks (6 mm) impregnated with a standard amount of antimicrobes were placed onto an agar plate to which bacteria have been swabbed by a bacterial suspension using distilled water comparable to 0.5 McFarland turbidity standard. The plates of Muller Hinton Agar were incubated overnight, and the zone of inhibition of bacterial growth was measured. An interpretation of intermediate is given for zones which fall between the accepted cutoffs for the other interpretations **(CLSI, 2007)**.

3.12 Ethical and administrative consideration

The necessary approval to conduct the study was obtained from Helsinki committee in the Gaza strip (annex 3). Helsinki committee is an authorized professional body for providing permissions to researchers to conduct their studies with ethical concern in the area. An official letter of request was sent to Palestinian Ministry of Health (annex 4) to obtain approval for swabs collection. Burn patients were given an explanation about the purpose of the study and assurance about the confidentiality of the information and that the participation was optional. HCWs also were given an explanation about the purpose of the study.

3.13 Statistical analysis

Data generated from this work were tabulated into Microsoft excel sheets and uploaded to SPSS version 13.0 (Statistical Package for Social Sciences) software.

Statistical comparison of bacterial isolates and their resistance pattern was done using Chi square test. Risk factors (age, gender, the admission day ,TBSA, duration of hospitalization, the mean admission days, surgical procedures, type of hospital, burn sites, and burn degree) for culture results and Chi square test was used for statistical significant testing. *P*-value of < 0.05 was considered as statistically significant cutoff.

Chapter 4
Results

4.1 Description of Study Sample

Different samples were collected from the burn units at Al-Shifa hospital and Nasser hospital. These samples were collected from burn patients admitted to these units, HCWs who are working in these units (nasal, throat, and fingers), environmental sources and air.

4.1.1 Patients Samples

Total patients investigated were 118 from the two burn units, Al-Shifa 94 (79.7%) and Nasser 24 (20.3%)(figure 4.1).

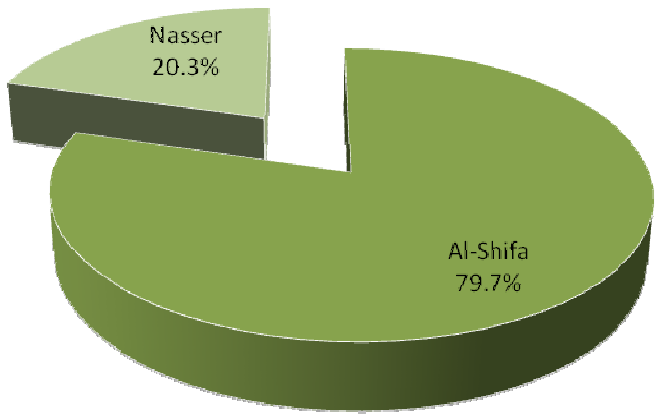

Figure (4.1): Cases distribution by hospitals

4.1.1.1 Age and Sex

The median age of patients was 4.5 years with a range of 1 to 74 years. Pediatric patients < 15 years account for 72% (85 cases) and adult patients ≥15 years were 28% (33 cases). There were 69 (58.5%) males and 49 (41.5%) females (figure 4.2).

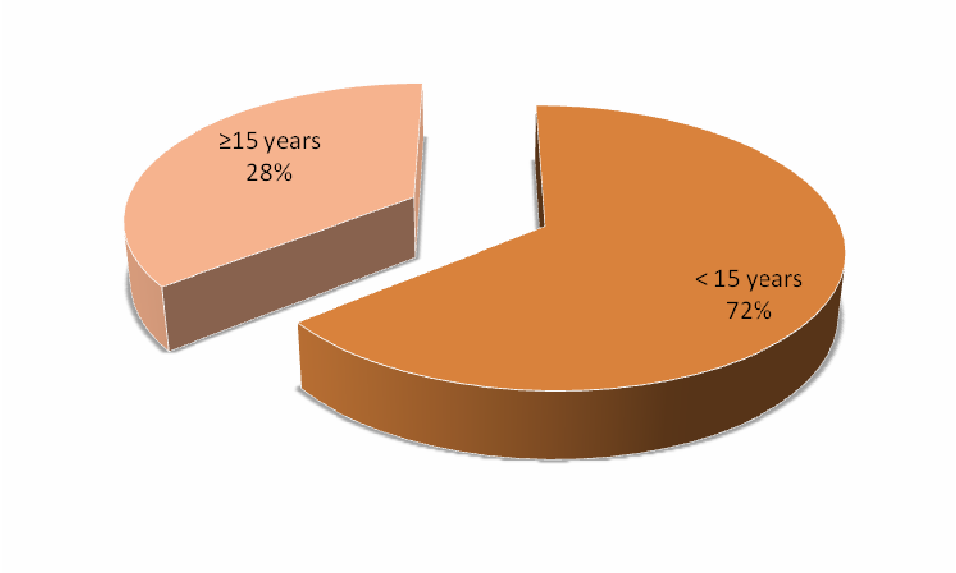

Figure (4.2): Patients distribution by age

4.1.1.2 Sites of burn

Studying the site of burn accident on the body of burned patients, the highest percentage of burn affected the trunk (39%), followed by lower limb (29.7%), and upper limb (17.8%). While the head and neck sites account for the lowest percentage (13.6%)(table 4.1).

Table (4.1): Patient distributions according to the burn sites

Burn site	Number	Percent
Head & Neck	16	13.6%
Upper Limb	21	17.8%
Lower limb	35	29.7%
Trunk	46	39.0%
Total	118	100.0%

4.1.1.3 Causes of burn

As shown in table 4.2, scald burns resulted in 78(66.1%) cases that admitted to both burn units during the study period, whereas open fire was responsible for the rest of cases (33.9%). There are significantly higher cases of burns due to scalds in comparison to open fire (*P*-value < 0.001). The majority (77.6%) of scald burns occurred in pediatric patients under 15 year-old. On

the other hand, the majority (63.6%) of open fire burns mainly occurred in adult patients (table 4.2).

Table (4.2): Association of burn causes with different age groups

Age Groups	Burn causes		Total
	Open fire	Scalds	
< 15 years	19	66	85
	22.4%	77.6%	100.0%
≥15 years	21	12	33
	63.6%	36.4%	100.0%
Total	40	78	118
	39.9%	66.1%	100%

P value < 0.0001

4.1.1.4 Extent of burns

The median total body surface area (TBSA) was 12% with a range of 1–90%**,** and (10-19%) category included the highest percentage of patients (47.5%)**,** and ≥30% category showed the lowest percentage of patients (7.6%)(table 4.3).

Table (4.3): Patient distribution according to burn extent

TBSA	Number	Percent (%)
1 – 9 %	38	32.2%
10 - 19%	56	47.5%
20 - 29%	15	12.7%
≥30%	9	7.6%
Total	118	100.0%

The degrees of burns as illustrated in figure (4.3) show that the second degree constituted 78% of cases while the third degree burn constituted only 22%.

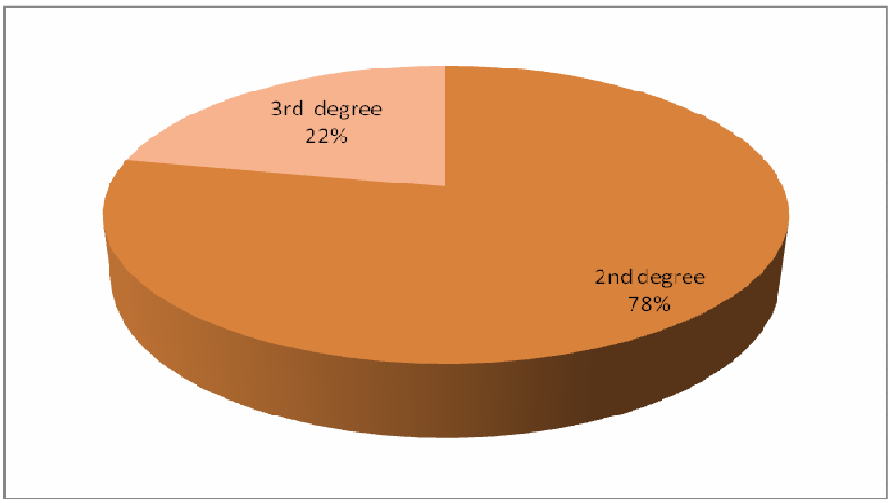

Figure (4.3): Cases distribution by degrees of burn

4.1.1.5 Number of operations

From the 118 studied burned patients, 68 operations were performed on 49 patients. Twenty patients (40.8%) had one operation and 29 (59.2%) patients had more than one operation. The types of these operations that performed included skin graft, escharotomies, debridement, plastic surgery and others.

4.1.1.6 Lengths of hospital stay

The median hospital stay for all studied patients was 11 days with a range of 3–60 days. Seventy eight (66.1%) of patients were discharged from burn unit within 14 days residency and 25.4% of them were discharged after about four weeks. However, 10 (8.4%) patient stayed over 30 days and as long as 60 days (table 4.4).

4.1.2 Health care workers (HCWs) samples

In this study, there are 31 HCWs members including medical doctors, nurses and other HCWs in burn units. In regard to the hand hygiene training, only 14 workers (45.2%) received hand hygiene training, in comparison to 17(54.8%) workers who did not receive it (table 4.5).

Table (4.4): Length of hospital stay

Hospital days	Number of patients	Percent (%)
1-14	78	66.1%
15 – 29	30	25.4%
30 – 44	7	5.9%
45 – 60	3	2.5%
Total	118	100.0%

Table (4.5): HCWs who received hand hygiene training

Hand hygiene training	Number of health workers	Percent (%)
Yes	14	45.2%
No	17	54.8%
Total	31	100.0%

Since there are no records on the extent of nosocomial infection in the studied burn units, we asked HCWs about their estimation of nosocomial infection among inpatients, the results showed that 29% of them believe that nosocomial infections is minimal (less than 25%), while 32.3% believed that it could affect up to 50% of patients and 38.7% of HCW reported that it may reach up to 75% of all admitted cases (table 4.6).

Asking the HCWs about the role of hand hygiene in preventing nosocomial infections in burn units, the results in table (4.7) shows that 13 of them (41.9%) said that hand hygiene prevents higher than 75% of these infections, while 10(32.3%) of them think that hand hygiene prevents between 50 to 74% of these infections in burn units.

Table (4.6): The HCWs estimation of nosocomial infection among burn patients in the studied burn units

Estimated nosocomial infections in burn units	Number of HCWs	Percent (%)
< 25%	9	29.0%
25 – 49%	10	32.3%
50 – 74%	8	25.8%
≥75%	4	12.9%
Total	31	100.0%

Table (4.7): HCWs opinions about the prevention of nosocomial infection by hand hygiene

The percent of infection prevention by hand hygiene	Number	Percent (%)
< 25%	4	12.9%
25 – 49%	4	12.9%
50 – 74%	10	32.3%
≥ 75%	13	41.9%
Total	31	100.0%

4.1.3 Environmental samples

Ninety seven different environmental samples were investigated. A total of 72(74.2%) were collected from Al-Shifa hospital and the rest 25 (25.8%) were from Nasser hospital (table 4.8).

Table (4.8): Environmental samples distribution by hospitals

Hospitals	Number	Percent (%)
Al-Shifa	72	74.2%
Nasser	25	25.8%
Total	97	100.0%

4.1.4 Indoor Air samples

Eighteen samples of indoor air were collected and analyzed for microbial contamination from the two burn units in Al-Shifa 14 (77.8%) and Nasser 4 (22.2%) hospitals (figure 4.4).

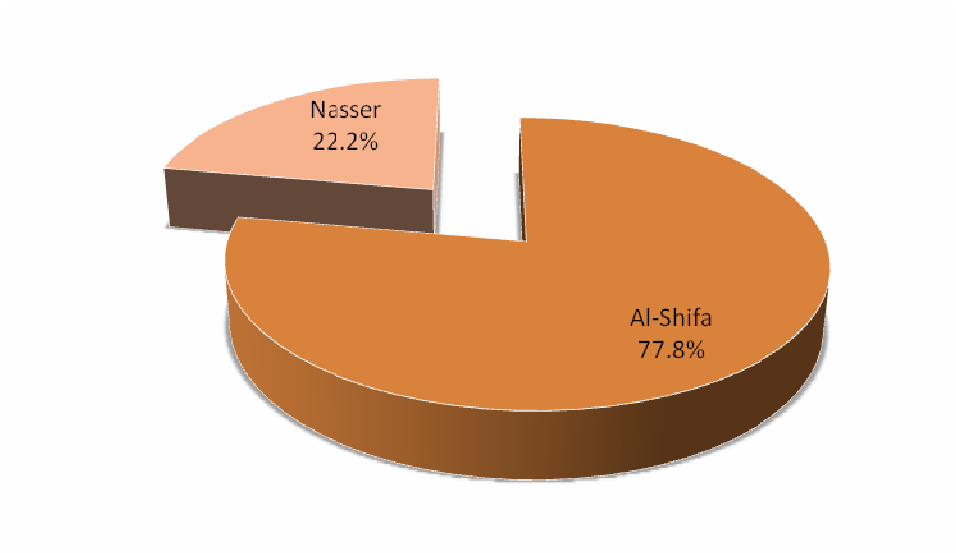

Figure (4.4): Distribution of air samples by hospitals

4.2 Microbiological investigation

Swabs samples that were collected from patients, HCWs, environment, and air were inoculated on blood and MacConkey agar. Standard biochemical tests and API 20E were used to identify bacterial isolates.

4.2.1 Culture results of patient's samples

The overall percentage of positive cultures from both hospitals was 45.8%. In Al-Shifa burn unit the negative cultures account for 60.6% in comparison to 39.4% positive cultures but Nasser burn unit showed higher percentage of

positive cultures (70.8%) in comparison to the negative cultures (29.2%). Positive cultures from Nasser hospital was higher than the positive cultures from Al-Shifa hospital (70.8% vs. 39.4%) and this difference reach statistical significance (*P* value = 0.006) (table 4.9).

Table (4.9): Distribution of culture results collected from patients by hospitals

Hospitals	Negative	Positive	Total
Al-Shifa	57	37	94
	60.6%	39.4%	100.0%
Nasser	7	17	24
	29.2%	70.8%	100.0%
Total	64	54	118
	54.2%	45.8%	100.0%

P value = 0.006

From the positive swabs that collected from burn patients in both hospitals, the most commonly isolated bacteria was *Pseudomonas* spp. 27(50%), followed by *Enterobacter* spp. 15(27.8%), *Staphylococcus* spp. 5(9.3%), and *Escherichia* spp. 3(5.6%). Meanwhile, *Citrobacter* spp., *Acintobacter* spp., *Klebsiella* spp., and Yeast represent the lowest isolated microorganisms and only account for one (1.9%) isolate for each (table 4.10).

4.2.2 Culture results of HCWs samples

More than two thirds 22(78.6%) of finger tips washing from HCWs hands were positive for bacterial contamination (table 4.11). However, the positive culture percentage from nasal and throat swabs (34.6%) was lower than that isolated from hands (78.6%).

Table (4.10): Types of pathogens isolated from patients samples

Pathogens isolates	Number	Percent (%)
***Pseudomonas* spp.**	27	50.0%
***Enterobacter* spp.**	15	27.8%
***Staphylococcus* spp.**	5	9.3%
***Escherichia* spp.**	3	5.6%
***Citrobacter* spp.**	1	1.9%
***Acinetobacter* spp.**	1	1.9%
***Klebsiella* spp.**	1	1.9%
Yeast	1	1.9%
Total	54	100.0%

Table (4.11): Culture results of samples that collected from HCWs

Culture result	Hand	Percent (%)	Nose & Throat	Percent (%)
Positive	22	78.6%	9	34.6%
Negative	6	21.4%	17	65.4%
Total	28	100.0%	26	100.0%

Table (4.12) shows that in the HCWs cultures, the highest bacterial genus isolated was *Pseudomonas* spp. 10(32.3%), followed by *Staphylococcus* spp. 9(29%), *Klebsiella* spp. and *Escherichia* spp. 3(9.7%). However, *Enterobacter* spp. and *Serratia* spp. represented 6.5% of the total positive culture for each. Meanwhile, the lowest isolated bacteria with only one isolate (3.2%) was from the genus Streptococci.

4.2.3 Culture results of environmental samples

From the 97 different environmental samples that have been investigated, there were 23 (23.7%) positive samples from both burn units and the rest were negative 74 (76.3%) (table 4.13).

Table (4.12): Types of bacteria isolated from HCWs

Bacterial isolate	Number	Percent (%)
***Pseudomonas* spp.**	10	32.3%
***Staphylococcus* spp.**	9	29.0%
***Klebsiella* spp.**	3	9.7%
***Escherichia* spp.**	3	9.7%
***Serratia* spp.**	2	6.5%
***Enterobacter* spp.**	2	6.5%
***Streptococcus* spp.**	1	3.2%
***Proteus* spp.**	1	3.2%
Total	31	100.0%

Table (4.13): Culture results of samples collected from burn units environment

Culture Result	Number	Percent (%)
Positive	23	23.7%
Negative	74	76.3%
Total	97	100.0%

As shown in table (4.14) which summarizes the environmental samples cultures results, the most commonly isolated bacteria was *Pseudomonas* spp. 9(39.1%), followed by *Staphylococcus* spp. 9(39.1%). *Pasteurella* spp. was isolated only from 2(8.7%) samples, where as *Enterobacter* spp., *Acintobacter* spp., and *Klebsiella* spp. represented the lowest isolated bacteria and each of them was found only in one sample with a percentage equal to 4.3%.

Table (4.14): Types of bacteria isolated from burn units environment

Bacterial isolate	Number	Percent (%)
***Pseudomonas* spp.**	9	39.1%
***Staphylococcus* spp.**	9	39.1%
***Pasteurella* spp.**	2	8.7%
***Enterobacter* spp.**	1	4.3%
***Acinetobacter* spp.**	1	4.3%
***Klebsiella* spp.**	1	4.3%
Total	23	100.0%

4.2.4 Culture results of air samples

The eighteen tested indoor air samples were all positive and yielded 31 bacterial isolates distributed on three bacterial genera. The range count of bacteria in these air samples was from 49 to 205 colony forming unit / 50 Liter (CFU/50L) with Mean of 140.56 CFU/50L and Median of 139 CFU/50L. In Al-Shifa hospital, the lowest count was found in samples collected from the intensive care unit (ICU) where the count ranged from 49 and 98 CFU/50L. However, the highest count was found in the samples that were collected from patient's rooms with an average of 165 and 205 CFU/50L. On the other hand, in Nasser hospital, the highest bacterial count was found in dressing room and patient's rooms, where the count was 156 and 180 CFU/50L respectively.

Table (4.15) shows that in the air samples cultures, the most common isolated bacteria were *Staphylococcus* spp. 17(54.8%), followed by *Pseudomonas* spp. 13(41.9%). However, only one isolate of *Enterobacter* spp. was found with a percentage of 3.2%.

4.3 Risk factors

Different risk factors were studied for their role in hospital acquired infections in burn units. According to the age of patients, there were higher positive cultures 42(49.4%) in the age group < 15 years in comparison to the positive

cultures 13(39.4%) in the age group ≥15 years old. However, this difference did not reach statistical significance where *P* value = 0.328 (table 3.16).

Table (4.15): Types of bacteria isolated from air samples

Bacterial isolates	Number	Percent (%)
***Staphylococcus* spp.**	17	54.8%
***Pseudomonas* spp.**	13	41.9%
***Enterobacter* spp.**	1	3.2%
Total	31	100.0%

Table (4.16): Relationship between culture results and patients age

Age Groups	Culture results		Total
	Negative	Positive	
< 15	43	42	85
	50.6%	49.4%	100.0%
≥15	20	13	33
	60.6%	39.4%	100.0%
	63	55	118
Total	53.4%	46.6%	100.0%

P value = 0.328.

In regard to the gender of our target population, males had more positive cultures 35(50.7%) than female patients 20(40.8%) but again there was no significant difference between males and females (*P* value = 0.288)(table 4.17).

In regard to the hospital, Nasser burn unit revealed higher positive cultures 17(68.0%) than Al-Shifa burn unit 38(40.9%). There is a significant statistical difference between both hospitals where *P* value = 0.016 (table 4.18).

Table (4.17): Relationship between cultures and patients gender

Gender	Culture results		Total
	Negative	Positive	
Male	34	35	69
	49.3%	50.7%	100.0%
Female	29	20	49
	59.2%	40.8%	100.0%
Total	63	55	118
	53.4%	46.6%	100.0%

P value = 0.288

Table (4.18): Relationship between cultures results and hospitals

Hospital	Culture results		Total
	Negative	Positive	
Al-Shifa	55	38	93
	59.1%	40.9%	100.0%
Nasser	8	17	25
	32.0%	68.0%	100.0%
Total	63	55	118
	53.4%	46.6%	100.0%

P value = 0.016

According to the site of burn in the body, we found that the trunk area was the most commonly infected area (56.5%) in comparison to other burn sites but this higher contamination did not reach statistical significance where *P* value = 0.302 (table 4.19).

Table (4.19): Relationship between cultures results and burn sites

Burn sites	Culture results		Total
	Negative	Positive	
Head & Neck	11	5	16
	68.8%	31.3%	100.0%
Upper Limb	12	9	21
	57.1%	42.9%	100.0%
Lower limb	20	15	35
	57.1%	42.9%	100.0%
Trunk	20	26	46
	43.5%	56.5%	100.0%
Total	63	55	118
	53.4%	46.6%	100.0%

P value = 0.302

In analyzing the results concerning burn degree, it was clear that patients with third degree burns had more positive cultures 16(61.5%) compared to the positive cultures 39(42.4%) isolated from patients with second degree burns. However, there was no statistical significant difference between burn degrees (*P* value = 0.084) (table 4.20).

Table (4.20): Relationship between cultures results and burn degree

Burn degree	Culture results		Total
	Negative	Positive	
2nd	53	39	92
	57.6%	42.4%	100.0%
3rd	10	16	26
	38.5%	61.5%	100.0%
Total	63	55	118
	53.4%	46.6%	100.0%

P value = 0.084

With regard to duration of hospitalization (admission days), the groups that were hospitalized for 30-44 days and 45-60 days were found to have higher positive cultures (71.4%, 66.7% respectively) than the groups that were hospitalized for only 1-14 days and 15-29 days (41.0%, 53.3% respectively). Again this difference did not reach statistical significance (*P* value = 0.291) (table 4.21).

Studying the risk factor TBSA, the groups with TBSA 20-29% and > 30% yielded higher positive cultures than the groups with TBSA 1-9 % and 10-19%. However, there was no statistically significant difference between different types of TBSA (*P* value = 0.101) (table 4.22).

Table (4.21): Relationship between cultures results and duration of hospitalization

Duration of Hospitalization	Culture results		Total
	Negative	Positive	
1-14	46 59.0%	32 41.0%	78 100.0%
15 – 29	14 46.7%	16 53.3%	30 100.0%
30 – 44	2 28.6%	5 71.4%	7 100.0%
45 – 60	1 33.3%	2 66.7%	3 100.0%
Total	63 53.4%	55 46.6%	118 100.0%

P value = 0.291

Table (4.22): Relationship between cultures results and TBSA

TBSA groups	Culture results		Total
	Negative	Positive	
1 - 9 %	25	13	38
	65.8%	34.2%	100.0%
10 - 19%	30	26	56
	53.6%	46.4%	100.0%
20 - 29%	5	10	15
	33.3%	66.7%	100.0%
> 30%	3	6	9
	33.3%	66.7%	100.0%
Total	63	55	118
	53.4%	46.6%	100.0%

P value = 0.101

Concerning the surgical procedures (as escharotomies, debridement, plastic surgery and others) as a risk factor, we found that patients who performed surgical procedures had higher positive cultures 28(60.9%) than the patients who did not perform any surgical procedures 27(37.5%). There was a significant statistical difference in the percentage of positive cultures between patients who had or had not surgery where *P* value = 0.013 (table 4.23).

Finally, in regard to skin graft as a risk factor, it was clear from table 4.24 that patients with skin graft had higher positive cultures (bacterial infection) in comparison to the patients without skin graft 16(84.2%) and 12(30%) respectively. The difference between the two groups was statistically significant where *P* value < 0.001(table 4.24).

Table (4.23): Relationship between cultures results and surgical procedures

Surgical Procedures	Culture result		Total
	Negative	Positive	
Yes	18	28	46
	39.1%	60.9%	100.0%
No	45	27	72
	62.5%	37.5%	100.0%
Total	63	55	118
	53.4%	46.6%	100.0%

P value = 0.013

Table (4.24): Relationship between culture results and Skin graft

Skin graft	Culture results		Total
	Negative	Positive	
Yes	3	16	19
	15.8%	84.2%	100.0%
No	60	39	99
	60.6%	39.4%	100.0%
Total	63	55	118
	53.4%	46.6%	100.0%

P value = 0.000

4.4 Antimicrobial susceptibility testing

To determine the antimicrobial resistance for the isolated bacteria from different collected samples, the isolated strains were divided into three groups according to CLSI:

1- Enterobacteriaceae (*Enterobacter* spp., *Escherichia* spp., *Citrobacter* spp., *Proteus* spp., *Serratia* spp .and *Klebsiella* spp.)
2- *Pseudomonas* spp.
3- *Staphylococcus* spp.

However, there were two isolates from the genus *Pasteurella*, *Acinetobacter*, and one isolate from *Streptococcus* that were not included in the antimicrobial sensitivity testing.

Table (4.25) shows that antimicrobial resistance of *Pseudomonas* isolates recovered from patients samples was higher than other isolates from other samples. *Pseudomonas* isolates of patients samples were found to be resistant to most of antimicrobials used except for piperacillin-tazobactam. However, it is interesting to find that all isolates of *Pseudomonas* irrespective of their isolation source were resistant to the azetreonam and most of them were sensitive to piperacillin-tazobactam. Finally, the second effective antimicrobials against these isolates were imipenem and amikacin.

Table (4.26) shows that the family Enterobacteriaceae isolated from patients and environmental samples were resistant to most of the tested antimicrobials. However, the Enterobacteriaceae isolates from air and HCWs samples were sensitive to the most of the tested antimicrobials.

However, it is interesting to find that all isolates of the family Enterobacteriaceae that were tested, irrespective of their isolation source were resistant to ampicillin and cefazoline, whereas most of them were sensitive to imipenem.

Finally, the second effective antimicrobial against these isolates were gentamycin and ciprofloxacin.

Table (4.25): Antimicrobial susceptibility for *Pseudomonas* spp. isolated from different samples

Antimicrobial	Patients samples			HCWs samples			Environmental samples			Air samples		
	S No. (%)	I No. (%)	R No. (%)	S No. (%)	I No. (%)	R No. (%)	S No. (%)	I No. (%)	R No. (%)	S No. (%)	I No. (%)	R No. (%)
Gentamycin	3 11.1	1 3.7	23 85.2	7 70.0	1 10.0	2 20.0	7 87.5	-	1 12.5	11 84.6	-	2 15.4
Piperacillin	5 18.5	3 11.	19 70.4	7 70.0	-	3 30	7 87.5	-	1 12.5	6 46.2	-	7 53.8
Ciprofloxacin	4 14.8	-	23 85.2	8 80.0		2 20.0	7 87.5	-	1 12.5	7 53.8	-	6 46.2
Cefapime	3 11.1	1 3.7	23 85.2	8 80.0	1 10.0	1 10.0	7 87.5	-	1 12.5	8 61.5	-	5 38.5
Imipenem	6 22.2	1 3.7	20 74.1	8 80.0	-	2 20.0	7 87.5	-	1 12.5	9 69.2	2 15.4	2 15.4
Amikacin	4 14.8	1 3.7	22 81.5	8 80.0	-	2 20.0	7 87.5	-	1 12.5	9 69.2	2 15.4	2 15.4
Ceftazidime	4 14.8	-	23 85.2	7 70.0	2 20.0	1 10.0	7 87.5	-	1 12.5	3 23.1	2 15.4	8 61.5
Norfloxacin	4 14.8	2 7.4	21 77.8	7 70.0	1 10.0	2 20.0	7 87.5	-	1 12.5	6 46.2	-	7 53.8
Aztreonam	-	-	27 100	-	-	10 100	-	-	8 100	-	-	13 100
Piperacillin-tazobactam	24 88.9	2 7.4	1 3.7	9 90.0	-	1 10.0	8 100	-	-	9 69.2	-	4 30.8

S= susceptible I= intermediate R= resistance

Table (4.27) shows that, penicillin and cefuroxime were the least effective drugs against most of *Staphylococcus* spp*.* isolated from all samples but linezolid and imipenem were the best effective drugs against most of *Staphylococcus* spp*.* from all samples. Totally, 74.62% of all *Staphylococcus* spp. from all samples were resistant to oxacillin. This is a high percentage of presumptive identification of methicillin resistant staphylococci.

Table (4.26): Antimicrobial susceptibility for Enterobacteriaceae isolated from different samples

Antimicrobial	Patients samples			HCWs samples			Environmental samples			Air samples		
	S No. (%)	I No. (%)	R No. (%)	S No. (%)	I No. (%)	R No. (%)	S No. (%)	I No. (%)	R No. (%)	S No. (%)	I No. (%)	R No. (%)
Gentamycin	8 40.0	1 5.0	11 55.0	7 63.6	-	4 36.4	-	-	2 100	1 100	-	-
Piperacillin	2 10.0	1 50.0	17 85.0	6 54.5	1 9.1	4 36.4	-	-	2 100	1 100	-	-
Ciprofloxacin	8 40.0	3 15.0	9 45.0	8 72.7	-	3 27.3	1 50	-	1 50	-	1 100	-
Ceftriaxone	5 25.0	1 5.0	14 70.0	8 72.7	-	3 27.3	-	-	2 100	1 100	-	-
Cefuroxime	2 10.0	4 20.0	14 70.0	2 18.2	3 27.3	6 54.5	-	-	2 100	-	-	1 100
Cefazoline	-	-	20 100	-	-	11 100	-	-	2 100	-	-	1 100
Cefapime	5 25.0	3 15.0	12 60.0	8 72.7	-	3 27.3	1 50	-	1 50	1 100	-	-
Ampicillin	-	-	20 100	-	-	11 100	-	-	2 100	-	-	1 100
Tetracycline	-	2 10	18 90.0	6 54.5	3 27.3	2 18.2	-	-	2 100	1 100	-	-
Chloramphenicol	-	4 20.0	16 80.0	7 63.6	-	4 36.4	-	-	2 100	1 100	-	-
Imipenem	14 70.0	2 10.0	4 20.0	11 100	-	-	-	-	2 100	1 100	-	-
Co-Trimoxazol	1 5.0	-	19 90.0	7 63.6	-	4 36.4	-	-	2 100	1 100	-	-

S= susceptible I= intermediate R= resistance

The incidence of methicillin-resistant Staphylococci according to oxacillin sensitivity test was 60% in patient's samples, 70.6% in air samples, 77.8% in HCWs samples and 90% in environmental samples.

Table (4.27): Antimicrobial susceptibility pattern of Staphylococci isolated from different samples

Antimicrobial	Patients samples			HCWs samples			Environmental samples			Air samples		
	S No. (%)	I No. (%)	R No. (%)	S No. (%)	I No. (%)	R No. (%)	S No. (%)	I No. (%)	R No. (%)	S No. (%)	I No. (%)	R No. (%)
Gentamycin	2 40.0	-	3 60.0	6 66.7	1 11.1	2 22.2	8 80	1 10	1 10	15 88.2	1 5.9	1 5.9
Tetracycline	3 60.0	-	2 40.0	2 22.2	3 33.3	4 44.4	8 80	-	2 20	14 82.4	1 5.9	2 11.8
Ciprofloxacin	2 40.0	1 20.0	2 40.0	7 77.8	-	2 22.2	5 50	5 20	3 30	9 52.9	5 29.4	3 17.6
Chloramphenicol	1 20.0	-	4 80.0	5 55.5	1 11.1	3 33.3	6 60	1 10	3 30	11 64.7	2 11.8	4 23.5
Ceftriaxone	1 20.0	1 20.0	3 60.0	1 11.1	-	8 88.9	1 10	1 10	8 80	13 76.5	1 5.9	3 17.6
Cefuroxime	1 20.0	1 20.0	3 60.0	3 33.3	-	6 66.7	-	-	10 100	6 35.3	3 17.6	8 47.1
Linezolid	4 80.0	-	1 20.0	9 100.0	-	-	9 90	-	1 10	16 94.1	-	1 5.9
Erythromycin	1 20.0	-	4 80.0	4 44.4	1 11.1	4 44.4	2 20	1 10	7 70	12 70.6	2 11.8	3 17.6
Pencillin	-	-	5 100.0	-	-	9 100	-	-	10 100	-	2 11.8	15 88.2
Oxacillin	1 20.0	1 20.0	3 60.0	-	2 22.2	7 77.8	1 10	-	9 90	4 23.5	1 5.9	12 70.6
Imipenem	3 60.0	-	2 40.0	-	-	9 100	9 90	-	1 10	17 100	-	-
Trimthoprim	2 40.0	-	3 60.0	3 33.3	-	6 66.7	4 40	1 10	5 50	13 76.5	-	4 23.5

S= susceptible I= intermediate R= resistance

Comparison of antimicrobial resistance patterns of isolated bacteria from patients with burns and from other sources was tabulated in table 4.28.

There is no significant difference in antimicrobial resistance patterns of *E. cloacae*, and *Staphylococci* spp. (CoNS) that were isolated from the burn patients samples and other samples ($P>0.05$). The difference between the two groups of *P. aeruginosa* was statistically significant ($P<0.05$).

A total of 53 bacterial species were isolated from 118 patients: *P. aeruginosa* accounts for the highest percentage 27(50.9%) from the burn patients followed by *E. cloacae* 15(28.3%), and *Staphylococcus* spp. 5(9.4%)(table 4.29).

Table (4.28): Comparison of antimicrobial resistance of isolated bacteria from patients and other sources

Antimicrobial agent	No. of resistant isolates (%)		
	Patients isolates	Other isolates	*P* value
	P. aeruginosa		
	N=27	N=31	
Gentamycin	24(88.9)	5(16.1)	0.000
Piperacillin	20(74.1)	11(35.5)	0.001
Ciprofloxacin	24(88.9)	9(29)	0.000
Cefapime	23(85.2)	7(22.6)	0.000
Imipenem	20(74.1)	5(16.1)	0.000
Amikacin	23(81.5)	5(16.1)	0.000
Ceftazidime	23(85.2)	11(35.5)	0.000
Norfloxacin	21(77.8)	10(32.3)	0.001
	Enterobacter cloacae		
	N=13	N=4	
Gentamycin	8(61.5)	2(50)	0.682
Piperacillin	11(84.6)	2(50)	0.143
Ciprofloxacin	6(46.2)	2(50)	0.849
Cefapime	8(61.5)	2(50)	0.607
Trimethoprim	12(92.3)	4(100)	0.567
Ampicillin	13(100)	3(75)	0.063
Cefuroxime	10(76.9)	3(75)	0.498
Ceftriaxone	9(69.2)	2(50)	0.539
	***Staphylococcus* spp.**		
	N=5	N=37	
Ceftriaxone	3(60.0)	19(51.4)	0.374
Ciprofloxacin	2(40)	8(21.6)	0.804
Cefuroxime	3(60.0)	24(64.9)	0.840
Penicillin	5(100)	34(91.9%)	0.804
Trimethoprim	3(60.0)	15(40.5)	0.843
Oxacillin	3(60.0)	27(73.0)	0.763

S= susceptible I= intermediate R= resistance

Table (4.29): Percent of bacteria isolated from burn patients

Bacterial isolates	Number	Percent (%)
P. aeruginosa	27	(50.9%)
E. cloacae	15	(28.3%)
***Staphylococci* spp.**	5	(9.4%)
E. coli	3	(5.7%)
Citrobacter freundii	1	(1.9%)
K. pneumonia	1	(1.9%)
***Acintobacter* spp.**	1	(1.9%)
Total	53	(100%)

Chapter (5)
Discussion

Nosocomial Infection is an important cause of mortality in burns. It has been estimated that 75% of all deaths following thermal injuries are related to infections. The rate of nosocomial infections are higher in burn patients due to various factors like nature of burn injury itself, immunocompromised status of the patient, invasive diagnostic, and therapeutic procedures and prolonged ICU stay. Moreover, cross-infection results among different burn patients due to overcrowding in burn wards. Complicating this high rate of infection is the fact that the spectrum of bacterial isolates varies with time and geographical area **(Mehta *et al.,* 2007)**.

In addition, the control and prevention of infectious diseases among burned patients present a greater and more specialized problem, because the skin barriers are disrupted, the environment in burn units can become contaminated with resistant organisms, and these organisms can be transferred easily from one patient to another. Thus, BCUs can be the site of explosive and prolonged outbreaks caused by resistant organisms **(Falk *et al.,* 2000 and Roberts *et al.,* 2001)**.

Although eradication of infection in burn patients is impossible, a well conducted surveillance, infection control and prevention program can help reduce the incidence. It is known that effective surveillance and infection control may reduce infection, mortality rates, length of hospitalization and associated costs **(Oncul *et al.,* 2000)**. The purpose of this cross sectional study was to identify the most common burn pathogens, antimicrobial resistance of bacteria that causing nosocomial infections in BUs at local hospitals in Gaza strip (Al-Shifa burn unit and Nasser burn unit), and to identify the sources of these pathogens. It also aimed at identifying the risk factors for acquisition of nosocomial infections in burn patients.

In this study patients were divided into two age groups depending on age: pediatric patients (<15 years) and adult patients (≥15 years) **(Wai-sun *&***

Ying, 2001). The main findings of this study were that pediatric burn patients (72%) are much more than adult burn patients (28%), this may be due to the fact that children have more mobility inside houses and have less sense and awareness of dangers. Most of the investigated children patients were exposed to boiling water and fire at their homes. Some houses in Gaza strip are small, unsuitable, overcrowded, and the kitchen is not separated from other rooms. It should be pointed that such a result was observed in a previous study **(Wai-sun *&* Ying, 2001)**.

In our study an increase burn number among males (58.5%) compared to females (41.5%) is also observed. This may be attributed to the fact that males in Palestine are involved more than females and responsible for most of duties outside home which increases risks of burn accidents. This is in agreement with similar studies in Iran **(Alaghehbandan *et al.,* 2001)** and occupied Palestine **(Silfen *et al.,* 2000)**. Our results were in contradiction with a similar study from Iran **(Panjeshahin *et al.,* 2001)** in which females were the victims of burns more frequently than males. They attributed the high number in females to the following reasons. First, most of Iranian females were housewives with low level of literacy, as these people mainly work at kitchen. Second, traditionally the style of females' clothes which has a higher volume compared to European females' clothes. Third, the material of females' clothes is mostly synthetic type comparing to the males' clothes suggesting that the females' clothes are more easily flammable **(Panjeshahin *et al.,* 2001)**. Other studies also reported that females were the victims of burns more frequently than males **(Cutillas *et al.,* 1998, Liu *et al.,* 1998 and Mzezewa *et al.,* 1999)**.

Studying of the site of burn accident on the body of burned patients, it was found that the highest percentage of burns affected in the trunk region (39%), followed by lower limb (29.7%), and upper limb (17.8%). While the head and neck sites accounted for the lowest percentage (13.6%) because the most burn etiology was hot liquid scalds and it pours on trunk and lower limb. Our results were in contradiction to another study **(Silfen *et al.,* 2000)** in which the highest percentage of burn happened in head and neck, followed by trunk,

upper limbs, and lower limbs. Silfen *et al.*, (2000) attributed their results to the reasons that most of the burns were scalds (boiling liquids), they compared that pattern of distribution to a "cascade" in which liquid was poured from above (head) towards distal areas. And they interpreted this to mean the minor surface areas affected; in other words, a flexion reflex hides the mobile parts and thus diminishes the probability of burns.

Regarding the burn etiology, we have observed that hot liquid (scalds) incidence (66.1%), followed by fire (33.9%) were the main reasons for burn accidents. This may be explained based on the fact that hot liquids are of high importance at our homes (where women and children usually exist) and most frequently used in many life aspects. This finding correlate with other studies in Egypt **(Nasser *et al.,* 2009)**, occupied Palestine **(Haik *et al.,* 2007)**, Iran **(Alaghehbandan *et al.,* 2001)**, and Hong Kong **(Wai-sun *&* Ying, 2001)**. Another study from Iran has reported that flame was the most common cause of burns followed by scalding **(Panjeshahin *et al.,* 2001)** and they explained their results based on the fact that flammable liquids such as kerosene and gas are nearly the most frequently used domestic fuels in Iran.

Cross tabulation of age groups by etiology of burns showed that flame was significantly the most common type of burns in adult patients (*P* value = 0.0001). This finding is in agreement with **(Barret *et al.,* 1999, Lari *et al.,* 2000, Alaghehbandan *et al.,* 2001, and Panjeshahin *et al.,* 2001)**. However, scalds were the most common etiology of burns among children. Children were at higher risk of burn injuries at their home environment by hot liquids (scalds). Children could be protected from burn accidents by restriction of causes and continuous parental supervision. Adults were mostly at risk of burn injuries at their work environment (open fire) and misuse of electrical generators and flammable liquids to make fire for warming in winter. They can be protected from burn accidents by increasing awareness on how to deal with flammable liquids.

The present study found that nearly 80% of patients had less than 20% TBSA burn which is lower than that found in Iran where the extent of the burn was

less than 40% of the TBSA in 55% of the patients **(Panjeshahin *et al.,* 2001)** and higher than that observed in occupied Palestine **(Silfen *et al.,* 2000)** in which 78% of patients had 5% TBSA burn. It is also found that almost 66% of the patients stayed less than 14 days in the hospitals. It is worth to mention that hospitalization duration is an important measurement of overall burn care. Factors such as severity of burn, patient's physiologic status, nursing care and surgical practice also affect the ultimate outcome, but the longer hospitalization duration is the more risk of infection.

The present study found that surgeries in the form of excision and skin grafting were performed in 59.2% of the admitted patients which is higher than recent studies in Egypt **(Nasser *et al.,* 2009)**, and occupied Palestine **(Haik *et al.,* 2007)**. Among these operations, skin graft, escharotomies, debridement, and plastic surgeries were the most common interventions.

Based on the HCWs questionnaire, 54.8% of them did not receive hand hygiene training; therefore, they could be one of the infection transmission sources to burn patients. Only, half of HCWs believed that hand hygiene of HCWs contributes considerably to prevention of nosocomial infection. Guidelines of CDC recommend handwashing with nonantimicrobial soap between the majority of patient contacts and washing with antimicrobial soap before and after performing invasive procedures or caring for patients at high risk. The hands of HCWs may become persistently colonized with pathogenic flora (e.g., *S. aureus*), gram negative bacilli, or yeast then transient to patients **(CDC & MMWR, 2002)**.

5.1. Risk factors

In our study no statistically significant relationship was found between age of patients and burn patients cultures. There were higher positive cultures (49.4%) in the children group in comparison to the positive cultures (39.4%) in the adult group. This may be attributed to the mobility of children compared to adults. Curiosity and hyperactivity and their continuing attempt to make a contact with many burn unit environment elements make children more prone to infections.

Moreover, there was no significant statistical difference between the results of patient cultures and sex of the patients. Males were found to have more positive cultures (50.7%) than females (40.8%). This could be due to the higher number of admitted male patients (58.5%) in comparison to the female patients (41.5%). This was in contradiction with a study in Turkey **(Oncul *et al.,* 2009)** in which the sex has been considered a risk factor.

A statistically significant relationship was found between hospitals and patient cultures. Nasser burn unit revealed higher positive cultures (68.0%) than Al-Shifa burn unit (40.9%). This may be because Nasser burn unit is a smaller one with single room containing five beds. The place is crowded with patients which may cause direct contact among them. Moreover, there is no special bathroom for patients in the unit so they have to use the bathrooms of other departments.

In our study no statistically significant relationship was found between degree of burn and TBSA with patient cultures. Third degree burns had more positive cultures (61.5%) comparing to the positive cultures of second degree burns (42.4%). The groups with TBSA 20-29% and > 30% have the highest positive cultures. The high burn percent size and degree increase the chance of pathogenic organisms colonization. In recent studies, it was demonstrated that a significant association between increasing burn size and increasing incidence of pathogenic organisms **(Komolafe *et al.,* 2003 and Oncul *et al.,* 2009)**. They also reported that the incidence of invasive-cultures increased as burn size increased.

In this study, the prevalence of nosocomial infection was increased with increasing the hospitalization days. It was found that groups of over 30 days of hospitalization have the highest positive cultures which is in agreement with a similar study **(Oncul *et al.,* 2009)**. Contact with other patients' cross-infection, contaminated environment, and contaminated air in burn units are the main reasons of increasing infection in such cases.

The results of this study showed statistically significant relationship between surgical procedures and increase infection incidence. This may be because protocol of early escharectomy, debridement and skin grafting has simultaneously an advantage of reducing the burn severity and a disadvantage of increasing the chance of contamination of burns. This is in agreement with a study conducted in Brazil where they concluded that one or more surgical procedures might also allow burn patient to be colonized with multi resistant organisms **(Soares *et al.,* 2006)**.

Among the HCWs hand samples, 78.6% of the cultures were positive. It means that they may play an important role in transmission of the infection to patients. So HCWs are considered a risk factor. Moreover, 54.8% of them did not receive hand hygiene training so they could be one of the infection transmission sources to burn patients by their hands.

5.2 Bacterial isolates from burn patients

The overall percentage of positive cultures from both hospitals was 45.8% which is in agreement to a study from Brazil where the positive cultures from burn unit was 44.8% **(Soares *et al.,* 2006)**. In environmental samples, 76.3% were negative cultures. This may be an indicator of the cleanliness in units and the use of suitable disinfectants. In air samples, the highest bacterial count was found in the samples collected from patients' rooms and dressing room because these places are crowded with patients and health workers.

The present study found that *P. aeruginosa* (50%) is the highest isolated bacteria from the burn patients followed by *E. cloacae* (27.8%), CoNS (9.3%), and *E. coli* (5.6%). Our results were compatible with those found in a study in USA **(Agata, 2004)** study in which *P. aeruginosa* was the highest isolated bacteria followed by *Enterobacter* species. Different results were obtained in Turkey **(Oncul *et al.,* 2009)**. They reported the following percents: *P. aeruginosa* (57%), *A. baumannii* (21%), and *S. aureus* (14%). Other studies showed that most commonly isolated organisms from burn patients were *Pseudomonas* species followed by *S. aureus* and *Klebsiella* species **(Lari *&***

Alaghehbandan, 2000, Ozumba *et al.,* 2000, Singh *et al.,* 2003, and Mehta *et al.,* 2007).

As we mentioned, in our study *P. aeruginosa* was the most predominant organism in the burn patients (50%). This result is similar to that found in other studies in Turkey (57%) **(Oncul et al., 2009)**, and Korea (45.7%) **(Song *et al.,* 2001)**. The remarkably high prevalence of *P. aeruginosa* in the burn wards may be due to the fact that the organism thrives in a moist environment **(Song *et al.,* 2001)**. *P. aeruginosa* is known for its ability to resist killing by a variety of antimicrobials. The minimal nutritional requirements of *Pseudomonas*, as evidenced by its ability to grow in distilled water and its tolerance to a wide variety of physical conditions, contribute to its ecological success and ultimately to its role as an effective opportunistic pathogen **(Parsnjothi *&* Dheepa*,* 2010)**. In contrast, there was a rise in the isolation rate of *Acinetobacter* species as an important cause of nosocomial infection in burn units **(Sengupta *et al.,* 2001 and Keen *et al.,* 2010)**. There are a number of factors which may contribute to this increase of *Acinetobacter* species like its presence as a normal skin commensal and its easy spread due to MDR in a hospital setting **(Vivian *et al.,* 1981)**.

The present study found that *E. cloacae* has the highest percent of Enterobacteriaceae that were isolated from patients samples followed by *E. coli*. This is in agreement with the results of a study in China **(Shi *et al.,* 2010)**. However, in a study conducted in Egypt, *K. pneumoniae* is found to be the highest isolated bacteria, followed by *E. coli* and then by *Enterobacter* species **(Nasser *et al.,* 2003)**.

The third isolated bacteria (9.3%) was CoNS. This is relatively low incidence and is in consistent with many previous reports on burn wound colonization in which the pathogenicity of this organism has been questioned **(Husain *et al.,* 1989, Vindenes *&* Bjerknes, 1995, and Nasser *et al.,* 2003)**. In view of the immunocompromise status of critically-ill burned patients, such centers have consistently stressed that CoNS should be considered a significant pathogen **(Nasser *et al.,* 2003)**.

5.3 Antimicrobial resistance

The antimicrobial resistance pattern of *Pseudomonas* spp. isolates that were recovered from patients samples was as follow: (gentamycin (85.2%), piperacillin (70.4%), ciprofloxacin (85.2%), cefapime (85.2%), ceftazidime (85.2%), norfloxacin (77.8%), imipenem (74.1%), amikacin (81.5%), and aztreonam (100%). So these isolates considered MDR because they are resistant to three or more antipseudomonal agents **(Defez *et al.,* 2004)**. This resistance could be due to overuse or misuse of these antimicrobials and acquisition of resistant genes from other MDR bacteria, however, this should be confirmed and approved by molecular techniques as pulsed field gel electrophoresis typing method and this study is beyond this work. MDR *P. aeruginosa* isolates were also found in previous studies **(Alaghehbandan *et al.,* 2001, Song *et al.,* 2001 and Oncul *et al.,* 2009)**.

P. aeruginosa was found to have the highest resistance for antimicrobials, followed by *Enterobacter* spp. This is in agreement with other findings in an American study **(Agata, 2004)**. All isolates of *Pseudomonas spp.* were resistant to most antimicrobials. The most effective antimicrobials against these isolates were piperacillin-tazobactam which also coincides with the results of **(Agata, 2004)**. Isolated members of the family Enterobacteriaceae were resistant to the most antimicrobial agents tested, whereas most of them were sensitive for imipenem. A similar report of MDR gram-negative bacilli was also reported by **(Singh *et al.,* 2003 and Mehta *et al.,* 2007)**.

Staphylococcus spp*.* strains isolated from patients' samples were sensitive to linezolid. This is in agreement with the a previous study in India **(Mehta *et al.,* 2007)**.

A marked increase in the number of hospital infections due to methicillin-resistant staphylococci has been reported in many countries **(Husain *et al.,* 1989 and Taylor *et al.,* 1992)**. In the present study, among the staphylococci strains isolated from patients the incidence of methicillin-resistant staphylococci was 60% based on oxacillin resistance test. However, the methillin-resistant in staphylococci should be confirmed by other molecular

techniques as PCR for *mec* gene and this is beyond this study. This percentage is similar to that reported in a study **(Kimura *et al.,* 1992)** in Japan where MRSA isolates accounted for 60% of all *S. aureus* strains. A higher incidence of MRSA was reported in Italy and France although some European hospitals reported no cases caused by MRSA **(Vincent *et al.,* 1995)**. But it is much lower than the mean incidence in Korea (98%) **(Song *et al.,* 2001)**.

5.4 Sources of infection

The results showed that *Pseudomonas* spp. and Enterobacteriaceae isolated from patients were resistant to the most of antimicrobials tested. However, the *Pseudomonas* spp. and Enterobacteriaceae isolated from air, environment, and HCWs were less resistant and more sensitive to the most of antimicrobials tested. So, it can be concluded that the main source of these bacteria may be from the endogenous flora (autoinfection) or cross-infection (direct cross-transmission from patient to patient or from HCWs to patient). HCWs hand samples within the burn units failed to demonstrate carriage of the MDR strain of *P. aeruginosa* except for one isolate. Hence, HCWs have no fundamental role as a source of contamination with *P. aeruginosa*. There was an evidence that cross-infection (direct cross-transmission patient to patient or HCWs to patient) was occurring in the burns and may be via transient HCWs hand contamination. These results are similar to those obtained in Concord hospital burn unit in Australia **(Douglas *et al.,* 2001)**.

P. aeruginosa from patients' samples have the same antimicrobial profile with one *P. aeruginosa* isolated from Al-Shifa environmental samples, and two from Nasser hospital: one from HCWs hands and the other from patient room air sample. *E. cloacae* from patients' samples have the same antimicrobial profile with one from Al-Shifa environment and another from HCWs hands. These results indicate that environment may be playing a role as a source of nosocomial infections and HCWs hands may be play a role in transmission of infection in these burn units. However, clonality depending on antimicrobial pattern as typing method is not enough alone and other molecular typing methods are necessary as pulsed field gel electrophoresis which is beyond this study.

In our study *staphylococcus spp.* strains that were isolated from patients (9.3%) were CoNS. The main source of this organism is the patient's own endogenous (normal) flora. But from the incidence of methicillin-resistant staphylococci which was 60% in patient's samples, 70.6% in air samples, 77.8% in HCWs samples and 90% in environmental samples, there was evidence that transmission of staphylococci to patient was occurring in the burns via transient HCWs, air contamination, and environmental contamination. Most of methicillin-resistant staphylococci strains isolated from HCWs were from nasal swabs**.** So the environment of the units and HCWs may be considered as source of nosocomial infection with CoNS. Again, MR-CoNS should be confirmed by other molecular methods beside the screening oxacillin sensitivity test.

Chapter (6)
Conclusions and Recommendations

6.1 Conclusions

This cross sectional study identified risk factors and risk groups to assess future efforts directed toward the prevention of infections among burn patients in Gaza, and to set the foundation to establish a prevention plan in order to minimize the infections and antimicrobial resistance among burn patients.

The study showed that the highest risk groups were pediatric patients and males. The main burn etiology was hot liquid (scalds) especially in pediatric patients while in adult patients the etiologic agent was open fire and flammable liquids.

In burn infections, the hospital and surgical procedures could be -considered as risk factors for the acquisition of nosocomial infections.

Our study showed that positive culture are related to many patient factors such as age, gender, burn degree, TBSA, and duration of hospitalization.

The infections in Nasser hospital burn unit were higher than those in Al-Shifa hospital burn unit with a significant statistical difference between both hospitals.

The most commonly isolated bacteria from the burn patients was *P. aeruginosa* (50%), followed by *E. cloacae* (27.8%) and CoNS (9.3%).

The results of this study confirmed that the most common route of infection was cross-infection.

The environment of the units could be considered as a source of infection and HCWs hand contamination play a role in the transmission of the infection as

proved by the findings of the antimicrobial resistance patterns of isolated pathogens.

Also, it was obvious that transmission of Staphylococci to patients was occurring through cross-infection, via HCWs, air contamination, and environment contamination.

In general, among all isolates, bacteria isolated from the burn patients exhibited the highest antimicrobial resistance rate.

The results revealed that *P. aeruginosa* is mostly susceptible to piperacillin-tazobactam, whereas imipenem is the most effective antimicrobials agents against Enterobacteriaceae. Meanwhile, the second effective antimicrobial agents against these isolates were gentamycin and ciprofloxacin. Linezolid was the best effective drug against most of *Staphylococcus* spp*.* and tetracycline, ciprofloxacin and imipenem were the second most effective antimicrobial agents.

6.2 Recommendations

1. Training for HCWs in nosocomial infections control programs. The continuous education of hospital authorities and HCWs on principles of infection control through training and re-training is advocated.

2. Application of infectious diseases control program which could improve hygiene, particularly hand washing and PPE (gowns, gloves, masks and caps).

3. The prevention of patients crowding in units and compliance with infection control guidelines by patient care personnel will prevent cross-transmission of MDR microorganisms in burn units. It is important to control the flow of human traffic in the burns unit as well as strictly enforcing hand washing both before and after handling a patient so as to curtail the risk of cross-infections and spread of drug-resistant bacteria.

4. The isolation care unit especially for the infected patients and the patients with high TBSA% is important in the prevention of nosocomial transmission of infection and decrease the mortality.

5. Infection control should be incorporated into universities and institution programs as an essential course for medical science students, medical doctors, nurses, and other paramedics.

6. Nasser burn unit must be an independent unit and equipped with all instruments to prevent the cross-infection and the contact of burn unit patients with others.

7. To avoid air contamination, the patient's and dressing rooms should be sterilized by U.V or any other available mean as providing high quality air filters.

8. Antimicrobial resistance must be avoided in order to control a hospital-acquired infection by more restricted antimicrobial prophylaxis use.

9. Making a database in Gaza strip for nosocomial and antimicrobial resistance in burn units can provide the authorities with an up-to-date status of burns and could be the base for future prevention programs that can be modified periodically. Furthermore, such a database can enable a comparison of the quality of treatment in the different burn units, which will enable improvements to be made and enable treatment burn patients and decrease antimicrobial resistance.

10. The use of alcohol-based hand rubs for HCWs can prevent health care–associated infections and the spread of nosocomial infections in these burn units.

11. The physicians have to be familiar and updated with the antimicrobial susceptibility profiles of pathogens, specially the MDR pathogens.

12. Bacterial colonization is very common in burn units which make infection control measures extremely important. Regular surveillance of bacterial profile and their antimicrobial susceptibilities should be encouraged to help guide first-line therapy for burns-related sepsis. The pattern of antimicrobial resistance of isolated bacteria observed in this study is very high. This could be an important ground for the development and spread of antimicrobial resistant bacteria that may have danger on their life.

13. We recommend other researchers to study the molecular typing methods as pulsed field gel electrophoresis for these pathogens to find out the clonality and to search for anaerobic bacteria in these burn units.

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(annex 1,2): These questionnaires for health-care workers (HCWs) and patients to determine the infection in the burn units at Al-Shifa and Nasser hospitals.

ANNEX 1

Questionnaire for Health-care Workers

You are in direct contact with patient on a daily basis and this is why we are interested in your opinion on health care-associated infections.

Data collected will be used for research purposes only. No names shall be mentioned

Hospital: ……..

1. DOB: ……..... **2. Gender:** ☐ Male ☐ Female

3. Profession:

☐ Nurse ☐ Auxiliary nurse ☐ Medical doctor

☐ Resident ☐ Technician ☐ Nurse student

☐ Therapist ☐ Others……

4. Did you receive formal training in hand hygiene in the last three years?

☐ yes ☐ no

5. Do you routinely use an alcohol-based handrub for hand hygiene?

☐ yes ☐ no

6. In your opinion, what is the average percentage of hospitalized patients in burn unit who will develop a health care-associated infection (between 0 and 100%)?

☐ % ☐ I do not know

7. In general, what the impact of health care-associated infection on a patient's clinical outcome?

……

8. In your opinion, what if the effectiveness of hand hygiene in preventing health care-associated infection?

……..

9. Hand washing basins (observation and staff):

☐ Hot and cold water ☐ Liquid hand wash ☐ Paper towels

☐ Soup ☐ Antiseptic products

10. Use of Personal Protective Equipment (PPE):

P Protective Eyewear	☐ yes ☐ no	O Protective Eyewear	☐ yes ☐ no
P Head cover	☐ yes ☐ no	O Head cover	☐ yes ☐ no
P Foot cover	☐ yes ☐ no	O Foot cover	☐ yes ☐ no
P Gloves	☐ yes ☐ no	O Gloves	☐ yes ☐ no
P Gown	☐ yes ☐ no	O Gown	☐ yes ☐ no

ANNEX 2

Questionnaire for Patient

1. DOB: ……**2. Gender:** ☐ Female ☐ Male

3. Residence: ……..

4. Hospital: ……..... ☐ Department ☐ BICU

5. Occupation:

☐ Student ☐ Housewife ☐ Unskilled

☐ Employee ☐ Others……..

6. Past History:

☐ Hypertension ☐ Heart diseases ☐ Hepatitis

☐ Tuberculosis ☐ Blood Transfusion ☐ Diabetes

☐ Drug & Allergy Others……

7. The cause of Burn:

☐ Open fire ☐ Hot water ☐ Scalds

☐ Flammable liquids ☐ Chemical ☐ Electrical

☐ Inhalation ☐ Radiation Other…….

8. The site of Burn:

☐ Head ☐ Neck ☐ Abdomen

☐ Trunk – anterior ☐ Trunk – posterior ☐ Buttocks

☐ Genitalia/perineum ☐ Arms ☐ Hands

☐ Legs ☐ Feet Others……

Burn degree: ☐**1st** ☐ **2nd** ☐ **3rd**

9. Admission:

Admission days (stay in the unit)…….

Transfer from other hospital: ☐ yes ☐ no

If yes, What hospital …….

Total body surface area (TBSA%)……

Did you have skin graft?

☐ yes ☐ no If yes, What facility_____?

Did the patient have any surgical procedures during admission?

☐ yes ☐ no If yes, please specify what procedures_____

10. Previous clinical history

Prior antimicrobes usage: ☐ yes ☐ no

If Yes:

a., how many different times were antibiotics prescribed? ____

b. How many months before your skin infection were antibiotics prescribed?

____ < ____1 – 3 ____3 – 6 ___ 6 – 12 ____ Don't know

c. Why did you receive antibiotics? ____ Skin infection ____ Other

……,……..,……,……

Had any type of surgery or invasive procedure (i.e., sutures, IV)?

☐ yes ☐ no

If Yes,

a. what facility _____

b. What type of surgery?

_____

Been hospitalized? ___ Yes ____ No

If Yes,

a. What hospital?

_____

b. Why were you hospitalized?

_____

The WBC……

10. Complications:

Hypothermia potential	☐ yes ☐ no	Electrolyte loss	☐ yes ☐ no
Hypovolemia	☐ yes ☐ no	Hypoxia	☐ yes ☐ no
Infection	☐ yes ☐ no	Organ Failure	☐ yes ☐ no
Urinary tract infection	☐ yes ☐ no	Pneumonia	☐ yes ☐ no
Bloodstream infection	☐ yes ☐ no		

ANNEX 3

Palestinian National Authority
Ministry of Health
Helsinki Committee

السلطة الوطنية الفلسطينية
وزارة الصحة
لجنة هلسنكي

التاريخ6/12/2010

Name:

الاسم: غسان عبد الرحمن عثمان تايه

I would like to inform you that the committee has discussed your application about:

نفيدكم علماً بأن اللجنة قد ناقشت مقترح دراستكم حول:-

Risk Factor and Antimicrobial Resistance of Pathogens Isolated from Burn Unit At Local Hospitals In Gaza Strip, Palestine

In its meeting on December 2010 and decided the Following:-

و ذلك في جلستها المنعقدة لشهر ديسمبر 2010

و قد قررت ما يلي:-

To approve the above mention research study.

الموافقة على البحث المذكور عاليه.

Signature
توقيع

Member
عضو

Member
عضو

Chairperson

Conditions:-

- Valid for 2 years from the date of approval to start.
- It is necessary to notify the committee in any change in the admitted study protocol.
- The committee appreciate receiving one copy of your final research when it is completed.

ANNEX 4

Palestinian National Authority
Ministry Of Health
Hospitals General Administration

السلطة الوطنية الفلسطينية
وزارة الصحة
الإدارة العامة للمستشفيات

التاريخ:2010-09-02

الرقم:أ.م

السيد/ مدير عام مجمع الشفاء الطبي
السيد/ مدير عام مجمع ناصر الطبي
المحترمين،،،

تحية طيبة وبعد ،،

الموضوع/ تسهيل مهمة باحث.

قادم إليكم الطالب/ غسان عبد الرحمن تايه من الجامعة الإسلامية وهو طالب في برنامج ماجستير العلوم الحياتية تخصص أحياء دقيقة حيث يقوم بإجراء بحث تخرج بعنوان:

"عدوى المستشفيات من وحدات الحروق في المستشفيات المحلية (مستشفى الشفاء وناصر)"

نأمل تسهيل مهمة الأخ المذكور للحصول على بيانات خاصة بالمرضى وكذلك أخذ مسحات ميكروبيولوجية من وحدات الحروق في المستشفيات لديكم مع العلم أن الطالب سيقوم بإجراء الفحوصات الخاصة ببحثه خارج مختبرات الوزارة وعلى نفقته الخاصة وذلك بما لا يتعارض مع مصلحة العمل وضمن ضوابط وأخلاقيات البحث العلمي.

وتفضلوا بقبول فائق الاحترام،،،

د. محمد الكاشف
مدير عام المستشفيات

الإدارة العامة للمستشفيات
صادر
رقم: ٤٠٥
التاريخ: ٩/١٠

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