دراسة بكتريولوجية سريرية

ومناعية

لمرضى الحروق

رسالة تقدمت بها

هدى عبد الوهاب جواد بلاكت

بكالوريوس طب وجراحة عامة إلى

مجلس كلية الطب / جامعة بابل

وهي جزء من متطلبات نيل درجة ماجستير علوم في علم الأحياء

المجهرية الطبية

صفر ١٤٢٨ هـ شباط ٢٠٠٧ م

وَمَا أَنزَلْنَا عَلَيْكَ الْكِتَابَ إِلَّا لِتُبَيِّنَ لَهُمُ
الَّذِي اخْتَلَفُوا فِيهِ وَهُدًى وَرَحْمَةً
لِّقَوْمٍ يُؤْمِنُونَ

﴿سورة النحل / الآية ٦٤﴾

Clinical Bacteriology and Immunology of Burn Victims

A Thesis

Submitted to the Council of the College of Medicine

University of Babylon in Partial Fulfillment of the Requirements for the Degree of Master of Science in

Medical Microbiology

By

Huda Abdul Wahab Jawad Balakit

M.B.Ch.B.

February ٢٠٠٧ A.D. **Safar ١٤٢٨ A.H.**

We, the examining committee, certify that we have read the thesis entitled ***(Clinical Bacteriology and Immunology of Burn Victims)*** and have examined the student ***(Huda Abdul Wahab Jawad Balakit)*** in its contents, and that in our opinion it is accepted as a thesis for the degree of Master of Science in Medical Microbiology.

Signature
Chairman
Dr. Muhammed Shamkhi Jabur
Professor
Department of Microbiology
Technical Medical Institute/ Baghdad

Signature
Member
Dr. Mahmood Muslih Jawad
Assistant Prof.
Department of Surgery
College of Medicine
Babylon University

Signature
Member
Dr. Salman Aziz Adoos
Assistant Prof.
Department of Microbiology
College of Medicine
Al-Kufa University

Signature
Member and Supervisor
Dr. Muhammed Sabri A. Razzak
Assistant Prof.
Department of Microbiology
College of Medicine
Babylon University

Signature
Member and Supervisor
Dr. Muhammed A. K. Al-Sa'adi
Assistant Prof.
Department of Microbiology
College of Medicine
Babylon University

Approved for the College Committee of Graduate Studies

Signature

Dr. Ali K. Al-Shaali

Assistant Prof.

Dean of College of Medicine

Babylon University

Abstract

This study aims to know some of the clinical parameters of the burn patients including age, sex and Boyd index, to identify the types of bacterial isolates associated with burn infections and to study the effects of some antibacterial agents on these isolates, in addition to the determination of the levels of some humoral immunological factors for burn patients.

During the period between ١st/ ١١ / ٢٠٠٥ and ١st/ ٥ / ٢٠٠٦ , a total of ٧٨ skin swabs, ٤٨ blood specimens from ٧٨ burn patients, ٣٠ swabs from the burn ward at Al-Hilla General Teaching Hospital, and ١٢ blood specimens from normal healthy subjects(controls) have been studied.

The results have revealed that most cases of burn injury are among females. The most frequent type of burn injury is flame. The most affected age group is that of (١–٥) years in whom scald injury is more than flame injury. It has been found that Gram negative bacteria are more frequent than Gram positive type in skin, blood and burn ward specimens. *Pseudomonas aeruginosa* is the most frequent species among the Gram negative bacteria in skin and burn ward. Whereas, in blood culture, *Enterobacter* spp. is the most frequent. On the other hand, *Staph. aureus* is the most frequent

isolate among Gram positive bacteria in skin , and in blood. In burn ward specimens, *Entercoccus faecalis* is the predominant type.

The level of Boyd index is more than (٨٠) for the dead cases. The mortality increases as the percentage of burn injury increases.

The effect of some antibiotics on each bacterial isolate has been studied. It has been found that amikacin and ciprofloxacin are the most effective antibiotics on all bacterial isolates, whereas carbenicillin and amoxicillin are the least effective. There is an evident similarity in the antibiogram profile of bacteria isolated from skin and burn ward and in that of bacteria isolated from skin and blood suggesting that the burn ward is an important source for burn infections. The effect of both silver sulphadiazin(١٪) and silver nitrate(٠.٥٪) is studied on bacterial isolates of skin by the agar well diffusion technique. It is observed that there is no significant difference($p>٠.٠٥$) between the effect of these two agents on bacterial skin isolates.The study of humoral immunological factors reveals that there is a significant decrease($p<٠.٠٥$) in the mean level of IgG and IgA. Whereas, the mean level of IgM is not increased($p>٠.٠٥$) during bacterial infection of burn victims. Likewise, $C^{٣}$ and $C^{٤}$ complement components are not increased as a mean level for burn victims when compared to that of controls($p>٠.٠٥$).

List of Abbreviations

ADCC	antibody-dependent-cell-cytotoxicity
AK	amikacin
AX	amoxicillin
AZM	azithromycin
BHS	β- hemolytic streptococci
C٣,C٤, andC٥	complement component٣,٤,٥ respectively
CIP	ciprofloxacin
CN	gentamicin
CoNS	coagulase negative staphylococci
CTX	cefotaxim
DNA	deoxy ribonucleic acid
EMB	eosin methylene blue
ESBL	extended spectrum beta lactamase
GFR	glomerular filteration rate
IFN-γ	interferon-gamma
Ig	immunoglobulin
IgA,IgG,andIgM	immunoglobulin A,G,and M
IL-٢,٦,١٠	interleukin-٢,٦,١٠
M	mean
MAC	membrane attack complex
MR	methyl red reagent
MRSA	methicilline resistant *Staphylococcus aureus*
PGE٢	prostaglandin E٢
Py	carbenicillin
SD	standard deviation
S-IgA	secretory immunoglobulin-A
SN	silver nitrate
SRID test	single radial immunodiffusion test
SSD	sulfadiazine silver
SXT	trimethoprim-sulfamethoxazole
TBSA	total body surface area
TEM, SHV-S	types of β-lactamase
Th-١ and Th-٢	T-helper-١ and T-helper-٢ lymphocytes
TNF-α	tumor necrosis factor-alpha
TSI	triple sugar iron
VP	Voges-Proskauer reagent

الخلاصة

تهدف هذه الدراسة إلى تحديد بعض المعايير السريرية مثل العمر, الجنس ومؤشر Boyd لمرضى الحروق كما تهدف إلى تحديد أنواع المسببات البكتيرية لاخماج الحروق ودراسة تأثير بعض المضادات الحياتية على هذه المسببات ، بالإضافة إلى تحديد مستويات بعض المعايير المناعية لهؤلاء المرضى.

شملت هذه الدراسة التي استمرت للفترة من تشرين الثاني (٢٠٠٥) إلى أيار (٢٠٠٦) (٧٨) مسحة جلد، (٤٨) عينة دم من مرضى الحروق و (٣٠) مسحة من ردهة الحروق التابعة الى مستشفى الحلة التعليمى العام, إضافة إلى (١٢) عينة دم من الأشخاص الأصحاء للمقارنة.

أظهرت هذه الدراسة بأن اغلب حالات الحروق كانت بين الإناث ٤٢ مقارنة بالذكور وأن أكثر طريقة للحرق كانت بواسطة اللهب مقارنة بالسمط. وكانت أكثر فئة عمرية معرضة للإصابة بالحرق(١– ٥) سنوات وكانت طريقة الحرق بالسمط هي الأكثر حدوثا بالنسبة لهذه الفئة العمرية وهي أكثر بكثير من اللهب(١.٣ %).

كانت البكتريا السالبة أكثر شيوعا من البكتريا الموجبة في عينات الجلد والدم وردهة الحروق كما وأن بكتريا(*Pseudomonas aeruginosa*) كانت أكثر الأنواع عدداً من بين البكتريا السالبة في عينات الجلد وعدد هذه البكتريا(٢٧ عزلة) وفي عينات ردهة الحروق كان عددها (٦ عزلات) بينما كانت بكتريا (*Enterobacter*) الأكثر عددا ضمن عينات الدم. أما بكتريا(*Staphylococcus aureus*) فهي الأكثر عدداً من بين البكتريا الموجبة حيث كان عددها في عينات الجلد(٤ عزلات)، و(٧ عزلات) في الدم بينما كانت بكتريا(*Entercoccus faecalis*) في ردهة الحروق هي الأكثر وبعدد (٤ عزلات) .

إن مستوى مؤشر (Boyd) لحالات الوفيات بسبب الحروق كان أكثر من (٨٠). وقد وجد بأن هناك تناسباً طردياً بين نسبة الوفيات ونسبة الحرق .

جرت دراسة تأثير بعض المضادات الحياتية على جميع العزلات البكترية. وقد تبين من خلال هذه الدراسة بأن المضادين الحياتيين: الاميكاسين والسبروفلوكسلسين هما الأكثر تأثيراً على كل العزلات البكترية بينما كان المضادان الحياتيان: الكاربنسيلين والاموكسيلين هما الأقل تأثيراً .

أبرزت الدراسة بأن هنالك تشابها واضحا في نمط استجابة العزلات البكترية المعزولة من الجلد وردهة الحروق للمضادات الحياتية وان هذا التشابه موجود أيضا بين

عزلات الدم والجلد لنفس المريض مما يدل على ان ردهة الحروق هى مصدر مهم لاخماج الحروق.

جرت دراسة تأثير كل من السلفرسلفاديازين(١%) والسلفرنايتريت(٠.٥%) على عزلات بكتريا الجلد باستخدام طريقة انتشار الحفر فى الوسط الزرعى. أظهرت الدراسة بأنه لا يوجد أي فرق معنوي (قيمة الاحتمالية > ٠.٠٥) بين تأثير السلفرسلفاديازين والسلفرنايتريت على عزلات بكتريا الجلد .

كان معدل مستويات الكلوبيولينات المناعية نوع IgG ، IgA قليلا في المرضى المصابين باخماج الحروق. أما معدل مستوى IgM فأنه لم يظهر أية زيادة فى حالات اخماج الحروق(قيمة الاحتمالية >٠.٠٥) وبالمثل فأن معدل مستويات مكونات نظام المتمم C٣ ، C٤ لم يزدد في مرضى الحروق بالمقارنة مع الأشخاص الأصحاء (قيمة الاحتمالية > ٠.٠٥).

Acknowledgements

To "**ALLAH**" and to his prophet "**Muhammad**", my praise and thanks are due for their blessings without which this research would not have been achieved.

Due thanks are to **Dr. Muhammad Sabri Abdul-Razak** and **Dr. Muhammad A. K. Al-Sa'adi** for their valuable advice, assistance, cooperation, and motivation throughout the course of preparing my thesis.

My special indebtedness also goes to Dr. Ahmad A. K. Norris, a specialist aplastic surgeon at Al-Hilla General Teaching Hospital for his valuable advice and endless help.

Thanks to the University of Babylon, College of Medicine and Department of Microbiology for providing the necessary facilities for completing this study.

Thanks to Dr. Haitham E. Baqir and Dr. Sanaa' Halabiya at the General Health Laboratories / Baghdad.

My greatest debt is to my brother, Mr. Sa'ed; to my sister Miss Nada and to Dr. Hatim A. Lattif for their help and support.

Last, but not least, I am really indebted to the staff members of the Burn Ward at Al-Hilla Teaching Hospital for their sincere cooperation and endless help.

Certification

We certify that this thesis was prepared under our supervision at the Department of Microbiology, College of Medicine, University of Babylon as partial fulfillment of the requirements for the Degree of Master of Science in Medical Microbiology.

Supervisor
Dr. Muhammad Sabri A. Razzak
M.Sc Ph.D.
Assistant Professor
College of Medicine
Babylon University
/ / ٢٠٠٦

Supervisor
Dr. Muhammad A. K. Al-Sa'adi
M.Sc Ph.D.
Assistant Professor
College of Medicine
Babylon University
/ / ٢٠٠٦

In view of the available recommendation, I present this thesis for evaluation by the Examining Committee.

Assistant Professor

Dr. Muhammed Sabri A. Razzak

M.Sc Ph.D.

Head of Department of Medical Microbiology

College of Medicine

Babylon University

/ / ٢٠٠٦

Contents

Dedication

To . . .

My first teachers: my father and mother ;

My support in life: my husband , Dr. Iyad ;

The tender hearts that surrounded me with care and courage: my husband's parents and his sisters; and all my brothers and sisters;

My hope in this world: my daughter,
Qamar.

Huda

List of Tables

List of Figures

List of Appendices

Appendix (١)

Morphological and Biochemical Features for Identification of Gram Negative Isolates

Tests	***E.coli***	***Klebsiella pneumoniae***	***Enterobacter spp.***	***Proteus spp.***	***P.* aeruginosa***	***Acinetobacter baumannii***
Gram's stain	G-ve, short rods	G-ve, short rods	G-ve rods	G-ve rods	G-ve rods	G-ve Coccobacilli (Diplococci)
Capsule	–	+	+	–	+	–
Oxidase	–	–	–	–	+	–
Catalase	+	+	+	+	+	+
Indole	+	–	–	–	–	–
MR	+	–	–	+	–	–
VP	–	+	+	–	–	–
Citrate	–	+	+	+	+	+
Urease	–	+	–	+	–	–
TSI	A/A with gas	A/A with gas	A/A with gas	Alk/A with gas	ALK/ ALK	ALK/ ALK
H٢S	–	–	–	+	–	–
Motility	+	–	+	+	+	–
Swarmig	–	–	–	+	–	–
Hemolysis	–	–	–	–	+(beta)	–
EMB	Metalic sheen	Centrally dark	Centrally dark	Pale	Pale	Pale
Lactose fermenter	+	+	+ (slow)	–	–	–

*The major diagnostic test for *P. aeruginosa* is the production of blue(pyocyanin) pigment with the yellow / green pyoverdin(fluorescein) giving the characteristic blue-green appearance of culture.

Appendix (٢)

Morphological and Biochemical Features for Identification

Test	*Staph. aureus*	*Staph. epidermidis*	*B-haemolytic Streptococci*	*Enterococcus faecalis*
Gram's stain	G+ve cocci (clusters)	G+ve cocci (clusters)	G+ve cocci (chains)	G+ve long cocci (often pairs)
Capsule	–	–	–	–
Oxidase	–	–	–	–
Catalase	+	+	–	–
Coagulase	+	–	–	–
Haemolysis	+(beta)	–	+(beta)	–
Esculin test	–	–	–	+
Urease	–	–	–	–
Growth on MacConkey	–	–	–	+
Mannitol fermenter	+	–	–	+
Motility	–	–	–	–

of Gram Positive Isolates

Appendix (٣)

Antibiotic Resistance of Bacterial Isolates of Skin

Type of bacterial isolates	Number of Isolates	Type of antibi				
		AX* (%)	Py* (%)	AZM* (%)	CTX* (%)	AK (%
Gram positive bacteria	٧	٧ (١٠٠٪)	٧ (١٠٠٪)	٤ (٥٧٪)	٥ (٧١.٤٪)	١ (١٤.٣
Staph. aureus	٤	٤ (١٠٠٪)	٤ (١٠٠٪)	٢ (٥٠٪)	٤ (١٠٠٪)	١ (٢٥٪
Staph. epidermidis	٢	٢ (١٠٠٪)	٢ (١٠٠٪)	١ (٥٠٪)	٠	٠
Enterococcus faecalis	١	١ (١٠٠٪)	١ (١٠٠٪)	١ (١٠٠٪)	١ (١٠٠٪)	٠
Gram negative bacteria	٧١	٧٠ (٩٨.٦٪)	٧١ (١٠٠٪)	٤٢ (٥٩.٢٪)	٧٠ (٩٨.٦٪)	٢٨ (٣٩.٤
Pseudomonas aeruginosa	٢٧	٢٧ (١٠٠٪)	٢٧ (١٠٠٪)	١٦ (٥٩.٢٥٪)	٢٧ (١٠٠٪)	٥ (١٨.٥
Enterobacter spp.	٢٠	٢٠ (١٠٠٪)	٢٠ (١٠٠٪)	١٠ (٥٠٪)	٢٠ (١٠٠٪)	١١ (٥٥٪
E. coli	١٢	١٢ (١٠٠٪)	١٢ (١٠٠٪)	٦ (٥٠٪)	١٢ (١٠٠٪)	٥ (٤١.٦
Klebsiella pneumoniae	٧	٧ (١٠٠٪)	٧ (١٠٠٪)	٧ (١٠٠٪)	٧ (١٠٠٪)	٥ (٧١.٤
Acinetobacter baumannii	٣	٣ (١٠٠٪)	٣ (١٠٠٪)	٢ (٦٦.٧٪)	٣ (١٠٠٪)	٢ (٦٦.٧
Proteus spp.	٢	١ (٥٠٪)	٢ (١٠٠٪)	١ (٥٠٪)	١ (٥٠٪)	

AX- Amoxicillin Py- Carbenicillin AZM- Azithromycin AK- Amikacin

CTX- Cefotaxim CIP- Ciprofloxacin SXT- Trimethoprim-Sulfamethoxazole CN- Gentamicin

Appendix (٤)

Antibiotic Resistance of Bacterial Isolates of Blood Culture

e of isolates	Number of Isolates	Type of antibiotics						
		AX (%)	Py (%)	AZM (%)	CTX (%)	AK (%)	CN (%)	C (%
ve bacteria	١١	١١ (١٠٠٪)	١١ (١٠٠٪)	٧ (٦٣.٦%)	١٠ (٩٠.٩%)	٥ (٤٥.٤٪)	٧ (٦٣.٦%)	(٦٦
taph. aureus	٧	٧ (١٠٠٪)	٧ (١٠٠٪)	٦ (٨٥.٧%)	٧ (١٠٠٪)	٥ (٧١.٤٪)	٦ (٨٥.٧%)	(٧١
Streptococci	٢	٢ (١٠٠٪)	٢ (١٠٠٪)	١ (٥٠%)	١ (٥٠٪)	٠	١ (٥٠٪)	(٥
epidermidis	٢	٢ (١٠٠٪)	٢ (١٠٠٪)	٠	٢ (١٠٠٪)	٠	٠	
ve bacteria	١٣	١٣ (١٠٠٪)	١٣ (١٠٠٪)	٥ (٣٨.٥%)	١٣ (١٠٠%)	٤ (٣٠.٨%)	١٣ (١٠٠٪)	(٢٣
s aeruginosa	٧	٧ (١٠٠٪)	٧ (١٠٠٪)	٣ (٤٢.٨%)	٧ (١٠٠٪)	٣ (٤٢.٨%)	٧ (١٠٠%)	
obacter spp.	٦	٦ (١٠٠٪)	٦ (١٠٠٪)	٢ (٣٣.٣%)	٦ (١٠٠٪)	١ (١٦.٧%)	٦ (١٠٠%)	(٥

Appendix (٥)

Antibiotic Resistance of Bacterial Isolates of Burn Unit

Type of bacterial isolates	Number of Isolates	Type of antibiot				
		AX (%)	Py (%)	AZM (%)	CTX (%)	AK (%)
Gram positive bacteria	٨	٨ (١٠٠٪)	٨ (١٠٠٪)	٥ (٦٢.٥%)	٧ (٨٧.٥%)	٠
Enterococcus faecalis	٤	٤ (١٠٠٪)	٤ (١٠٠٪)	٣ (٧٥%)	٤ (١٠٠٪)	٠
Staph. aureus	٣	٣ (١٠٠٪)	٣ (١٠٠٪)	٢ (٦٦.٧%)	٣ (١٠٠٪)	٠
Staph. epidermidis	١	١ (١٠٠٪)	١ (١٠٠٪)	٠	٠	٠
Gram negative bacteria	١٦	١٦ (١٠٠٪)	١٦ (١٠٠٪)	٧ (٤٣.٧٥%)	١٤ (٨٧.٥%)	٥ (٣١.٢٥%
Pseudomonas aeruginosa	٦	٦ (١٠٠٪)	٦ (١٠٠٪)	٢ (٣٣.٣%)	٦ (١٠٠٪)	١ (١٦.٦%
Enterobacter spp.	٣	٣ (١٠٠٪)	٣ (١٠٠٪)	١ (٣٣.٣%)	٣ (١٠٠٪)	٣ (١٠٠%
E. coli	٣	٣ (١٠٠٪)	٣ (١٠٠٪)	o	١ (٣٣.٣%)	o
Acinetobacter baumannii	٢	٢ (١٠٠٪)	٢ (١٠٠٪)	٢ (١٠٠٪)	٢ (١٠٠٪)	١ (٥٠٪)
Klebsiella pneumoniae	٢	٢ (١٠٠٪)	٢ (١٠٠٪)	٢ (١٠٠%)	٢ (١٠٠٪)	o

١.١ Introduction

Burns are one of the most common and devastating forms of trauma. They induce a state of immunosuppression that predisposes burn victims to infectious complications(Church *et al.*, ٢٠٠٦). The latter is the primary cause of morbidity and mortality in burn victims (Wibbenmeyer *et al.*, ٢٠٠٦) .

The cause and risk of burn injury as well as the risk of burn death are influenced by the age of the patient, economic circumstances, season of the year and occupation; i.e., the risks of burn injury and fire death are greatest in the extremes of age, the low socioeconomic class and in winter months (Pruitt and Mason, ١٩٩٦; Kobayashi *et al*., ٢٠٠٢).

Burn injury destroys the physical skin barrier that normally prevents the invasion of microorganisms and consequently, this injury provides novel sites for bacterial colonization, infection and clinical sepsis(Vindenes and Bjerknes, ١٩٩٥).

It is clear that large open wound areas containing necrotic tissue and the general state of immunosupression that is caused by impaired cellular and humoral immunity make burn victims more susceptible to infection (Gang *et al*., ١٩٩٩). In these conditions, microorganisms can easily multiply and colonize the wounds to high densities(Hodle *et al*., ٢٠٠٦). 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 blood vessels and urinary bladder; also in these units, both the air and environmental surfaces are heavily contaminated. This is why burn victims are high risk groups for infections(Rastegar Lari *et al*., ٢٠٠٥).

Measures to prevent and treat infections are essential for the survival of patients with extensive burns(Ahmad *et al.,* ٢٠٠٦). Initially, the burned area is considered free of major microbial contamination. However, Gram positive bacteria in the depth of sweat glands and hair follicles may survive the impact of initial injury and unless topical antimicrobial agents are applied, these bacteria heavily colonize the wounds within the first ٤٨ hours post-injury. The organisms that predominate as causative agents of burn wound infection in any burn unit change over time where Gram positive organisms are initially prevalent and then gradually superseded by Gram negative opportunists(Pruitt and McManus, ١٩٩٢).

To minimize the development of antibiotic resistance of the bacteria present at the burn wound and in other parts of the body, systemic antibiotic should not be given prophylactically but should be administered only on the basis of a secure clinical or laboratory diagnosis of infection (Pruitt *et al.,* ١٩٩٧).

Aims of The Study

Up to our knowledge, there is no recorded studies about the immune status, the bacterial aetiology and antibacterial treatment of burn patients who are admitted to the burn unit at Al-Hilla General Teaching Hospital, hence this study aims to:

١- Isolate and identify the aerobic bacteria from the skin and blood of burn patients as well as from burn unit, then to identify the relation among these bacterial isolates.

٢- Study the effect of some antibiotics on bacterial isolates.

٣- Study the effect of silver sulfadiazine and silver nitrate on bacterial skin isolates.

٤- Clarify some clinical parameters including age, sex of burn patients and the Boyd index for the dead cases.

٥- Study some aspects of the humoral immunological factors in burn patients.

١.٢ Literature Review

١.٢.١ Anatomy and Physiology of Skin

Skin, the largest organ of the body, serves as a protective barrier preventing internal tissues from exposure to trauma, ultraviolet radiation, temperature extremes, toxins and bacteria, and it has other important functions which include sensory perception, immunologic surveillance, thermoregulation and control of insensible fluid loss(Hettiaratchy and Dziewulski, ٢٠٠٤).

The skin consists of three layers: epidermis, dermis, and subcutaneous tissue. Epidermis, the outermost layer, is composed of stratified epithelium and has two components: an outer layer of anucleate cornified cells called stratum corneum that covers an inner layer of viable cells which is the Malpighian layer, from which cornified superficial cells arise by differentiation(Palastanga *et al.*, ٢٠٠٠).

Beneath the epidermis is the dermis which is composed of dense fibroelastic connective tissue stroma containing collagen elastic fibers and an extracellular gel termed the ground substance. The dermis contains an extensive vascular and nerve network and special glands and appendages communicating with the overlying epidermis (Klein *et al*., ٢٠٠٤). The dermis is divided into two parts; the first is the most superficial portion, the papillary dermis, is

moulded against the epidermis and contains superficial elements of the microcirculation of the skin and it consists of relatively cellular, loose connective tissue with smaller, fewer collagen and elastic fibers than the second underlying portion, which is the reticular dermis(Marks, ٢٠٠٣).

Beneath the dermis is the third layer of the skin which is the subcutaneous tissue. It is composed of areolar and fatty connective tissue and contains skin appendages. It supports the dermis and the epidermis and provides an important source of stored energy(Hettiaratchy and Papini, ٢٠٠٤).

١.٢.٢ Mechanisms of Burn Injury

١.٢.٢.١ Scald Injury

In children under ٨ years of age, the most common burns are scalds(Herendon and Spies, ٢٠٠١). In other words, about ٧٠٪ of burns in children are caused by scalds, however; they also occur in elderly people. The common mechanisms are spilling hot drinks or liquids or being exposed to hot bathing water(Hettiaratchy and Dziewulski, ٢٠٠٤).

Scald burns are the leading cause of pediatric burn admission and related morbidity, as well as a major cause of pediatric death (Haziniski *et al*., ١٩٩٣). Exposed areas of skin tend to be burned less deeply than clothed areas, as the clothing retains the heat and keeps

the hot liquid in contact with the skin for a longer period of time (Yeoh *et al.,* ١٩٩٤).

١.٢.٢.٢ Flame Injury

Flame burns are the second most common mechanism of thermal injury(Munster, ١٩٩٦). They comprise ٥٠٪ of adult burns and are often associated with inhalational injury and other concomitant trauma(Hettiaratchy and Dziewulski, ٢٠٠٤). Although the incidence of injuries caused by house fires has decreased with the use of smoke detectors, smoking-related fires, improper use of flammable liquids, motor-vehicle collisions and ignition of clothing by stoves or space heaters also are responsible for flame burns. Patients whose bedding or clothes have been on fire rarely escape without some full-thickness burns(Pruitt *et al.,* ١٩٩٨).

١.٢.٢.٣ Other Mechanisms of Burns

Flash burns are caused by intense heat for a brief time e.g., explosions of natural gas, propane, butane, petroleum distillates, alcohols and other combustile liquids(Yarbrough, ١٩٩٨). They are typically epidermal or partial thickness and their depth depends on the amount and kind of fuel that explodes and time of exposure (Pruitt *et al.,* ١٩٩٧).

Contact burns result from contact with hot metals, plastics, glass, or hot coals(Margulies *et al*., ١٩٩٨). They are usually limited in extent, but are invariably deep(Ahuja and Bhattacharya, ٢٠٠٤).

Other burn injuries include the electrical injuries in which the electric current will travel through the body from one point to another(Still *et al*., ١٩٩٧). While the chemical burns are usually the result of industrial accidents and may occur with household chemical products, they tend to be deep as a corrosive agent continues to cause coagulative necrosis until completely removed. There are two aspects of a chemical injury, the first is the physical destruction of the skin and the second is any poisoning caused by systemic absorption(Wassermann, ٢٠٠٢).

١.٢.٣ Pathophysiology of Burn Injury

Thermal injury causes coagulative necrosis of the skin and underlying tissues to variable depth and it exerts deleterious effects on all other organ systems with the extent and duration of organ dysfunction proportional to the extent of burn(Hettiaratchy and Dziewulski, ٢٠٠٤).

١.٢.٣.١ Local Response of Skin to Burn Injury

Regarding the local response of skin to burn injury, there are three zones of a burn first described by Jackson in ١٩٤٧ as follows(Klein *et al.,* ٢٠٠٤):

a. Zone of coagulation: it occurs at the point of maximum damage and in this zone there is an irreversible tissue loss due to coagulation of the constituent protein.

b. Zone of stasis: it surrounds the previous zone and is characterized by decreased tissue perfusion and the tissue in this zone is potentially salvageable and the main aim of burn resuscitation is to increase tissue perfusion here and prevent any damage becoming irreversible.

c. Zone of hyperaemia: in this outermost zone tissue perfusion is increased and the tissue will invariably recover unless there is severe sepsis or prolonged hypoperfusion.

١.٢.٣.٢ Systemic Response to Burn Injury

Regarding the cardiovascular response, the changes that occur in this system are of vital importance and require treatment priority in order to limit volume deficits and prevent the development of hypovolaemic shock(Pruitt *et al*.,١٩٩٧). The cardiovascular response has two separate phases: the first is the acute or resuscitative phase

which immediately follows burn trauma and lasts for about ٤٨ hours. It is characterized by hypoperfusion of tissues and organs and is thought to be caused by hypovolemia following injury(Pruitt and Mason,١٩٩٦). The second phase is the hypermetabolic phase characterized by increased blood flow to the tissues and organs and increased internal core temperature. During this phase rapid edema formation occurs and is attributed to hypoproteinaemia which favours the outward movement of water from the capillary to the interstitium (Demling, ٢٠٠٥).

The pulmonary response is characterized by an increasing evidence of lung inflammation(pneumonitis) beginning in the first several hours after a local burn injury and lasting for at least ٥ days and these processes are initiated by oxidants, especially hydroxyl radicals. Moreover; systemic activation of complement may initiate the process of lung inflammation(Sener *et al.,* ٢٠٠٢). The inflammatory mediators cause bronchoconstriction and, in severe burns, adult respiratory distress syndrome can occur(Hettiaratchy and Dziewulski, ٢٠٠٤). Inhalational injury is caused by the minute particles within thick smoke, which, because of their small size, are not filtered by the upper airway, but are carried down to the lung parenchyma and stick to the moist lining causing an intense reaction in the alveoli leading to chemical pneumonitis. The presence of

inhalational injury has a very significant effect on the mortality of any burned patient(Wassermann, ٢٠٠٢).

Regarding the renal response, there is a post burn decrease in renal blood flow and glomerular filteration rate(GFR) because of the diminished blood volume and cardiac output, the resulting oliguria if untreated may progress to acute renal failure. However, following resuscitation, renal blood flow and GFR increase to suprarenal levels in concert with the increase in cardiac output characteristic of post burn hypermetabolism(Pruitt *et al*., ١٩٩٧). It is quite clear that acute renal failure is rare if prompt, adequate resuscitation is accomplished. Although the GFR may decrease in the first few hours following burn injury, it rapidly returns to a normal level with adequate resuscitation(Dimick, ١٩٨٨). There is another form of delayed renal failure which develops later and has a more complex pathogenesis because it is related to sepsis and multi-organ failure and is most often fatal, this is attributed to hypovolemia and ischemia secondary to multi-organ failure and sepsis, in addition there may be precipitation of myoglobulin in the renal tubules leading to tubular necrosis with resultant renal failure (Planas *et al*., ١٩٨٢).

While the gastrointestinal response includes the following conditions: adynamic ileus, gastric dilatation, increased gastric secretion and ulcer incidence, gastrointestinal hemorrhage and

decrease mesenteric blood flow which are among the important effects of thermal injury on gastrointestinal system(Smith *et al*., ١٩٩٨). Increased bacterial translocation and macromolecular leak have been well documented after burn injury(Herek *et al*., ٢٠٠٠). Intestinal ischemia resulting from decreased splanchnic blood flow may activate the neutrophils and tissue bound enzymes such as xanthin oxidase and those factors destroy the gut mucosal barrier and result in bacterial translocation; i.e., there is a gut barrier leak after burn which may be the source of circulating endotoxins. Endotoxin, a lipopolysaccharide (LPS) derived from the outer membrane of Gram negative bacteria, translocates across the gastrointestinal tract barrier within one hour of thermal injury (Alexander *et al*.,١٩٩٠). Endotoxins are potent activators of the macrophages and neutrophils, this leads to the release of the massive amounts of oxidants; arachidonic acid metabolites and proteases, which cause further local and systemic inflammation in burn-induced tissue damage(Youn *et al.,* ١٩٩٥).

١.٢.٤ Immune Response Secondary to Thermal Burns

Major injury due to trauma and burns has been demonstrated to increase susceptibility to infectious complications and related multi-organ failure because of a suppressed immune system (Atiyeh and Al-Amm, ٢٠٠١). Various components of immune system have been incriminated, including loss of barrier function of skin, tissue

ischemia and destruction(Mack *et al.*,١٩٩٦), neutrophil dysfunction, abnormality in opsonic activity, depletion of complement cascade, helper cell dysfunction, macrophage dysfunction and increase in the prostaglandin E٢(PGE٢) level(Decker *et al.,* ١٩٩٦). Furthermore, Atieyh and Al-Amm (٢٠٠١) suggest that the immune suppression is the result of T-cell dysfunction with failure of interleukin-٢(IL-٢) production. Kelly *et al.*(١٩٩٧) point out that susceptibility to sepsis after severe burn injury is correlated with reduced production of T-helper-١(Th-١) cytokines(IL-٢) and interferon-gamma(IFN-γ). Likewise, they mention that burn injury induces the loss of antigen-specific Th-١ cell function and IL-١٠ acts as a trigger to down-regulate Th-١ activity after injury. It seems that the normal immune defense mechanisms start to become suppressed in burn injuries of around ٢٥٪ of total body surface area(TBSA)(Di Piro *et al.,* ١٩٩٥). Robins(١٩٩٠) point out that neutrophil intracellular killing power is reduced as oxygen delivery to the wound is decreased. Besides, cell-mediated immunity is depressed and T-cell lymphocyte counts are decreased. Meakins (١٩٩٠) suggests that activation of a pro-inflammatory cascade plays an important role in the development of major complications associated with burn trauma. With regard to this, macrophages are major producers of pro-inflammatory mediators, namely PGE٢, IL-٦ and tumor necrosis factor-alpha(TNF-α)(MacMicking *et al.,* ١٩٩٧). Dysregulation of macrophage activity leading to increased release of pro-inflammatory factors appears to

be of fundamental importance in the development of the post-burn immune dysfunction, in addition to the T-cell dysfunction, glucocorticoids and T-helper(Th)٢ cytokines which are also regarded as causative factors for post-burn immune dysfunction(Schwacha and Somers, ١٩٩٨). Barlow(١٩٩٤) stated that thermal trauma resulted in impaired immune function which had been attributed to reduction in T lymphocyte numbers, increased suppressor cell activity, serum suppression factors and related cytokine synthesis and receptor expression on T-cells. Messingham *et al.* (٢٠٠٢) found that the loss of lymphocyte production of IL-٤ after burn injury may contribute to the exaggerated production of IL-٦, a known mediator of immune suppression after injury. IL-٢ is the primary cytokine required for T-lymphocyte activation and proliferation. It has been well established that one of the main factors contributing to decrease T-lymphocyte function, and thus cellular immunity, after burn injury is a dramatic decline in IL-٢ production(Messingham *et al.,* ٢٠٠١). Another important immunological aspect of thermal injury is the increased production of eicosanoids, which are metabolites of arachidonic acid(e.g. prostaglandins, leukotrienes, thromboxanes). Prostaglandins, which are elevated in burned patients, are considered important immunosuppressive mediators and macrophages from burned hosts exerting an enhanced prostaglandin productive capacity(Schwacha *et al.*, ٢٠٠٢). Since cyclo-oxyginase enzyme is responsible for some of the deleterious consequences

associated with thermal injury, cyclo-oxyginase enzyme inhibitors are capable of restoring the various aspects of immune function and improve survival after thermal injury(Strong *et al*., ٢٠٠٠).

It was concluded that severe burn injury regularly induces an early transient increase in circulating suppressor cells accompanied by a depression of lymphocyte activation. A later (greater than ١٤days post-burn) increase in suppressor cells to levels detectable by functional assays is closely correlated with mortality from sepsis (McIrvine *et al*., ١٩٨٢). Prolonged skin allograft survival in burned patients is a good indicator for the suppressed cellular immune responsiveness in these patients(Demling, ٢٠٠٥).

١.٢.٤.١ Effect of Thermal Burns on Humoral Immune Response

Immunoglobulin G (IgG) accounts for approximately ٧٥٪ of the total serum immunoglobulins in normal adults and is the most abundant antibody produced during secondary humoral immune responses in the blood. It is the only antibody to pass through the placenta and is therefore the most abundant immunoglobulin in newborns. Immunoglobulin M(IgM), which constitutes approximately ١٠٪ of normal serum immunoglobulins, is the main immunoglobulin produced early in the primary immune response and is the most efficient complement fixing immunoglobulin.

Immunoglobulin A(IgA) is the predominant immunoglobulin produced by B-cells in Peyer's patches, tonsils, and other submucosal lymphoid tissues and it accounts for only ١٠-١٥٪ of serum immunoglobulin, it is by far the most abundant antibody class found in saliva, tears, intestinal mucus, bronchial secretion, milk, prostatic fluid and other secretions, so it is called the secretory immunoglobulin A(S-IgA)(Brooks *et al*., ٢٠٠٤). Roberts and Steihm(٢٠٠١) point out that in severe thermal burns, acquired immuno-deficiency occurs as a result of protein-losing states, this reflects the decrease in the level of IgA after burn injury. Serum IgG levels are decreased after burn injury and gradually return to normal over the next two to four weeks(Pruitt *et al*., ١٩٩٧). Dimick (١٩٨٨) points out that the depression of immunolobulins both IgG and IgM occurs and persists for ٢-٣ weeks following burn injury. Robins(١٩٩٠) mentioned that humoral immunity is altered with the drop in the IgG levels. Tabata *et al*.(١٩٩٦) clarify that there is decreased IgM synthesis after burn injury which may represent continued immune impairment in burn patients. Moreover, the immunoglobulin levels including IgA are decreased in patients with burn injury(Maitra, ٢٠٠٣; Demling, ٢٠٠٥).

١.٢.٤.٢ Effect of Thermal Burns on Complement Activation

Complement component ٣(C٣) is the critical component for the activation of all pathways of complement activation, thus this

component is associated with specific(classical) and nonspecific (lectin and alternative) pathways. Complement component ٤(C٤) is the second component of complement system in the classical pathway which is activated via antibody-dependent pathway; this means that this component is associated with specific immunity (Brooks *et al*., ٢٠٠٤). The final step of complement activation in all pathways is the formation of membrane attack complex(MAC)which makes holes and destroys membrane of bacteria(Roitt *et al*., ٢٠٠١). Magliacani and Stella(١٩٩٠) pointed out that massive activation of the alternative complement pathway was seen in burn injured patients. This activation was associated with the generation of neutrophil aggregating activity in the plasma, neutrophil aggregates in the lungs, increased pulmonary vascular permeability, and increased lung edema formation. Furthermore, they mentioned that decomplementation with analogue of cobra venom factor or genetic C٥ deficiency diminished these pathologic changes and cobra venom factor pretreatment substantially reduced burn mortality in the first ٢٤ hours after injury. During burn injury, complement disappears rapidly but is also reconstituted rapidly (Dimick, ١٩٨٨).

١.٢.٥ Burn Severity

There are many factors that determine the severity of a burn injury including the size of the burn which is the major factor, the depth of the injury, the age and general health of the patient and the

presence or absence of inhalational injury(Glasheen *et al*., ١٩٨٣). A general idea of the burn size can be made by using the rule of nines for adult patients and by Lund-Browder charts that are particularly helpful in assessing pediatric burns. For smaller burns, an accurate assessment of burn size can be made by using the patients palmer hand surface including the digits, which accounts to approximately ١٪ of TBSA (Hettiaratchy and Papni, ٢٠٠٤).

Burn wound depth is a significant determinant of patient's treatment and morbidity. According to their increasing depth, burn wounds are classified into ٤ categories: epidermal(first degree burn), superficial and deep partial thickness(second degree burn), full thickness (third degree burn) and fourth degree burns(Johnson and Richard, ٢٠٠٣). Superficial burns and superficial partial thickness burns typically heal without any need for surgical excision and grafting. Dressing changes and daily wound care can remove necrotic debris and facilitate healing with minimal scaring. For deep partial thickness and full thickness burns, however, operative debridement with subsequent skin graft coverage is necessary(Klein *et al*., ٢٠٠٤). The four degrees of burns(Kao and Garner, ٢٠٠٠; Hettiaratchy and Papni, ٢٠٠٤) are :

a-First degree burns: in these burns, minor epithelial damage of epidermis exists with redness, tenderness and pain but without blistering.

b-Second degree burns are superficial partial and deep partial thickness burns in which some portion of the skin appendages remains viable, allowing epithelial repair of the burn wound without skin grafting. The superficial partial thickness burn involves the epidermis and superficial (papillary) dermis. They are pink, moist, soft and tender, healing in approximately ٢-٣ weeks usually without scarring by outgrowth of epithelial buds from the viable pilosebaceous units and sweat glands residing in papillary and reticular dermis. Deep partial thickness burns extend into the reticular dermis and heal within ٣-٦ weeks, but with scarring. Skin graft is prefered for this type of burn.

c-Third degree burns are full-thickness that destroy both epidermis and dermis. Skin grafting is always necessary to resurface the injured area.

d-Fourth degree burns cause full thickness destruction of skin and subcutaneous tissue with involvement of underlying fascia, muscle, bone or other structures.

١.٢.٦ Burn Wound Infection

Infection is a major complication of burn injury and is responsible for ٥٠-٦٠٪ of deaths in burn patients. It is promoted by loss of epithelial barrier of skin, malnutrition induced by the

hypermetabolic response to burn injury and by a generalized post-burn immunosuppression (Abston *et al*., ٢٠٠٠).

The burned tissue, which is rich in protein and moist by virtue of the trans-eschar movement of fluid and serum serves as an excellent microbial culture media(Pruitt *et al*., ١٩٩٧).

Microbial colonization of the open wound, primarily from an endogeneous source begins within ٢٤ hours and is usually established by the end of first week after burn injury (Noronha and Almeida, ٢٠٠٠).

Since there is a variability of both local and systemic clinical manifestations of invasive burn wound infection, great emphasis is placed on the proper identification of burn wound microbial flora by the clinicians treating burn wound sepsis(Ahmad *et al.,* ٢٠٠٦).

Infection risk is directly correlated with the area of burn injury; the larger the injured area, the higher the risk of infection(Wibbenmeyer *et al*., ٢٠٠٦). Other contributors to infection include comorbid conditions, the use of invasive devices such as catheters and poor hand hygiene among health care staff(Hodle *et al.,* ٢٠٠٦).

Because of the immunocompromized state of burn patients as well as their intense longstanding hypermetabolism, they do not exhibit the usual clinical parameters of infection. Thus the surgeon

must be constantly aware of the clinical state of the patient and be alert for any subtle changes which are often the first indicators of incipient sepsis e.g., any changes in burn wound appearance, softening of the eschar or surrounding cellulitis, beginning of purulent material to issue from the wound or once-healthy granulation tissue may begin to deteriorate (Press, ١٩٩٧). In other words, the occurrance of local, multifocal or generalized dark brown, black or violaceous discoloration of the burn wound is one of the earliest and most frequently observed signs of burn wound infection(El Morsi, ١٩٩٠).

١.٢.٦.١ Mode of Transmission of Infection

There are numbers of ways in which microorganisms can gain access to a wound either by direct contact by transfer of microorganism from equipment or hands of health care workers, or by air born dispersal in which microorganisms deposited from the surrounding air, or lastly by self-contamination from the patient's skin or gastrointestinal tract(Collier, ٢٠٠٣).

١.٢.٦.٢ Diagnosis of Burn Wound Infection

The diagnosis of infection in burn patients is confounded by the fact that most of the systemic and many of the laboratory signs of infection are mimicked by the physiologic response to severe injury

i.e. burn patients have an inflammatory state from the injury itself that can mimic infection(Ansermino and Hemsley, ٢٠٠٤).

Because clinical and laboratory signs are not reliable in making the diagnosis of infection in severely burned patients, reliance must be placed on daily examination of the entire burn wound to identify changes indicative of local infection. The examination of the wound is best carried out at the time of daily cleansing when all dressings and topical applications have been removed (Pruitt *et al.,* ١٩٩٨). Local dark red, brown or black discolouration of the burn wound is the most common tinctorial change associated with burn wound infection(Pruitt and McManus, ١٩٩٢). Conversion of an area of partial-thickness injury to full-thickness necrosis is the most reliable sign of burn wound infection(Al-Akayleh, ١٩٩٩).

Wound biopsy, followed by histological examination and quantitative culture, is the definitive method for the diagnosis of burn wound infection. However, it is time-consuming and expensive, making it impractical as a routine diagnostic technique. Therefore, diagnosis of infection relies strongly on clinical parameters, with the aid of blood, skin surface cultures to identify likely pathogens(Ansermino and Hemsley, ٢٠٠٤).

١.٢.٧ Systemic Sepsis

As local microbial growth increases, the potential for invasion to subjacent viable tissue and penetration into circulation increases. The burn wound, especially when covered with necrotic eschar, is a common site for primary infection in burned patient; other sites are common including the upper and lower respiratory tracts, urinary tracts and less frequently infections from osteomyelitis or suppurative phlebitis (Rabson, ١٩٨٨).

Many organisms that are normal flora on the skin and in the intestine are beneficial and pose no threat, but when they spread throughout the body by way of blood stream, they can progress to overwhelming infection unless the body defenses destroy them. In other words, if the microorganisms from the burned wound invade the unaffected tissue, local sepsis develops; if they invade the lymphatic and vascular systems, systemic sepsis develops(Komolafe *et al.,* ٢٠٠٣). Even so, when sepsis ensues, while awaiting the results of blood cultures, knowledge of the organisms that colonize a burn wound can facilitate prompt and appropriate antibiotic treatment that is based on the expected sensitivity of the identified microorganism, rather than initiating a purely empirical therapy. So there is need for qualitative and quantitative tests that are more rapid than bacterial culture (Devos *et al*., ١٩٩٧).

Although effective topical antimicrobial chemo-therapy and early excision of burn wounds have significantly reduced the

occurrence of invasive burn wound infections, sepsis is still a major problem(De Macedo *et al*., ٢٠٠٣). The risk of septicaemia increases in proportion to the degree of cutaneous infection(Pirnay *et al*., ٢٠٠٣).

Clinically, any change in the patient's general health should lead to a high suspicion of sepsis. Possible changes include unexplained hypotension, tachypneoa, spiking fevers above the patient's daily baseline, tachycardia, new onset of ileus, altered mental status, thrombocytopenia, hyper or hypoglycemia, hypoxia or hypothermia and decreased urine output or progressive leucocytosis and/ or leucopenia(Press, ١٩٩٧).

١.٢.٨ Nosocomial Infection

A nosocomial infection is one that is acquired in a hospital or health care facility. In the same manner, the infection was not present or incubating at the time of admimission (Diekema and Pfaller, ٢٠٠٣).

Nosocomial infections are considerable problem for health services in all countries, with serious effects on the survival of high risk patients, such as burn patients. In burn ward, primary blood stream infections, pneumonia and infection of burn sites are very dangerous complications that can compromise the patients survival and the outcome of reconstructive treatment (Torregrossa *et al.,*

٢٠٠٠). The microbiological profile studies reflect the hospital environment and vary from hospital to another(Ogunsola *et al*., ١٩٩٨). In general, there has been a change in the main infective organisms over time from β-hemolytic streptococci to resistant Gram negative organisms including *Pseudomonas*, resistant Gram positive organisms, and fungi. However, infection control measures help to minimize cross infection between patients and acquisition of nosocomial pathogens such as methicilline-resistant *Staphylococcus aureus*(MRSA) or multi-resistant Gram-negative bacteria(Ansermino and Hemsley, ٢٠٠٤).

The medical and nursing staff can also be responsible for cross-contamination between patients simply by moving from one room to another-they are an involuntary vehicle for germs. So good hygienic and environmental control can prevent the diffusion and the development of multi-resistant germs(Bollero *et al.,* ٢٠٠٣). Clinical trials demonstrate that hand washing can decrease approximately ٣٠٪ of the incidence of nosocomial infections(Doebbeling *et al.,* ١٩٩٢).

١.٢.٩ Bacterial Aetiology

١.٢.٩.١ *Pseudomonas aeruginosa*

It is a non-fermentative aerobic Gram negative rod that is prevalent in hospital environment and can cause severe nosocomial

infections besides its ability to cause disease in particular susceptible individuals(Passador *et al.,*١٩٩٣).

Despite the introduction of a wide variety of antimicrobial agents with antipseudomonal activities, life threatening infections caused by *Pseudomonas aeruginosa* continue to be a common complication in burn patients(Estahbanati *et al.,* ٢٠٠٢) and to contribute substantially to burn related morbidity and mortality world-wide(Mayhall, ١٩٩٦).

Numerous *Pseudomonas aeruginosa* virulence factors contribute to the pathogenesis of burn wound infections. Pilli and flagella are essential for their ability to persist in the burn wound and cause disseminated infections (Gillespie and Bamford, ٢٠٠٣). Protease promotes infection and dissemination. Elastase degrades collagen and non-collagen host proteins. Therefore, elastase disrupts the host physical barriers which inhibit the spread of infections(Lyczak *et al*., ٢٠٠٠). Elastase also inhibits monocyte chemotaxis which can clear the infections by phagocytosis as well as antigen presentation to the host immune system(Kharazmi and Nielsen, ١٩٩١). Multi-drug resistant *P. aeruginosa* has frequently been reported as the cause of nosocomial outbreaks of infection in burn units or as colonizers of the wounds of burn patients(Richard *et al*., ١٩٩٤).

١.٢.٩.٢ *Staphylococcus aureus*

It is a Gram positive spherical non-motile bacterium usually arranged in grape-like irregular clusters(Mims *et al.,* ٢٠٠٤). It is one of the most virulent and common nosocomial pathogens. The colonization with *Staphylococcus aureus* is associated with more operative procedures and prolonged admissions(Reardon *et al*., ١٩٩٨). The mechanism of antimicrobial resistance in *Staphylococcus aureus* is an alteration in the penicillin-binding proteins of the organisms and it confers cross-resistance to all other β-lactam antimicrobials including cephalosporins (Lowy, ١٩٩٨).

The risk factors associated with MRSA infections are age, ward type, previous hospitalization, invasive procedures and length of hospitalization(Asenssio *et al*., ١٩٩٦). MRSA can be transmitted by hands and environmental routes. MRSA colonization/ infection in a burn unit has an important implication because it provides reservoir within the unit and increases the risk of infection for other patients. Infection with MRSA increases the morbidity and mortality in burn patients including the risks for bacteremia and loss of skin grafts(Cook, ١٩٩٨).

The pathogenicity of staphylococci contributes to hemolysis of the blood, coagulation of the plasma and production of extracellular enzymes and toxins which act on host cell membrane and mediated the cell destruction(Mims *et al*., ٢٠٠٤).

١.٢.٩.٣ *Enterococcus faecalis*

They are Gram positive cocci, facultative anaerobe, catalase-negative, hydrolyze esculin and they grow readily on ordinary nutrient media and on MacConkey agar(Collee *et al*., ١٩٩٦).

Enterococci have emerged as an important nosocomial pathogen (Murray, ٢٠٠٠). They are the third most common cause of primary blood stream infections in medical-surgical intensive care units in the United States of America(Kao and Garner, ٢٠٠٠). About ٨٠-٩٠٪ of entrococcal infections are caused by *E. faecalis* (Cetinkaya *et al.,* ٢٠٠٠).

The major reasons that enterococci stay in the hospital environment are their intrinsic resistance to commonly used antimicrobials and their ability to develop resistance either by mutation or by receiving foreign genetic materials(Jones *et al*., ١٩٨٦). Enterococci can spread resistant genes to other bacteria by conjugation and genetic exchange through plasmids and transposons. Vancomycin-resistant enterococci spread rapidly and have become a major problem in many institutions during the last decade(Franz *et al*., ١٩٩٩; Bonten *et al.,* ٢٠٠١).

١.٢.٩.٤ *Streptococcus pyogenes*

It was the most common cause of burn wound and systemic infections in the pre-antibiotic era and is still to be feared in burn wounds(Al-Akayleh, ١٩٩٩). It is usually seen within the first few days

of injury and it is characterized by rapid deterioration in the state of burn wound and invasion of neighboring healthy tissue. Penicillins are the drug of choice for treatment and erythromycin or vancomycin can be used for penicillin allergic patients(Mims *et al*., ٢٠٠٤).

١.٢.٩.٥ *Acinetobacter baumannii*

They are strictly aerobic, Gram negative bacilli or coccobacillary rods (often diplococco-bacilli), catalase-positive, oxidase-negative, non-lactose fermenter (Brooks *et al.,* ٢٠٠٤). *Acinetobacter* species are one of the causes of persistant nosocomial infections. They are carried on the hands of hospital personnel(Hsueh *et al.,* ٢٠٠٢) and are most likely to involve the respiratory tracts, urinary tracts and wounds and may progress to septicaemia(Cisneros *et al*., ١٩٩٦). Isolates of *A.baumannii*, particularly those recovered from patients with nosocomial infections, are frequently resistant to multiple antimicrobial agents, including cephalosporins, aminoglycosides and quinolones(Pandey *et al.,* ١٩٩٨).The pathogenicity of bacteria is related to the presence of small capsule and production of β-lactamase similar to that of MRSA bacteria with high antibiotic resistance(Iskandar *et al*., ٢٠٠٣).

١.٢.٩.٦ *Klebsiella pneumoniae*

Bacteria belonging to the genus *Klebsiella* frequently cause human nosocomial infection, in particular the medically most important species, *K.pneumonia* which accounts for a significant proportion of nosocomial infection like urinary tract infection, pneumonia, septicaemia and soft tissue infection (Podschun and Ullmann, ١٩٩٨). Most of the clinical isolates of *K.pneumoniae* are fully encapsulated and adhere *in vitro* to intestinal cell lines with aggregative patterns. Its polysaccharide capsule plays an active role during the initial steps of pathogenesis by protecting the bacterium from phagocytosis by the polymorph-nuclear granulocytes and prevents killing of the bacteria by bactericidal serum factors(Podschun *et al*., ١٩٩٢). Several pilli are also involved in the adherence of this bacterium to the host cell (Tarkkamen *et al*., ١٩٩٠).

١.٢.٩.٧ *Enterobacter* spp.

It is a motile member of enterobacteriaceae. It rarely causes disease in a healthy individual(Alhambra *et al*., ٢٠٠٤). Patients most susceptible to acquire infections with this opportunistic pathogen are those who stay in the hospital, especially the intensive care units for prolonged periods, those using foreign devices such as intravenous catheter and those with serious underlying conditions including burns and immunosupression(Clark *et al*., ٢٠٠٣). Most isolates involved in nosocomial infections are resistant to multiple antibiotics(Arpin *et.al.,*١٩٩٦).

١.٢.١٠ Burn Wound Care

Attention should be directed to the burn wound itself only after resuscitation has been initiated and hemodynamic and respiratory stability are being restored(Pruitt *et al.,* ١٩٩٧).

It is important that the treatment of burn infection includes antibiotic therapy, removal of necrotic tissues, ensuring the blood and oxygen supply to the wound, the augmentation of the immune state of the burned patient and the adequate diet (Bowler *et al*., ٢٠٠١).

One aim of initial wound management is to prevent invasive infection. So aggressive surgery and the use of topical antimicrobial agents are effective. The latter slows wound colonization and is of use before definitive surgery(Ansermino and Hemsley, ٢٠٠٤).

١.٢.١٠.١ Systemic Antibiotics and Topical Antimicrobial Treatment in Burn

When properly used, systemic antibiotics are considered as a valuable therapeutic modality in the burn patient; however, injudicious use may not only fail to be beneficial to the patient but may also produce harmful effect either through direct toxicity or by contributing to the emergence of resistant strain of microorganism(Lesseva and Hadjiiski, ١٩٩٨).

Combination of antibiotics in treatment of burn infections are not always synergistic or even additive in effect, they may have a predisposing effect to superinfection by yeast or resistant organisms. In general, prophylactic systemic antibiotics for burned patients are indicated in only few clinical situations including the immediate preoperative and postoperative periods associated with excision and auto grafting and possibly in the early phases of burn in children(Dasco *et al.,* ١٩٨٧).

Imipenem/ cilastatin has been used during the last years for the treatment of life threatening infections caused by multi-drug resistant nosocomial strains in patients with severe burns(Lesseva and Hadjiiski, ١٩٩٨). It is active against Gram negative aerobic and anaerobic pathogens including *Enterobacter* spp., S*eratia* spp*., P. aeruginosa, Acinetobacter* spp., and also(unlike third generation cephalosporin) against Gram positive organisms like coagulase-negative and positive Staphylococci, and Streptococci(Wilson, ١٩٩٥). Antibiotic usage patterns that are effective in one ward may not be effective in another ward or at another time in the same ward(Sanyal *et al*., ١٩٩٨).

Selective decontamination of the digestive tract using non-absorbable antimicrobial agents that spare the anaerobic flora was shown to reduce colonization and infection rates in intensive care unit patients including trauma patients(Reidy and Ramsy, ١٩٩٠).

This approach was of value in the management of severe burns (>٣٠٪ TBSA). The regimen for adults consisted of tobramycin (٨٠mg), polymyxin E(١٠٠mg), and amphotercin B (٥٠٠mg)given orally or through the nasogastric tube four times a day (Kanchannapoom and Khardori, ٢٠٠٢).

In order to prevent the emergence of resistance to antibiotics, the current recommended treatment protocols suggest withholding the administration of antibiotics until there is sufficient evidence of infection in the burn wounds and focus on preventive measures such as debridement and cleansing of the affected area. The antibiotics normally employed in the treatment of burn infection are ampicillin, amoxicillin and cephalosporins which are used in the first week whereas carbencillin, gentamicin, amickacin, one of the third generation cephalosporins, are administered in the second week, given as single agent or in combination(Mansour and Enayat, ٢٠٠٤). From historical point of view, the topical antimicrobial agents, silver products have been used many years ago for their beneficial effects, often for hygiene and in more recent years as antimicrobials on wounds due to burns, trauma and diabetic ulcers(Silver *et al.,*٢٠٠٦). The basis for the use of topical antimicrobial agents in burn patients is simple. Eschar is formed on both partial and full-thickness burns. With increasingly thick eschar

over deeper burns, the distribution of systemically administered antibiotics to eschar is not reliable.

Silver sulfadiazine (SSD) is the most frequently used topical antimicrobial agent that was introduced in the early ١٩٧٠s by Fox (Japoni *et al.,* ٢٠٠٥). It is used in a ١٪ concentration and acts via inhibition of microbial DNA replication and altering the cell membrane structure of microorganism. It is a bactericidal and acts as a broad spectrum agent against Gram positive and most of Gram negative bacteria and some yeasts(Abston *et al.,* ٢٠٠٠). Minimal pain is associated with its application. It is non-staining and its wound penetration is intermediate between rapidly absorbed mafenide and poorly absorbed silver nitrate(Press, ١٩٩٧). Some patients treated with this agent develop transient leucopenia that does not result in cessation of treatment. Systemic absorption may produce reactions characteristic to sulphonamides, including crystaluria or methaemoglobinaemia (Noronha and Almeida, ٢٠٠٠).

Silver nitrate solution (SN) was introduced in ١٩٦٥ by Moyer. It is not toxic in a ٠.٥٪ concentration, but it has a significant antimicrobial effect. Its effect may result from silver ions that readily combined with sulphydryl, carboxy, phosphate, amino and other biologically important chemical groups in the microorganisms(Carcin *et al,* ٢٠٠٤). It is a broad spectrum agent, bacteriostatic at ٠.٥٪; development of resistance to the silver ion is distinctly uncommon.

There is minimal absorption from the wound making toxicity virtually unknown(Press, ١٩٩٧). The solubility properties of SN mandate preparation in distilled water, therefore a ٠.٥٪ solution is markedly hypotonic so results in substantial leaching of sodium, potassium and other plasma solutes from the burn wound. It does not penetrate the eschar and needs bulky and frequent dressing changes; besides, SN stains every thing it touches brown or black. These side effects limit the use of SN(Noronha and Almeida, ٢٠٠٠). Other topical antimicrobial agents include mafenide which is a topical sulfonamide that diffuses freely into the eschar and has a broad antibacterial spectrum. Its mechanism of action is unknown. It has the best eschar penetration of any agent and it also efficiently penetrates cartilage(Press, ١٩٩٧). It is a strong carbonic anhydrase inhibitor and its use results in an alkaline diuresis and can lead to acid-base abnormalities especially metabolic acidosis when used on >٢٠٪ of the body surface area. Significant pain results from its application probably due to its high osmolarity(Honari, ٢٠٠٤). Chlorhexidin(hibitane) and organic iodine preparations (povidone-iodine-betadine) are also used as topical treatment of burn wound(Silver *et al.,*٢٠٠٦).

Acticoat is a nanocrystalline silver-containing dressing. It has beneficial antimicrobial properties of the silver ion by coating the dressing material with a thin, soluble silver film. This dressing appears to maintain antibacterial levels of silver ions in the wound

for up to ٥ days. Because it remains on the burn wound for up to ٥ days, the patient spared the pain associated with dressing changes as well as the expense(Yin *et al*., ١٩٩٩). Topically applied aztreonam alone and in conjunction with its systemic administration is used in the control of Gram negative burn wound infection(Kamel and El Megeed, ١٩٩٧).

١.٢.١٠.٢ Surgical Management of Burn Wounds

Burn wound excision removes necrotic tissue that serves as a nidus for microbial proliferation and the development of burn wound sepsis (Drost *et al.*, ١٩٩٣). The advantages of burn wound excision include a lesser incidence of invasive burn wound infection and fewer complications, decreased hospital stay and associated lesser costs, an earlier return to work, and a greater likelihood that the patient will return to work(Pruitt *et al*., ١٩٩٧).

The best replacement for lost skin is skin itself (Bollinger and Delford,١٩٩١). Autografting is the best procedure for the replacement of skin defect due to deep dermal burn; hence, the skin grafting is the standard care in deep dermal burns and also decreases burn-related mortality and morbidity(Deved *et al*., ١٩٩٨). Besides autografting, biological dressings are also used. These include allografts, xenografts and amniotic tissue. More recent procedures are the use of synthetic skin as epidermal sheets to cover the burned wounds(Pruitt *et al*., ١٩٩٧).

٢.١ Materials:

٢.١.١ Laboratory Equipments and Apparatuses

Table (٢-١) Equipments and Aapparatuses Used Throughout The Study

Equipment	Company (Origin)
Autoclave	Stermite- Japan.
Bunsen burner	Germany
Centrifuge	Hermle- Japan
Hot air oven	Memmert-Germany
Hot plate	Classico-India
Incubator	Memmert- Germany
Light microscope	Olympus-Japan
Micropipette	Oxford, USA
Millipore filter paper	Satorius membrane filters GmbH- W. Germany
Ocular lens	Olympus-Japan
pH meter	Hoeleze&Cheluis,KG-Germany
Refrigerator	Concord- Italy
Sensitive electric balance	A & D-Japan
Sterile syringe	Discardit-Spain
Water bath	Memmert- Germany
Water distillator	GFL- Germany

٢.١.٢ Chemical and Biological Materials

Table (٢-٢) The Chemical and Biological Materials Used Throughout The Study

Material	Company (Origin)
A-Chemical Materials	
- α-naphthol, KOH, esculin, ferric ammonium citrate, HCl, isopropyl alcohol, methyl red, tetramethyl-P-paraphenylene diamine dihydrochloride,	B.D.H.- England.
-٩٩٪ ethanol, glucose, H_2O_2, *p*-dimethylaminobenzaldyde, ٩٩٪ methanol, urea solution.	Fluka chemika-Switzerland.
-Silver nitrate	Radian dehyene-England Jorden- Sahab
-Silver sulfadiazine	
B-Biological Materials:	
- Culture media: Agar-agar , Blood agar base, Brain heart infusion agar, MacConcky agar, Mannitol salt agar, Nutrient agar, Müller-Hinton agar, Nutrient broth, peptone broth.	Mast Lab.- England
Triple sugar iron agar(TSI agar), MR-VP broth, Simmon's citrate agar, Urea agar base .	Diffco-USA
-IgG, IgA, IgM, C_3, C_4 endoplates	Biomaghreb-Tunisia

C-Stains: -Gram stain: crystal violet, iodine, safranin	BDH- England

٢.٢ Patients and Methods

٢.٢.١ Patients

Seventy eight(٧٨) burn patients(٣٦ males and ٤٢ females) whose ages range between(٢-٦٥) years were included in this study which lasted from November/٢٠٠٥ to May/٢٠٠٦. Those patients were clinically diagnosed by specialist doctor as having burn wound infection and were admitted to the burn unit at Al-Hilla General Teaching Hospital. They were suffering from second to third degree (flame and scald) burn injury and their burn percentage was ranging from ١٠-٨٠٪ TBSA.

٢.٢.٢ Controls

Twelve apparently healthy subjects (clinically assessed by specialist doctor) were included as controls in this study. Their ages range between (١٠-٤٠) years.

٢.٢.٣ Specimens Collection

٢.٢.٣.١ Skin and Burn Unit Swabs

Skin swabs were taken from the pus of the burned area of all patients before the bathing of the affected area(before hydrotherapy). Thirty swabs were taken from the burn unit(medical appliances). Each swab was placed in a sterile tube containing normal saline till reaching the laboratory to be inoculated on culture media(Blood agar, MacConkey agar and Nutrient agar) and incubated aerobically for ٢٤- ٤٨ hours at ٣٧C°(Collee *et al*., ١٩٩٦).

٢.٢.٣.٢ Blood Samples

Out of the (٧٨) burned patients, ٤٨ patients were subjected for blood sampling. Blood samples were collected twice from the same patients to reduce the probability of contamination by commensal types of skin flora, in addition to the precautions taken to achieve aseptic technique. Blood culture bottles were incubated aerobically at ٣٧C° for ٢-٧ days with frequent manual shaking of the bottles during the incubation period and then to be cultured on different types of culture media to detect the bacterial growth(Collee *et al*., ١٩٩٦). Those who were positive regarding blood culture were included in the immunological assays.

Three ml of blood were taken from those patients and from the controls: ١ml for blood culture and the other two ml were used for the separation of sera involved in immunological assays. The two ml were put in sterilized plane tube to clot at ٣٧C° for (٣٠-٤٥) minutes,

then sera were separated by centrifugation for ١٥ minutes at ٣٠٠٠ rpm. Haemolysed serum is avoided (Lewis *et al.*, ٢٠٠١).

٢.٢.٤ Methods

٢.٢.٤.١ The Preparation of Reagents

I-Methyl red(MR) reagent: ٠.١ gram of methyl red was dissolved in ٣٠٠ ml of ٩٩٪ ethanol and then completed the volume to ٥٠٠ ml by distilled water, it was used for differentiation of organism's ability to produce acid as an end product when fermenting dextrose (MacFaddin, ٢٠٠٠) .

II-Voges –Proskauer(VP) reagent:

Reagent A- Five gram of alpha-naphthol was dissolved in ١٠٠ ml of ٩٩٪ ethanol .

Reagent B- Forty gram of KOH was dissolved in ١٠٠ ml of distilled water; it was used for differentiation of organisms produced acetylmethylcarbinol end products when fermenting dextrose (Collee *et.al.,*١٩٩٦) .

III-Oxidase reagent : It was prepared by dissolving ٠.١ gram of tetramethyl-paraphenylene diamine dihydrochloride in ١٠ ml of distilled water and then was stored in a dark container. It was used

for the detection of the ability of bacteria to produce oxidase enzyme(Baron *et.al.,*١٩٩٥) .

IV-**Catalase reagent :** ٣٪ solution of H_2O_2 was used to detect the ability of bacteria to produce catalase enzyme (Baron *et. al.,*١٩٩٥). It was stored in a dark container.

V-Kovac's reagent:

It was prepared by dissolving ٥ gram of (*P*-dimethyl-aminobenzaldehyde) in ٧٥ ml amyl alcohol, and then ٢٥ ml of concentrated hydrochloric acid was added. This reagent was used for detection of indole production(MacFaddin, ٢٠٠٠).

٢.٢.٤.٢ The Culture Media

All culture media were prepared according to the instructions of manufacturer manual:-

٢.٢.٤.٢.١-Esculin medium: The esculin is ٦,٧-dihydroxycoumarin ٦-oxidase xanthine on effect inhibitory has which glucoside enzyme(Capell *et al.,* ١٩٩٥). Esculin medium was prepared from nutrient agar with ٠.٤ gm ferric ammonium citrate and ٤ gram esculin; then the volume was completed to ١٠٠٠ ml with distilled water. Afterwards the medium was distributed in tubes and sterilized by autoclave; it was prepared as slants (MacFaddin, ٢٠٠٠).

It was used for differentiation of group D streptococci from other streptococci.

٢.٢.٤.٣ Stains

Gram's stain: This stain was used to differentiate Gram-negative from Gram-positive bacteria and was carried out according to Collee *et al*., (١٩٩٦).

٢.٢.٤.٤ Identification of Bacteria

A single colony was taken from each primary positive culture on blood agar and on MacConckey agar and it was identified depending on its morphology(colony shape, size, colour, borders, and texture) and then it was examined by the microscope after being stained with Gram's stain. After staining, the biochemical tests were done on each isolate to complete the final identification (Collee *et al.,* ١٩٩٦; Baron *et al.,* ١٩٩٥; Benson, ١٩٩٨; MacFaddin, ٢٠٠٠; and Murray *et al.,* ٢٠٠٣).

٢.٢.٤.٥ Biochemical Tests

٢.٢.٤.٥.١ Catalase Test: A colony of the organism is transferred by sterile wooden stick to the surface of a clean, dry glass slide, and one drop of ٣٪ H_2O_2 is added to it. The formation of gas bubbles indicates the positive result(Baron *et al.,*١٩٩٥).

٢.٢.٤.٥.٢ Oxidase Test : A piece of filter paper was saturated with oxidase reagent; then a colony of organism was spread onto the filter paper. If the color turned rose to purple, the oxidase test would be positive(Baron *et. al.,*١٩٩٥).

٢.٢.٤.٥.٣ Coagulase Test is an important method for differentiation between pathogenic and non-pathogenic strains of staphylococci. The test was read by tilting the tube and observing for clot formation in the plasma (Murray *et al.,* ٢٠٠٣).

٢.٢.٤.٥.٤ Indole Test is used for the determination of the organisim's ability to produce indole from deamination of tryptophan by tryptophanase. The formation of red color ring at top of broth indicates for a positive reaction while a yellow color ring indicated a negative reaction(MacFaddin, ٢٠٠٠).

٢.٢.٤.٥.٥ Methyl Red Test: It is employed to detect the production of sufficient acid during the fermentation of glucose. The change of color to orange was a positive reaction(Murray *et al.,* ٢٠٠٣).

٢.٢.٤.٥.٦ Voges-Proskauer(acetoin production) Test: The VP test is used to detect acetoin(acetyl-methyl-carbinol), which is produced by certain bacteria during growth in peptone glucose broth(MR-VP broth) The positive result was changing of the color of the medium to red. (MacFaddin, ٢٠٠٠).

٢.٢.٤.٥.٧ Simmon's Citrate Test: The citrate test was used to determine the ability of a bacterium to utilize citrate as its only source of carbon. The positive result was a change of the color of media from green to blue (Baron *et al.,*١٩٩٥).

٢.٢.٤.٥.٨ Triple Sugar Iron(TSI) Test: The aim is to differentiate the enterobacteriaceae according to carbohydrate fermentation and hydrogen sulfide production(Murray *et al.,* ٢٠٠٣).

٢.٢.٤.٥.٩ Urease Test: Urease is an enzyme that breaks the carbon-nitrogen bond of amides to form carbon dioxide, ammonia and water. The urea base agar was sterilized by autoclave, after that it was cooled to ٥٠ C^{o} and urea substrate was added to it and it was poured in sterile tubes; then it was inoculated by bacterial cultures, which were incubated for (٢٤ - ٤٨) hours at ٣٧C^{o}. When urea was broken down, ammonia was released and the pH of the medium increased. This pH change was detected by a pH indicator that turned pink in a basic environment.

A pink medium indicated a positive test for urease. Failure of deep pink color to develop was a negative reaction(Collee *et al.,* ١٩٩٦).

٢.٢.٤.٥.١٠ Esculin Test: The organism was grown in an esculin slants. The dark brown color was the positive result. The unchanging color was a negative reaction. This reaction presumptively identifies group D streptococci (Capell *et al.,*١٩٩٥).

٢.٢.٤.٥.١١ Mannitol Salt Agar: The medium was inoculated with bacterial colonies then incubated at ٣٧C° for ٢٤ hours. The color changed from pink to bright yellow when the bacteria was lactose fermenter and meant positive result, while unchanging color of the medium was a negative result(Collee *et al.,* ١٩٩٦).

٢.٢.٤.٥.١٢ Eosin Methylene Blue(EMB) Agar: Lactose fermenting colonies were either dark or possessed dark centres with transparent colorless peripheries, while organisms that did not ferment lactose remained uncolored(Murray *et al.,* ٢٠٠٣).

٢.٢.٤.٥.١٣ Motility Test(Semisolid Media): Non motile bacteria gave growth that is confined to the stab-line and had sharply defined margins leaving the surrounding medium clearly transparent. Motile bacteria typically gave diffuse hazy growth that spread throughout the medium rendering it slightly opaque(Murray *et al.,* ٢٠٠٣).

٢.٢.٤.٦ Antibiotics Sensitivity Test: -

Antibiotic diffusion test (Kirby-Bauer susceptibility test) was carried out according to (MacFaddin, ٢٠٠٠). Antibiotics discs potency was supplied from Bioanalyse(Turkey) (Table٢-٣).

Antibiotics	AX	Py	CTX	CN	AK	CIP	SXT	AZM
Disc potency µg/ml	٢٥	١٠٠	٣٠	١٠	٣٠	٥	١.٢٥/٢٣.٧٥	١٥

Table (٢-٣): Antibiotic Disc Potency

AX: Amoxicillin; Py: Carbenicillin; CTX: Cefotaxim; CN: Gentamicin; AK: Amikacin; CIP: Ciprofloxacin; SXT: Trimethoprim-Sulphamethoxazole; AZM: Azithromycin.

٢.٢.٤.٧ The Effect of Silver Sulphadiazin and Silver Nitrate on Bacterial Growth:

This is done according to Starodub and Trevors(١٩٨٩).

a. Plates of Müller Hinton agar with ٥mm diameter wells made by cork porer were prepared under aseptic conditions(٢ holes were made in each agar plate).

b. Bacterial suspension of previously identified bacterial isolates was made by adding growth from (٥) isolated pure colonies grown on a blood agar plate to (٥ml) nutrient broth. This culture was then incubated for ٢ hours to produce a bacterial suspension of moderate turbidity(MacFaddin; ٢٠٠٠).

c. The most frequent skin bacteria obtained in this study were included in this test: two types belonging to Gram positive bacteria: *Staph. aureus* and *Staph. epidermidis* and four types of Gram negative bacteria: *P. aeruginosa, Enterobacter, Klebsiella* and *E.coli*.

d. A sterile swab was used to obtain an inoculum from each type of bacterial suspensions and then to streak with the swab on the surface of Müller Hinton plates.

e. The two wells in Müller Hinton agar were filled, one with ١٪ silver sulphadiazin cream and the other with ٠.٥٪ silver nitrate solution by using sterile micropipette and under aseptic conditions.

f. The plates were incubated aerobically in darkness for ٢٤hours at ٣٧C°.

g. Resistance and susceptibility was defined as no zone of inhibition or significant inhibition zone respectively.

٢.٢.٤.٨ Determination of Immunoglobulin and Complement Levels:

Principles of single radial immunodifution(SRID) test:

Equal volumes of control and test serum samples were added to wells in an agarous gel-containing a mono-specific antiserum. The sample diffuses radically through this gel and the substance being assayed(antigen) forms a precipitation ring with the mono-specific antiserum(Lowell, ٢٠٠١). Ring diameters were measured by viewing device(ocular). Unknown concentrations were determined from the tables supplemented with each type of endoplate which contains ١٢ wells(Lewis *et al.*, ٢٠٠١).

Procedure

A- Endoplates and the serum(of patients and control) were removed from refrigerator. Reagents were equilibrated to room temperature.

B- Plate was removed from ziplock bag. After lid removed, the wells were inspected for moisture. If moisture was present, plates were left uncovered to remain at room temperature(approximately ١٥ minutes) until moisture evaporated.

C- Sera of patients were thoroughly shaken(in their own containers) by inversion. Each patient's sample was dispensed onto the appropriate wells. Each well required ٥ µl of serum.

D- After lid was replaced, it was incubated at room temperature on a level surface. Incubation times were ٤٨ hours for IgG, IgA, C٣ and C٤ tests and ٧٢ hours for IgM test.

E- Immunopreciptin ring diameters were microscopically measured by ocular lens to the nearest ٠.١mm. The calculated diameters were compared to the standard diameter to calculate the concentrations of serum humoral factors(Lowell, ٢٠٠١).

٢.٣ Statistical analysis:

Mean, standard deviation, and T-test (p-value (٠.٠٥)) were carried out according to Bowers (١٩٩٧).

٣.١ Clinical Study

٣.١.١ Sex Influence and Burn Injury

A total of ٧٨ burn cases were included in this study(Table ٣-١). It was seen that ٤٢ cases (٥٣.٨٪) were noticed among females and ٣٦ cases(٤٦.٢٪) among males, also flame burns were clearly more frequent ٤٧:٧٨(٦٠.٣٪) than scald burns ٣١:٧٨(٣٩.٧٪). Likewise, flame burns were the major cause of burn injuries in females ٢٨(٣٥.٩٪) when compared with that of scald injuries١٤ (١٧.٩٪). Meanwhile, this difference(between flame and scald injury) was slightly noticed among males ١٩ (٢٤.٤٪) and ١٧ (٢١.٨٪) respectively.

Table(٣-١) Sex Distribution and Type of Burn

Sex	Total Cases	Type of Burn	
		Scald	Flame
Male	٣٦ (٤٦.٢٪)	١٧ (٢١.٨٪)	١٩ (٢٤.٤٪)
Female	٤٢ (٥٣.٨٪)	١٤ (١٧.٩٪)	٢٨ (٣٥.٩٪)
Total	٧٨ (١٠٠٪)	٣١ (٣٩.٧٪)	٤٧ (٦٠.٣٪)

Traditionally women do all cooking for the family and have higher incidence of domestic burns (Nega and Lindtjorn, ٢٠٠٢). The results of this study agree with Mercier and Blond(١٩٩٦) in fact that

the domestic burn injuries were common among females more than males. In this study, flames are considered as the major cause of injuries. This may be attributed to the accidents of home generators, kerosene stoves, lanterns and portable kerosene heaters. Wassermann(٢٠٠٢) found that burns by flame are most common in severely burned adult patients. El-Sonbaty and El-Oteify(١٩٩٠); Attia *et al*., (١٩٩٧) found that flame injury represented the most common cause of burn injuries(٦٦.٨٪) and tended to affect females more than males. Moreover, they clarified that kerosene stoves were the most common source of flame injury. Similar results have been reported by

In this study, scalds were responsible for ٣٩.٧٪ of burn injuries. By contrast, in Ethiopia, Nega and Lindtjorn(٢٠٠٢) found that scald (٥٩٪) was the leading cause of burn followed by flame (٣٤٪). So the causes of burn injury are highly individualized in each country, largely depending on the standard of living and life style as well as the developmental stage of country.

٣.١.٢ Age and Burn Injury

The results in Table (٣-٢) showed that the most affected age group was that under five years ٢٠:٧٨ cases (٢٥.٦٪) in whom scald

injury is the major cause ١٩:٢٠ in comparison with flame injury ١:٢٠.

Age Groups (years)	Type of Burn				Total Cases	
	Scald		Flame		No.	%
	No.	%	No.	%		

In other words, ٩٥٪ of burns under five years of age were due to scald injury. This indicates that there is a good association between age and cause of burn injury. These results are in agreement with that recorded in Egypt(Massoud and Mandil, ١٩٩٢) and Lybia (Shahin *et al.,*١٩٩٨).

Table(٣-٢): Distribution of Burn Patients According to Age and Type of Burn Injury

١ – ٥	١٩	٢٤.٣	١	١.٣	٢٠	٢٥.٦
٦ –١٠	٦	٧.٧	٦	٧.٧	١٢	١٥.٤
١١ –١٥	٣	٣.٨	١١	١٤.١	١٤	١٧.٩
١٦ – ٢٠	٠	٠	١١	١٤.١	١١	١٤.١
٢١ – ٢٥	٠	٠	٣	٣.٨	٣	٣.٨
٢٦ –٣٠	١	١.٣	٦	٧.٧	٧	٩
٣١ –٣٥	١	١.٣	٥	٦.٤	٦	٧.٧
٣٦ – ٤٠	٠	٠	٢	٢.٦	٢	٢.٦
٤١ –٤٥	١	١.٣	١	١.٣	٢	٢.٦
>٤٦*	٠	٠	١	١.٣	١	١.٣
Total	**٣١**	**٣٩.٧**	**٤٧**	**٦٠.٣**	**٧٨**	**١٠٠**

*one case aged ٦٥ years

Age is an important epidemiological determinant for injuries including burn(Alden *et al.,* ٢٠٠٥). This study reveals that about one fourth of the studied sample is children of less than five years, nearly half of the cases are between (١٠-٤٠)years old. While those aged(٤٠) years and over represent only (٤٪) of cases. This age distribution is similar to that found by Subrahmmangam(١٩٩١). However, the discrepancy between the relatively low percentage of injury in the old age group in the present study and the higher percentage (١٦.٧٪) reported by Glasheen *et al.,* (١٩٨٣) in the United States of America

might be explained by the social structure in Iraq, where older individuals usually live within the family and are served by younger members thus decreasing their probability of exposure to hazardous situation and hence their liability to injury is decreased. This pattern means that burns tend to occur more in certain age group reflecting the particular behavioral patterns associated with age. In children, the lack of coordination and unawareness of dangerous substances play important roles in the increasing rate of burn exposure. In addition, young children are not adequately supervised because of large families and lack of domestic safety measures (Attia *et al*., ١٩٩٧).

٣.١.٣ Burn Injury and Mortality

The results expressed in Table(٣-٣) shows that both the positivity of skin culture in relation to the total number of cases for each percentage of burn and the number of the deaths increase with the increment of the percentage of burn. The number of dead cases is increased when burn percentage is equal or more than ٤٠٪ and reaching maximum when burn percentage is ٦٠٪ and more. Reig *et al*.(١٩٩٢) indicate that the extensive burns are more likely to be colonized and invaded by microorganisms and they demonstrate a significant association between increasing burn size and increasing incidence of Gram negative pathogenic bacteria.

Table(٣-٣) Relation Between Burn Percentage, Skin Culture and Number of Deaths

Burn Percentage	No. of Cases	No. of Positive Skin Culture	No. of Deaths during Hospitalization
١٠٪	١٨	١٢	٠
١٥٪	١٠	٨	٠
٢٠٪	١٩	١٦	٠
٢٥٪	٩	٩	١
٣٠٪	١	١	٠
٣٥٪	٤	٤	٠
٤٠٪	٨	٨	٢
٥٠٪	٤	٤	١
٦٠٪	١	١	١
٧٠٪	١	١	١
٧٥٪	١	١	١
٨٠٪	٢	٢	٢
Total	٧٨	٦٧	٩

In this study, all the dead cases were symptomatic and have positive blood culture for bacteria namely *Enterobacter*, *Pseudomonas* and *Staph. aureus*. The death occurs during hospitalization and specifically in the second week of admission to the hospital. Their symptoms were fever more than ٣٨.٥ C°, hypotension, pain, tachycardia, tachypnea, oliguria and disorientation. They were suffering from second degree deep partial thickness to third degree full thickness burns. The Boyd index (Table ٣-٤), which is the result of the summation of the age of burn patients

in years and the percentage of the burn, is a good predictor for the severity of burn injury. If the Boyd index is ٨٠ or more means that there is a high probability of death from burn injury (McGregor,١٩٩٨).

No. of Deaths	Bacterial Blood Isolates	Age (year)	Percentage of burn	Boyd Index
١	***Enterobacter*** spp.	٢	٢٥	٢٧
٢	***P. aeruginosa***	٩	٤٠	٤٩
٣	***Staph. aureus***	١٣	٤٠	٥٣
٤	***Staph. aureus***	٦٥	٥٠	١١٥
٥	***P. aeruginosa***	٢٠	٦٠	٨٠
٦	***P. aeruginosa***	١٨	٧٠	٨٨

Table(٣-٤) Boyd Index for The Dead Cases

٧	***Enterobacter*** spp.	٢٩	٧٥	١٠٤
٨	***Enterobacter*** spp.	١٨	٨٠	٩٨
٩	***Enterobacter*** spp.	١٨	٨٠	٩٨

The Boyd index explains that both the age and the percentage of the burn are important risk factors for mortality. Alden *et al.*(٢٠٠٥) point out that socioeconomic factors, especially poverty, are important determinant of the occurrence of burn injury, but patient factors such as age are the major determinant of survival.

Hodle *et al*. (٢٠٠٦) mention the risk factors for septicemia in burned patients including patient factors like extent and depth of burn, the age of patient, pre-existing diseases and the physical environment of the wound itself. They point out that an increased risk of septicemia is associated with burns of more than ٣٠٪ from the total body surface area, full thickness skin destruction, extremes of age i.e. infancy and old age, diabetes, cardiopulmonary disease, inhalation injury, malnutrition, a poor blood supply to the wound and a moist warm wound environment, and they explain that microbial factors influence the balance between resistance and susceptibility to infection. Furthermore, they notice that by the second week of the injury, the predominant bacteria is usually Gram negative and these are able to proliferate in necrotic tissue and by entering blood vessels, spread to remote tissues and organs. Moreover, invasiveness and virulence are related to the ability of organisms to produce endotoxin, exotoxin, permeability factors and

enzymes and to their intrinsic characteristic such as the capsule composition .

In this study only one case aged ٦٥ years suffered burn injury then died; she had a history of chronic disease(hypertension and heart failure). This agrees with Koupil *et al*.(٢٠٠٠) who state that age and concomitant chronic disease contribute to the high mortality and higher frequency of complications in geriatric patients who suffer burn injuries.

All the dead cases were females. This may be attributed to the immunosuppression effect of female sex hormones because estrogen mediates a sex difference in the post burn period; this immunosuppression increases the susceptibility to sepsis(Gregory *et al*., ٢٠٠٠), in addition to the pattern of clothing of the females when they are doing the domestic work and to the mechanism of burn injury they suffer(Ahuja and Bhattacharya, ٢٠٠٤). Only two dead cases had been injured by scald, whereas the other seven dead cases had suffered from flame injury. The flame injury is more dangerous than the scald. This agrees with De Macedo(٢٠٠٣) who stated that the flame burn was the predominant cause of burn amongst patients who had sepsis due to the fact that this agent produces deeper and more extensive lesions than other agents leading to more colonization of burn wound and then to sepsis and death.

٣.٢ Isolation of Bacteria

A total of ١٥٦ samples consisting of ٧٨ skin swabs, ٤٨ blood specimens and ٣٠ burn ward swabs were subjected for culturing on bacterial culture media. The results shown in Table(٣-٥) revealed that ١٠٨ samples(٦٩.٢٪) gave positive bacterial culture whereas ٤٨(٣٠.٨٪) showed no bacterial growth. Regarding skin swabs, ٦٧:٧٨ (٨٥.٩٪) were positive bacterial cultures consisting of single growth ٥٦(٨٣.٦٪), and mixed bacterial growth١١(١٦.٤٪). Meanwhile, no bacterial growth was found in ١١:٧٨ (١٤.١٪) of skin swab cultures. The single and mixed bacterial growth results of skin swabs are shown in Table(٣-٦). These results agree with that obtained by Bagdonas(٢٠٠٤) who found that ٨٦.٥٪ of skin swabs were positive for bacterial growth. Also, Al-Akayleh, (١٩٩٩) reported that negative bacterial growth was found in approximately ١٨٪ of the cultures of skin swab.

Table(٣-٥) Number and Percentage of Bacterial Isolates from Burn Patients and Burn Unit

Result	Source of Culture			
	Skin No. (%)	Blood No. (%)	Burn Unit No. (%)	Total of Samples No. (%)
Culture Positive	٦٧ (٨٥.٩٪)	٢٤ (٥٠%)	١٧ (٥٦.٧٪)	١٠٨ (٦٩.٢٪)
Single Growth	٥٦	٢٤	١٠	٩٠ (٨٣.٣٪)
Mixed Growth	١١	٠	٧	١٨ (١٦.٧٪)
Culture Negative	١١ (١٤.١٪)	٢٤ (٥٠٪)	١٣ (٤٣.٣٪)	٤٨ (٣٠.٨٪)
Total	٧٨ (١٠٠٪)	٤٨ (١٠٠٪)	٣٠ (١٠٠٪)	١٥٦ (١٠٠٪)
Single Growth		Mixed Growth		

Table (٣-٦) Bacterial Isolates Obtained from Skin Swab Culture

Bacterial Isolate	No. (%)	Bacterial Isolate	No. (%)
Staph. aureus	٢ (٣.٦ %)	*P.aeruginosa*+ *Enterobacter* spp.	٣ (٢٧ %)
Staph. epidermidis	٢ (٣.٦ %)	*P.aeruginosa + E.coli*	٢ (١٨.٢ %)
P.aeruginosa	١٨ (٣٢.١ %)	*P.aeruginosa + S.aureus*	٢ (١٨.٢ %)
Enterobacter spp.	١٥ (٢٦.٨ %)	*P.aeruginosa + Enterococcus faecalis*	١ (٩.١ %)
E.coli	٩ (١٦ %)	*P.aeruginosa + A.baumannii*	١ (٩.١ %)
K. pneumoniae	٧ (١٢.٥ %)	*Enetrobacter* spp.+ *Proteus* spp.	١ (٩.١ %)
A.baumannii	٢ (٣.٦ %)	*Enterobacter* spp.+ *E.coli*	١ (٩.١ %)
Proteus spp.	١ (١.٨ %)	—	—
Total	**٥٦(١٠٠٪)**	—	**١١(١٠٠٪)**

The high percentage of positive bacterial cultures of the skin swab may be attributed to the fact that the burn wound has a much higher incidence of infections compared with other forms of trauma because of extensive skin barrier disruption as well as alteration of cellular and humoral immune responses(Sanyal *et al.* ,١٩٩٨).

Regarding the blood culture(table٣-٧), results showed that positive bacterial cultures were found in ٢٤:٤٨ (٥٠٪)of burned patients. This result is higher than that obtained by De Macedo *et al*., (٢٠٠٣) in Brazilia who showed that (١٩.٤٪) of burned patients developed proven sepsis. In this study, the high rate of positive blood culture may be attributed to the high level of nosocomial infections acquired from medical devices in burn wards, the crowding of wards

with burned patients and the unavailability of recent techniques in the

Bacterial Isolate	No. (%)

sterilization of burn wards.

In this study, all the controls were negative for bacterial blood culture.

The burn unit samples showed positive cultures in ١٧:٣٠(٥٦.٧٪). Ten of them were of single growth, the other ٧ were mixed bacterial cultures(Table٣-٨). These findings reflect the higher percentage of bacterial contamination of the burn unit which explains the higher percentages of positivity of both skin and blood cultures found in this study. Torregrossa *et al*., (٢٠٠٠) observe that nosocomial infections are now clearly in a phase of expansion as testified by statistical findings and particularly in intensive care units including burn units.

Table (٣-٧) Bacterial Isolates Obtained from Blood Culture

Staph.aureus	٧ (٢٩.٢ %)
Staph.epidermidis	٢ (٨.٣ %)
β.haemolytic streptococci	٢ (٨.٣ %)
Enterobacter spp.	٧ (٢٩.٢ %)
P. aeruginosa	٦ (٢٥ %)
Total	**٢٤ (١٠٠٪)**

Table (٣-٨) Bacterial Isolates Obtained from Burn Unit

٣.٣ Types of Bacterial Isolates

Single Growth		**Mixed Growth**	
Bacterial Isolate	**No. (%)**	**Bacterial Isolate**	**No. (%)**
Staph. aureus	٢ (٢٠ %)	*P. aeruginosa+ Enterobacter* spp.	١ (١٤.٢٨٥ %)
Enterococcus faecalis	٢ (٢٠ %)	*P. aeruginosa +E.coli*	١ (١٤.٢٨٥ %)
P. aeruginosa	٢ (٢٠ %)	*P. aeruginosa +Klebsiella*	١ (١٤.٢٨٥ %)
Enterobacter spp.	٢ (٢٠ %)	*P. aeruginosa +A. baumannii*	١ (١٤.٢٨٥ %)
K. pneumoaiae	١ (١٠ %)	*Staph.aureus + E.coli*	١ (١٤.٢٨٥ %)
A. baumannii	١ (١٠ %)	*Enterococcus faecalis + Staph.epidermidis*	١ (١٤.٢٨٥ %)
—	—	*Enterococcus faecalis +E.coli*	١ (١٤.٢٨٥ %)
Total	**١٠(١٠٠٪)**	—	**٧(١٠٠٪)**

As shown in Tables(٣-٩) and (٣-١٠) which showed the frequency of bacteria in skin swab, blood and burn ward cultures, it is clear

from the total number of isolates that Gram negative bacteria are more frequent than Gram positive type. This agrees with Kamel and El-Megeed (١٩٩٧) who found that Gram negative bacteria represent about ٦٥٪ of micro-organisms that cause burn wound infection and that this type of bacteria has assumed a primary lethal role among the cases of burn wound infection and septicaemia.

The Gram negative bacteria cause trouble in three different ways: first, they produce a large quantity of pus-containing toxins which may kill surviving skin cells and thus convert an initially partial thickness burn into a full thickness burn, and absorption of toxins may cause general illness. Secondly, the large amount of pus produced may float off skin and cause great difficulty in success of skin graft. And thirdly, the invasive properties of these organisms are generally considered to be low, but a large burn causes such debility that septicemia can easily occur, especially with *P. aeruginosa*, and this condition will be fatal unless it is treated energetically (Muir *et al*,١٩٨٧). The predominance of Gram negative bacteria is clear from the high frequency of *P.aeruginosa* in each source of the cultures. This agrees with Maitra(٢٠٠٣) who states that after burn injury, the most common isolated microorganism is the opportunistic type like *P.aeruginosa* and MRSA. Mousa(١٩٩٧) found that *P. aeruginosa* is the most frequent bacteria in burn wound infections. The reasons for this high prevalence may be due to factors associated with the

acquisition of nosocomial pathogens in patients with recurrent long term hospitalization complicating illnesses, prior administration of antimicrobial agents and the immunosuppressive effects of burn trauma(Arslan *et al.,* ١٩٩٩; and Yotis, ٢٠٠٥). In the blood cultures(Table ٣-١٠), *Enterobacter* is slightly more frequent than *Pseudomonas* (٧ and ٦ isolates), respectively. The source of *Enterobacter* bacteraemia is either from burn wound or as a result of bacterial translocation which is caused by failure of the gut barrier, the imbalance of intestinal flora and impaired host immune defenses, all these conditions are caused and enhanced by burn injury (Herek *et al.*; ٢٠٠٠). It is clear that the burn wound can be contaminated by microorganisms that migrate from the gastrointestinal, urinary and respiratory tracts (Bowler *et al.,* ٢٠٠١). This indicates the idea of autoinfection that the burn patients suffer from in addition to the infection acquired from the burn unit itself(Collier, ٢٠٠٣). In the skin swab cultures, other Gram negative bacteria rather than *Pseudomonas* are *Enterobacter, E.coli*, *Klebsiella*, *Acinetobacter* and *Proteu*s, whereas in the burn ward, *Enterobacter* is followed by *E.coli*, *Acinetobacter* and *Klebsiella*. In this study, the last two bacteria have the same frequency in burn unit. Revathi *et al*.(١٩٩٨); and Mansour and Enayat(٢٠٠٤) both isolated each of *Klebsiella*, *Proteus*, *Enterococcus faecalis, E.coli*, *Acinetobacter* and others in burned patients in frequencies less than that of *Pseudomonas* and S*taph. aureus*.

Table (٣-٩)Frequency of Gram Positive Bacteria

Skin		Blood		Burn Unit	
Bacterial Isolates	**Frequency**	**Bacterial Isolates**	**Frequency**	**Bacterial Isolates**	**Frequency**
S. aureus	٤	*S. aureus*	٧	*Enterococcus faecalis*	٤
S.epidermidis	٢	β-haemolytic streptococci	٢	*S. aureus*	٣
Enterococcus faecalis	١	*S.epidermidis*	٢	*S.epidermidis*	١
Total	٧	**Total**	١١	**Total**	٨

Table (٣-١٠)Frequency of Gram Negative Bacteria

Skin		Blood		Burn Unit	
Bacterial Isolates	**Frequency**	**Bacterial Isolates**	**Frequency**	**Bacterial Isolates**	**Frequency**
P.aeruginosa	٢٧	*Enterobacter spp.*	٧	*P.aeruginosa*	٦
Enterobacter spp.	٢٠	*P.aeruginosa*	٦	*Enterobacter* spp.	٣
E.coli	١٢	ـــ	ـــ	*E.coli*	٣
K.pneumonae	٧	ـــ	ـــ	*A. baumannii*	٢
A.baumannii	٣	ـــ	ـــ	*K.pneumonae*	٢
Proteus spp.	٢	ـــ	ـــ	ـــ	ـــ
Total	**٧١**	**Total**	**١٣**	**Total**	**١٦**

Many other studies found that the most common pathogen in burn wound infection is *Pseudomonas* like those in developing countries as shown by Song *et al.*(٢٠٠٠) in South Korea, Al-Akeyleh, (١٩٩٩) in Jordan, Husain *et al.*(١٩٨٩) in Libya, and Rastigar Lari *et al*.(٢٠٠٥) in Iran. Although *P. aerugnosa* is not a classic pathogen of burn wound infection in developed countries, a few burn centers in Canada and U.S.A in a study done by Shankowsky *et al.*, (١٩٩٤), had reported *P. aeruginosa* as an important microorganism in burn units.

It is extremely likely that burn patients will be challenged with this organism because *P. aeruginosa* is so common in the environment. It can contaminate the floors, bed rails, sinks of hospitals and hands of health care workers. Once established, *P.*

aeruginosa tends to persist within the unit (Hsueh *et al*., ١٩٩٨; and Hauser and Sriram, ٢٠٠٥). *Pseudomonas* colonized in the intestinal tract of burn patients is noxious and can be fatal as a pathogen of digestive selective Furthermore infection. post-burn decontamination (decontamination of endogenous pathogens in the intestinal tract) is essential in preventing post-burn infection associated with bacterial translocation (Hatano *et al*., ١٩٩٦).

In this study the more frequent Gram positive bacteria isolated from the blood is *Staphylococcus aureus* followed by β-hemolytic streptococci and then *Staphylococcus epidermidis*. These results were approximately fitted with that of Sanyal *et al*.,(١٩٩٨) who found that MRSA comprised ٩٢٪ of the Gram positive bacteria isolated from blood of burn patients. Whereas De Macedo *et al.*(٢٠٠٣) showed that the most common bacteria isolated from blood culture were *Staph.* a*ureus* followed by coagulase negative Staphylococci(CoNS).

Furthermore, CoNS should be considered as an important pathogen for sepsis in burns. The organism, being ubiquitous in a hospital environment, and burn wounds being the ideal medium for its multiplication, it is hardly surprising that this bacteria would be the cause of ٢٠.٧٪ of septic episodes (De Macedo *et al*., ٢٠٠٣).

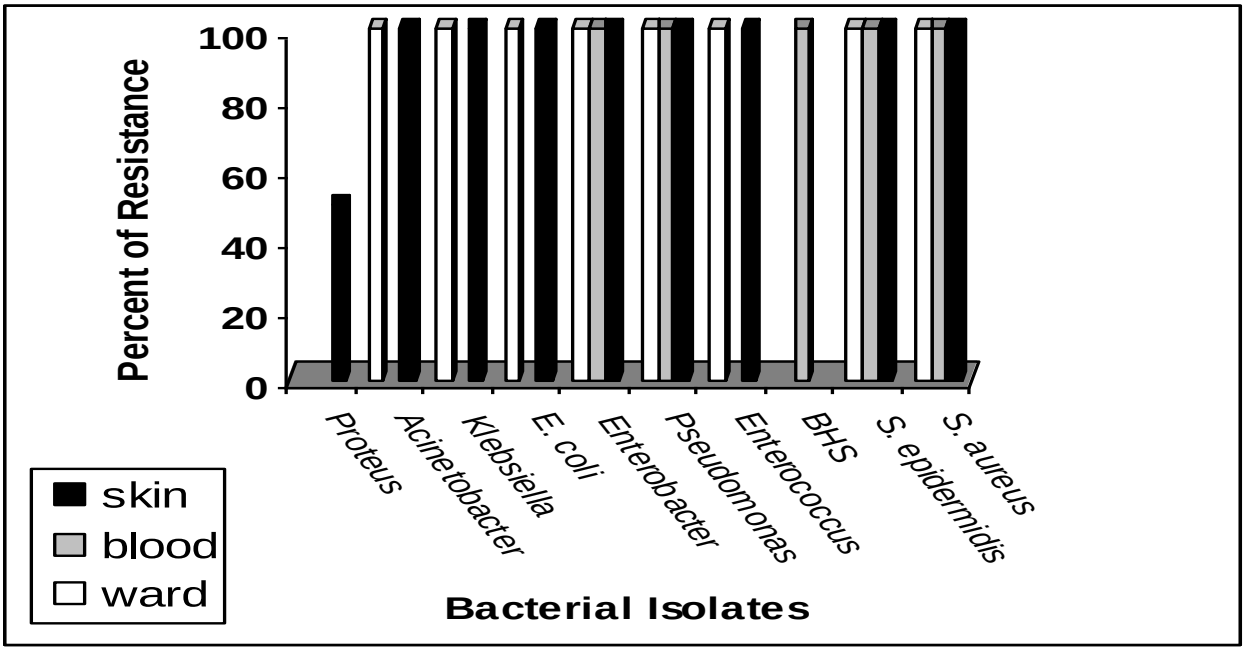
Emmerson (١٩٩٤) noted that *Staphylococcus aureus* is still one of the most frequently encountered single bacterial species in hospitals and continues to be a frequent cause of burn wound sepsis.

The invasiveness of the organism is related to the degree of injury, disturbance of normal flora, virulence of the organism, extent of external contamination and invasiveness of the patients endogenous gastrointestinal tract and upper respiratory tract flora (Kanchanapoom and Khardori, ٢٠٠٢).

٣.٤ Effect of Some Antibiotics on Bacterial Isolates

The results of this study reveal that there is a remarkable increase in bacterial resistance to β-lactam antibiotics: penicillins(amoxicillin and carbenicillin) and cephalosporins(cefotaxime). As shown in Figure(٣-١), nearly all the bacterial isolates were completely resistant(١٠٠٪) to amoxicillin. This result of high resistance to amoxicillin is nearly compatible with that of Kehinde *et al*.(٢٠٠٤) who found that *S. auerus* were resistant exclusively to ampicillin and cloxacillin(β-lactam antibiotics), Al-Saedi(٢٠٠٠) who found that ٩٨.٢٪ of *K. pneumoniae* were resistant to amoxicillin due to the production of β-lactamase: TEM(Temonera family) and SHV(sulfohydryl variable). This result is also higher than

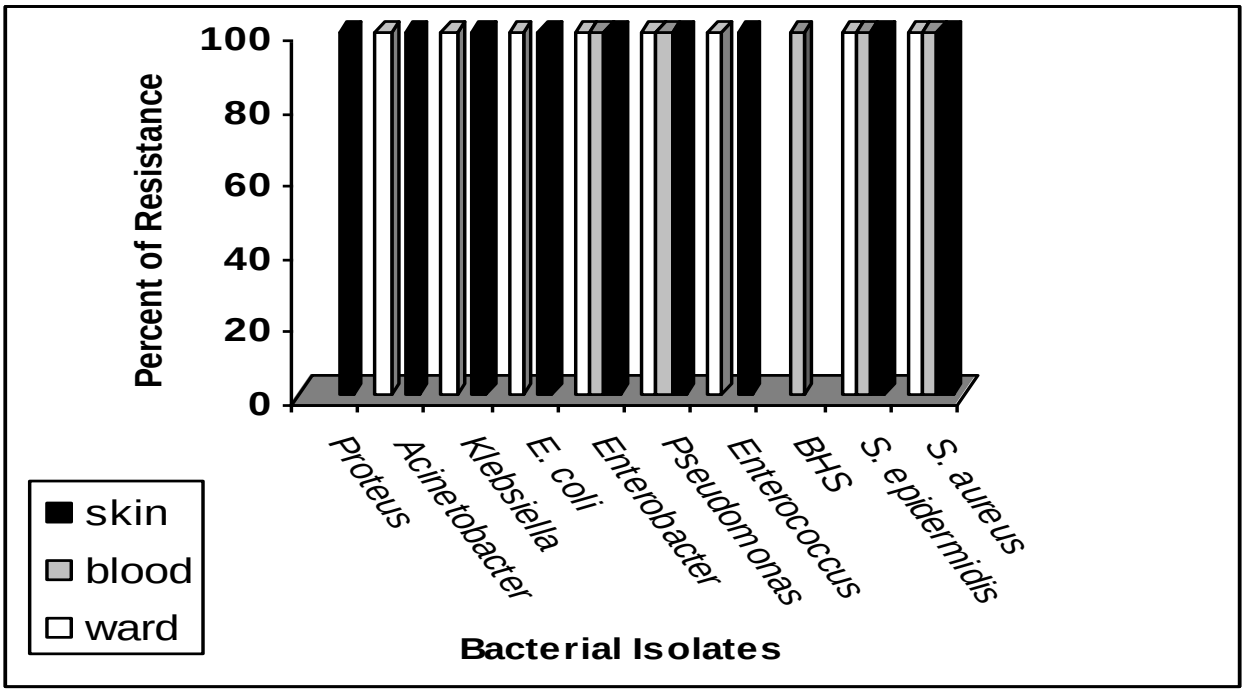
that obtained by Al-Shukri(٢٠٠٣) who clarified that the resistance rate of *Acinetobacter* to ampicillin was ٦٣.٦٪. Enterococcal resistance to different types of antimicrobial agents such as β-lactam drugs was observed by Weinstein *et al*.(١٩٩٦).



Figure(٣-١) Percentage of Bacterial Resistance to Amoxicillin

Gold and Moellering(١٩٩٦) notice that there is a rise in enterococcal prevalence as hospital pathogens which is attributed to their natural resistance to most commonly used antibiotics and their capacity to acquire resistance to penicillin by mutations. Reardon *et al*.(١٩٩٨) assert that there is no benefit from using penicillin, ampicillin, and amoxicillin alone in treating patients with septicaemia caused by *Staph. aureus* because of the high resistance to these antibiotics.

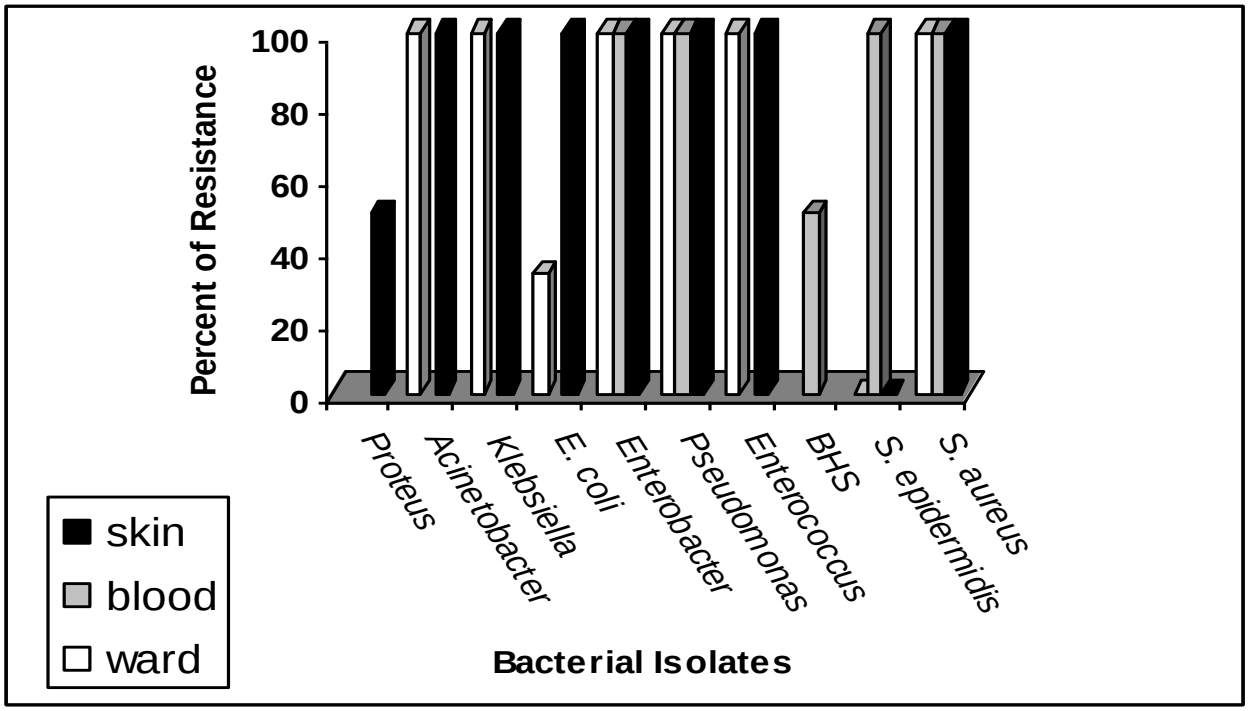
As shown in Figure (٣-٢) all the bacterial isolates in this study are completely resistant(١٠٠٪) to carbenicillin which is an anti-pseudomonal penicillin (Brooks *et al*., ٢٠٠٤). This result agrees with Al-Shukri(٢٠٠٣) who found that ١٠٠٪ of *Acinetobacter* were resistant to carbenicillin.



Figure(٣-٢) Percentage of Bacterial Resistance to Carbenicillin

Whereas, cefotaxime which is a third generation cephalosporin, its bactericidal activity results from inhibition of cell wall synthesis(Howland and Mycek, ٢٠٠٦). As shown in Figure (٣-٣) that cefotaxime was effective against *S. epidermidis* from burned skin and burn unit whereas that of blood was resistant(١٠٠٪). One isolate of β-hemolytic streptococci(٥٠٪), one isolate of *Proteus*(٥٠٪), ٣٣.٣٪ of

E.coli from the ward and (١٠٠٪) of all other bacterial isolates were resistant to this antibiotic. This agrees with Wiener *et al.*(١٩٩٩) who demonstrate that there is high resistance rate to several antimicrobials including cephalosporin both second and third generations. Arslan *et al.*(١٩٩٩) found that nearly ٧٤٪ of the Gram negative isolates were organisms associated with the production of inducible Richmond Sykes type one cephalosporinase and those organisms produce large quantities of type one cephalosporinase when exposed to first generation cephalosporin, ampicillin and pencillin G. As these antimicrobials are readily hydrolyzed by this enzyme, inducible organisms are intrinsically resistant to these agents.



Figure(٣-٣) Percentage of Bacterial Resistance to Cefotaxime

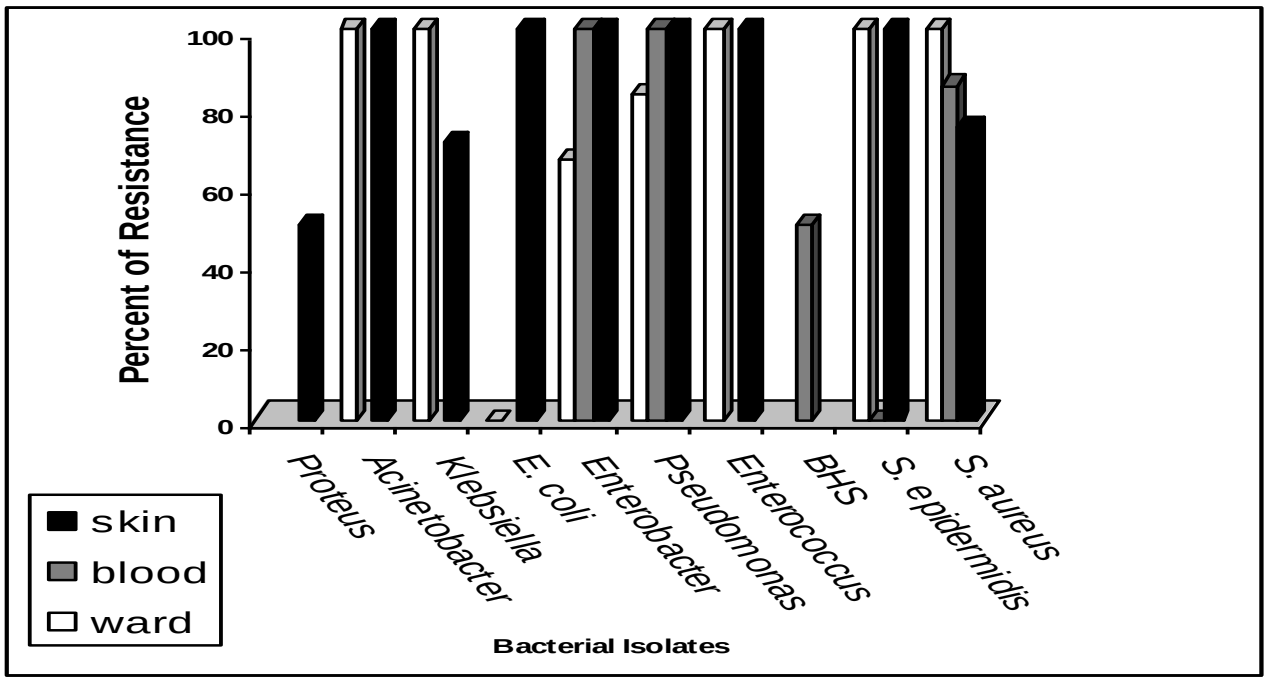
Eggman *et al*.(١٩٩٧) and Brooks *et al*.(٢٠٠٤) stated that the strains of *Enterobacter, Klebsiella, Proteus* and *Pseudomonas* isolated from hospitals had multiple antibiotic exposure were almost always resistant to cephalosporins because they possess inducible chromosomally determined β-lactamase with high affinity for cephalosporines. The antibiotic resistance phenomenon is widely common among clinical isolates of *Acinetobacter* which are often inherently resistant to many antimicrobial agents including β-lactams(Hpa, ٢٠٠٣). Zhang *et al.*(٢٠٠٣) mentioned that by forming biofilms, *S.epidermidis* is not only resistant to antibiotics, but also can be the reservoir for antibiotic resistant genes which can be transferred to other bacteria. There is a significant variance in resistance pattern which existed in different geographic regions. The longer antibiotics are in use the greater the resistance ratio appears in time. Resistance to multiple antibiotics including penicillin, cephalosporin and others, has gradually increased among a number of Gram negative hospital pathogens, especially *Klebsiella pneumoniae*(Gold and Moellering, ١٩٩٦), *Enterobacter* spp., *P. aeruginosa* (Richard *et al*., ١٩٩٤), and *Acinetobacter baumannii* (Towner, ١٩٩٧). Pechere and Kohler(١٩٩٩) pointed out that all known mechanisms of resistance to β-lactam antibiotics can be found in *P. aeruginosa*. These mechanisms are also explained by

Thomson and Amyes(١٩٩٣) as β-lactamase production that hydrolyzes the β-lactam ring which is controlled by plasmid or chromosomal regulation like the plasmid-encoded β-lactamase TEM-١, or lack of protein receptors on cell wall and alteration in their permeability to β-lactam antibiotics and preventing uptaking of antibiotics by blocking the pores of outer membrane. The resistance of *S. epidermidis* to β-lactams is mediated by β-lactamase production under chromosomal control(Humphreys *et al*., ٢٠٠٤). Furthermore, Thomson and Amyes(١٩٩٣) expressed that *S. epidermidis* exhibits resistance to many types of antibiotics and this resistance may be attributed to the R-plasmid acquired from pathogenic bacteria present in the site of infection. Moreover, Lowy(١٩٩٨) shows that *Staph*. *aureus* caries a wide variety of multi-drug resistant plasmids which can spread among different species of staphylococci mainly *Staph*. *epidermidis*. Resistance mediated by *P.aeruginosa* can be attributed to both an inducible, chromosomally mediated β-lactamase that can render broad spectrum cephalosporin inactive and to a plasmid mediated β-lactamase that can lead to resistance to several penecillins and older cephalosporins(Reardon *et al*., ١٩٩٨). Resistance of *P.aeruginosa* to carbapenems that is selected by prolonged imipenem use can be acquired by plasmid-mediated imipenemase or alteration of outer membrane protein. The resistance mechanism of *P.aeruginosa* to many antibiotics(multi-drug efflux pump) plays a major role in the resistance of

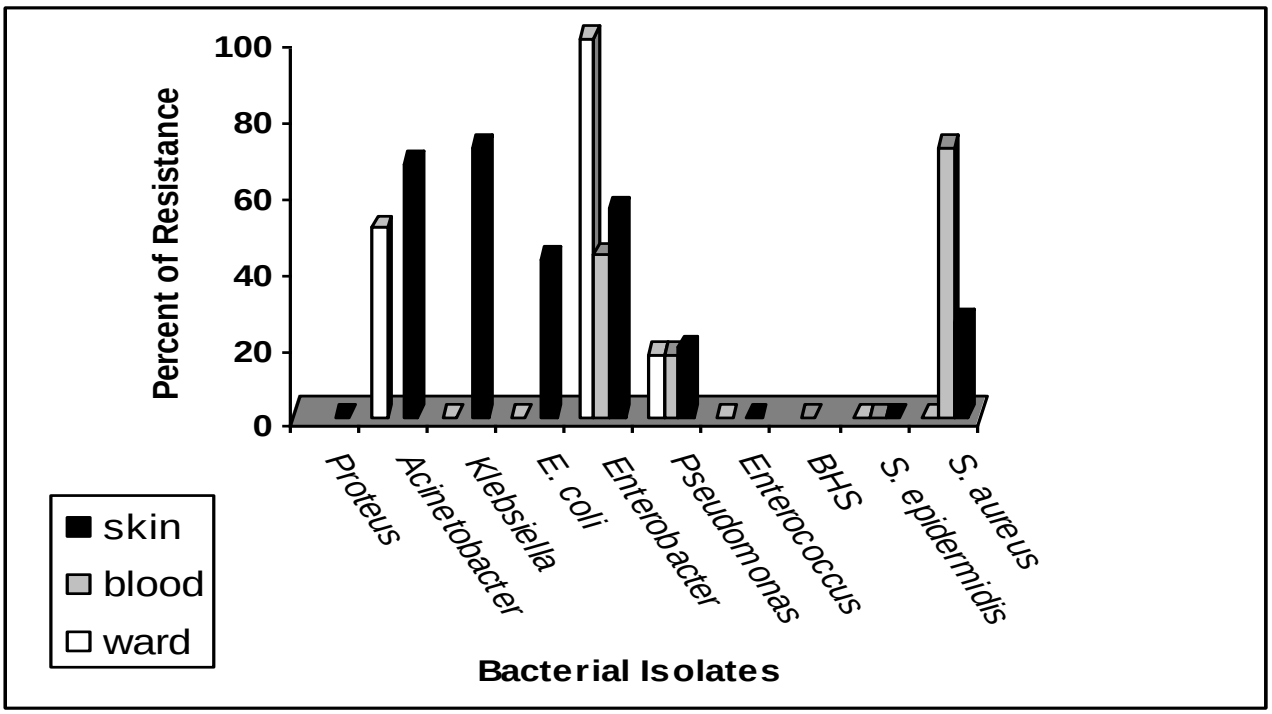
P.aeroginosa to carbapenems as well(Kanchannapoom and Khardori, ٢٠٠٢). Christian *et al.* (٢٠٠٣) noticed that the cause of high resistance of *Enterobacter* isolated from blood of septicaemic patients could be attributed to the production of extended spectrum beta lactamase enzyme(ESBL) which is very effective against third generation cephalosporin. This is also asserted by Kim *et al*. (٢٠٠٣) who found that *Enterobacter spp.* isolated from blood of septicaemic patients were resistant to third generation cephalosporin. Reynoldes *et al*.(٢٠٠٤) found that *E. coli, K.pneumoniae* and *Enterobacter* causing septicaemia were resistant to amoxicillin. The β-lactam antibiotics are widely used in treatment of various infections occurred in man, especially these infections caused by *S. aureus, S. epidermidis,* so, new generations of β-lactam antibiotics are better to be used instead of ampicillin and amoxicillin like those containing β-lactamase inhibitors, namely clavulanic acid(Brooks *et al*., ٢٠٠٤). As shown in Figure (٣-٤), complete resistance to gentamicin is expressed by each of the following bacteria isolated from burn ward: *S. aureus, S. epidermidis, Enterococcus, Acinetobacter, Klebsiella,* relatively less resistance rate is shown in *Pseudomonas* (٨٣.٣٪) and *Enterobacter* (٥٠٪) whereas only *E.coli* was sensitive to this antibiotic. Bacterial isolates from the skin showed the following resistance rate: ١٠٠٪ of each of *S.epidermidis, Enterococcus faecalis, P.aeruginosa, Enterobacter* spp., *E.coli, A.baumannii* were resistant, ٧٥٪ of *S. aureus*, and ٧١٪ of *Klebsiella*. Results of bacterial isolates of

blood showed ١٠٠٪ resistance for each of *Pseudomonas* and *Enterobacter.* About ٨٦% of *Staph. aureus and* ٥٠٪ of β-haemolytic streptococci were resistant while *Staph. epidermidis* was sensitive. Rashmi *et al.*(٢٠٠٥) noticed that *Acinetobacter,* and *Staph. auerus* showed high resistance rate to gentamicin.

Tonkic *et al*. (٢٠٠٥) found that *E.coli* was sensitive to this antibiotic. As shown in Figure (٣-٥), lesser resistance rates to amikacin were expressed by nearly all bacterial isolates. *S.aureus, S.epidermidis, Enterococcus, E.coli, Klebsiella*, from the burn ward, those from skin(*S.epidermidis*, *Enterococcus and Proteus)* and those from blood(β-haemolytic streptococci and *Staph. epidermidis*) were completely sensitive to amikacin.



Figure(٣-٤) Percentage of Bacterial Resistance to Gentamicin



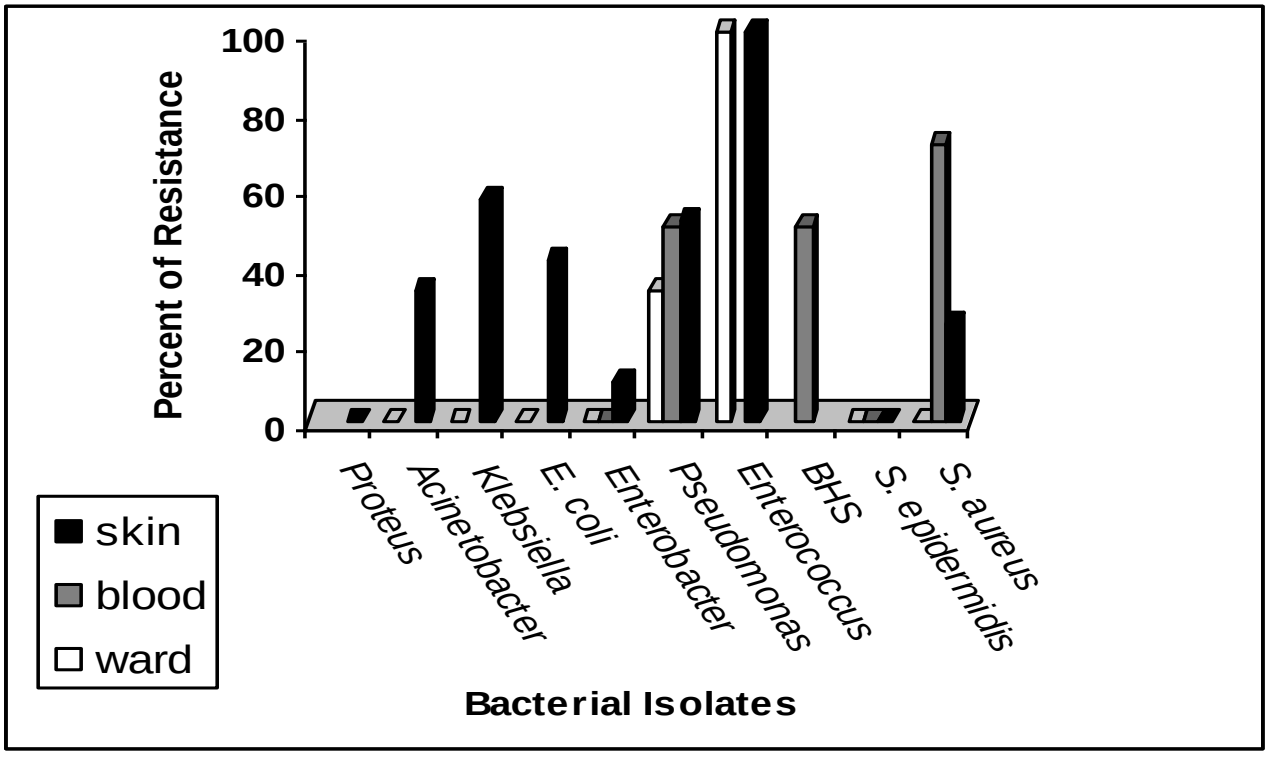
Figure(٣-٥) Percentage of Bacterial Resistance to Amikacin

Others showed lesser resistance rate in comparison to gentamicin. This agrees with Zahac *et al*.(٢٠٠٣) who revealed that *Enterobacter* was resistant to gentamicin, but it is sensitive to amikacin. Karlowsky *et al.* (٢٠٠٤) found the same result of high resistance to gentamicin and they pointed out that this resistance was due to the production of antibiotic modifying enzymes and they noticed that the previous exposure to antibiotics often leads to multi-drugs resistant *P.aerugenosa*.

Resistance to aminoglycoside in all types of bacteria, including *P.aerugenosa* has become more complex with increased time of aminoglycoside usage(Shahid and Malik, ٢٠٠٥). This resistance is commonly acquired by plasmid-mediated modifying enzymes(Karlowsky *et al.,*١٩٩٧). Kucers *et al*.(١٩٩٧) explained that enzymatic modifications, the most common type of aminoglycoside resistance, resulted in high level of resistance in *P.aerugenosa*. Therapy with aminoglycosides enhances the production of enzymes such as transacetylase, adenyltransferase and phosphotransferase which are known to be capable of inducing resistance(Magliacani *et al*., ١٩٩٠). Amikacin, a semisynthetic derivative of kanamycin, is relatively resistant to several enzymes that inactivate gentamicin and tobramycin and therefore amikacin can be employed against some microorganisms resistant to the latter drugs. However, bacterial resistance due to impermeability to amikacin is slowly increasing (Brooks *et al*., ٢٠٠٤).

Regarding ciprofloxacin, (Figure ٣-٦), most bacterial isolates were sensitive to this antibiotic. *Enterococcus faecalis* isolated from skin and burn ward showed ١٠٠٪ resistance to ciprofloxacin. *S. aureus* isolated from the blood showed ٧١.٤٪ resistance followed by *Klebsiella* ٥٧.١٪ which is isolated from skin, β-haemolytic streptococci(٥٠٪) and *P.aeruginosa* isolated from blood(٥٠٪), *E.coli* from skin (٤١.٦٪), *P.aeruginosa* of burn ward (٣٣.٣٪), *Acinetobacter*

baumannii isolated from the skin (٣٣.٣٪). This agrees with Al-Shukri(٢٠٠٣) who found that ٩١٪ of *Acinetobacter baumannii* were sensitive to ciprofloxacin, and Fernandez-Cuenca *et al*.(٢٠٠٤) who observed that ٢٠٪ of *Acinetobacter* were resistant to ciprofloxacin. Moreover, Robin(٢٠٠٥) found that ١٠٠٪ of *Enetrococcus faecalis* were resistant to ciprofloxacin. Rachid *et al*.(٢٠٠٠) observed that in staphylococci there were increased numbers of resistant strain to ciprofloxacin.

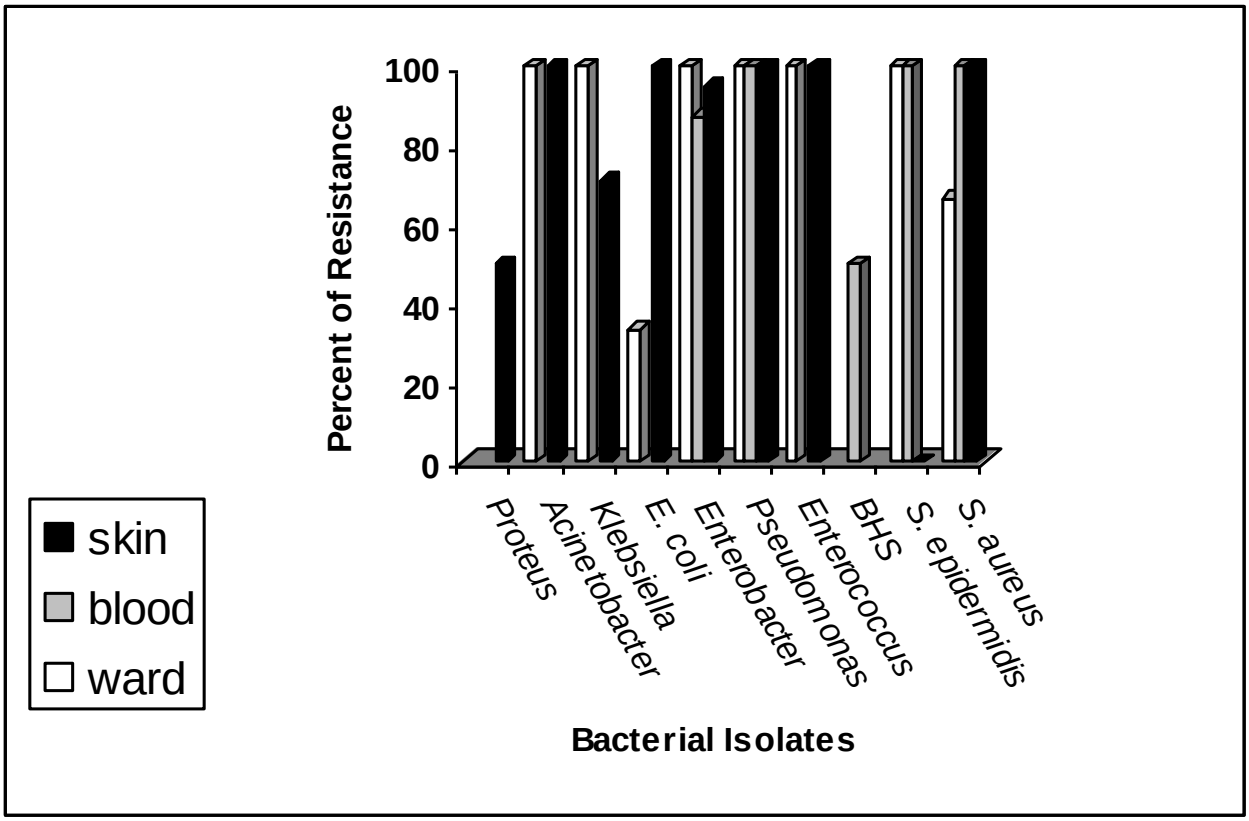


Figure(٣-٦) Percentage of Bacterial Resistance to Ciprofloxacin

Tonkic *et al.*(٢٠٠٥) found that ٤.٤٪ of *E.coli* and ٤٪ of *Klebseilla pneumoniae* were resistant to ciprofloxacin, and Oteo *et al.*(٢٠٠٥) showed that ١٩.٣٪ of *E.coli* was resistant, and Rashmi *et al.*(٢٠٠٥)

found that ciprofloxacin was the only drug of choice against *Staph. aureus.* Richard *et al.*(١٩٩٤) pointed out that *P. aerugenosa* was less resistant to ciprofloxacin (٢٦.٧٪). Ciprofloxacin inhibits bacterial DNA synthesis, an event that is followed by rapid bacterial cell death. It is active against Gram negative bacteria and less effective against Gram positive types(Howland and Mycek, ٢٠٠٦). Jalal and Wretlind (١٩٩٨); and Donnel and Gelone(٢٠٠٠) report that the resistance to flouroquinolones is through chromosomal mutations or alternations affecting the ability of flouroqinolones to permeate the bacterial cell wall.

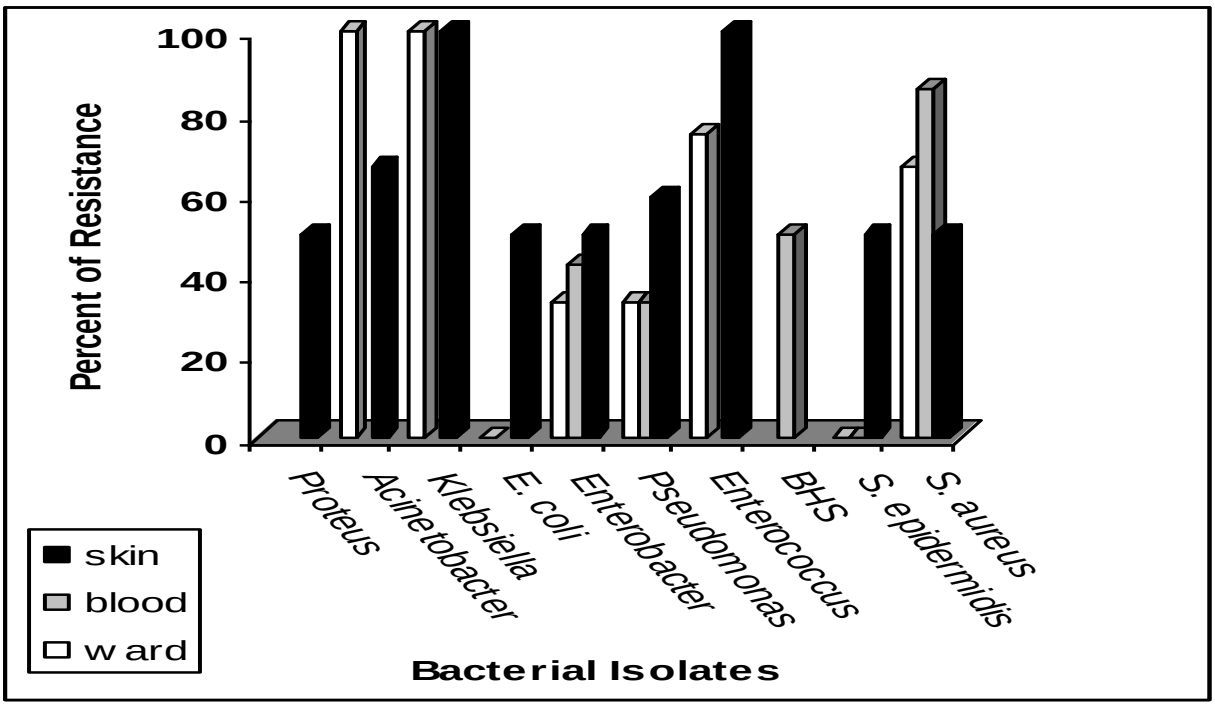
Regarding trimethoprim-sulphamethoxazol(Figure ٣-٧), most bacterial isolates showed ١٠٠٪ resistance to this antibiotic and only *Staph. epidermidis* isolated from skin was completely sensitive. About ٣٣٪ of *E. coli* and ٦٧٪ of *Staph*. *aureus* were resistant to this antibiotic. ٥٠٪ of β-haemolytic streptococci isolated from burn ward were resistant. This result agrees with Huda *et al.* (٢٠٠١) who found that ٤٠ % of *E. coli* were resistant. Bonnet (٢٠٠٤) found that *Enterobacter* was completely resistant to this antibiotic. Al-Shukri(٢٠٠٣) found that ٧٢.٢٪ of *A. baumannii* were resistant to trimethoprim. Oteo *et al*.(٢٠٠٥) stated that ٣٢.٦٪ of *E. coli* were resistant to trimethoprim. Trimethoprim is active against many Gram positive and Gram negative aerobic bacteria excepting *P. aeruginosa*(Brooks *et al*., ٢٠٠٤).



Figure(٣-٧) Percentage of Bacterial Resistance to Trimethoprim-sulphamethoxazol

Regarding azithromycin,(Figure ٣-٨) ١٠٠٪ of *Enterococcus faecalis*, *K. pneumoniae* and *A. baumannii* were resistant. About ٥٠٪ of *S. aureus, S. epidermidis*, *P.aeruginosa, Enterobacter* spp., *E. coli, Proteus* spp., and β-haemolytic streptococci were resistant. Other isolates show resistance in lesser degrees. Fairuze-Ali, (٢٠٠٦) found that Gram negative bacteria (*K. pneumoniae*, *E.coli, Enterobacter*

spp., *P. aeruginosa*) isolated from blood of septicaemic patients were resistant to erythromycin(٧٧.٥٪).



Figure(٣-٨) Percentage of Bacterial Resistance to Azithromycin

Azithromycin, from the macrolides (azalides) group of antibiotics is chemically related to erythromycin and it is active against staphylococci and streptococci, interferes with bacterial ribosomal function and inhibits protein formation. It is active against

Gram-negative bacteria but is relatively less effective against Gram-positive type than erythromycin(Howland and Mycek, ٢٠٠٦).

٣.٥ Sources and Modes of Infection

As mentioned above(page ٥٧ , in Tables(٣-٩) and(٣-١٠)), there is some similarity in the types of bacteria found in each of skin, blood and burn ward. This may indicate that the bacteria isolated from the burn unit can affect the burn patients causing burn wound infection and even can invade the viable tissue of the wound to cause bacteraemia. This agrees with Song *et al.*(٢٠٠١) who found that the main source of sepsis in burn patients was the burn wound. One reason for this might have been the fact that multi-drug resistant nosocomial bacteria present in the burn ward replace the original endogenous flora of burn patient within a few days of admission.

There are numerous ways in which microorganisms can gain access to a wound either by direct contact by transfer of microorganism from the equipment or hands of nursing staff or by air-born dispersal in which microorganisms deposited from the surrounding air, or by self- contamination from the patients skin or gastrointestinal tract (Collier, ٢٠٠٣). Factors predisposing to this transmission include, the length of stay in hospitals, intensity and duration of exposure to broad spectrum antibiotics, severity of underlying illness and the use of invasive devices (Lortholary *et al*., ١٩٩٥). As shown in Table (٣-٩) the Gram positive bacteria isolated in

this work were *Staph. aureus*, *Staph. epidermidis, Enterococcus faecalis* and β-haemolytic streptococci. The latter are the main pathogens associated with local and systemic invasion(Brooks *et al*., ٢٠٠٤).

Although *Staph. epidermidis* is usually non-pathogenic, it is an important cause of infection in patients whose immune system is compromised, or who have indwelling catheters(Goldmann and Pier, ١٩٩٣). Hall(١٩٩١) reveals that CoNS have emerged as pathogens in a growing numbers of serious nosocomial infections in intensive care units particularly blood stream infection.

Bagdonas *et al.*(٢٠٠٣) found that *Staph. aureus* was the most common pathogen in burned patients. Likewise, Cook(١٩٩٨) mentioned that colonization with MRSA increases the morbidity and mortality in burn patients including the risk for bacteraemia and loss of skin grafts. On the other hand, Fowler *et al.*(٢٠٠٣) clarified that *Staph. aureus* bacteraemia led to several complications including infective endocarditis, sepsis or metastatic foci of infection.

As shown in Table (٣-١٠), the high frequency of *P.aeruginosa* may be explained by the fact that it is regarded as an opportunistic pathogen. This agrees with Gillespie and Bamford(٢٠٠٣) who asserted that after burn injury, skin became highly susceptible to microbial infections and the greatest challenge came from the opportunistic bacteria which can infect and thrive as a result of

limited immunity of skin together with the lack of efficient supply of orally given antibiotics at the infection sites due to the loss of blood capillaries.

The *Pseudomonas* and *Acinetobacter* are widely distributed in soil and water. *P. aeruginnosa* sometimes colonizes humans and is the major human pathogen of the group. It is invasive and toxigenic, produces infections in patients with abnormal host defenses and is an important nosocomial pathogen(Brooks *et al.*, ٢٠٠٤). Other types of bacteria found in this study are the *enterobacteriaceae*, namely *Enterobacter* spp*., E.coli, K. pneumoniae* and *Proteus* spp.; this agrees with Mayhall(٢٠٠٣) who states that the gastrointestinal tract continues to be the potential reservoir for microorganisms that colonize the burn wound surface. There is also a high risk of contamination of the burn patients when they are wrapped in unsterilized blankets(Muir *et al*., ١٩٨٧). *Enterobacteriaceae* are also encountered in causing sepsis in burn patients. This is explained by Lenguas *et al*.(١٩٩١) who pointed out that the principal microorganisms which provoke sepsis in burn patients are staphylococci, *P.aeruginosa* and entrobacteriaceae group. The antibiogram profile is one of the methods used for biotyping bacteria isolated from two different sources; however, DNA techniques are used to confirm that the bacterial type which is isolated from one source is the same as that of the other (Al-Saeed, ١٩٩٧). In this

Bacterial Isolate	Source of Bacteria	AX	Py	AZM	CTX	AK	CN	CIP	SXT

study, generally, the antibiogram profile of the bacterial isolates obtained from the skin is similar to some extent to that of the burn unit isolates. Specifically, the antibiogram profile is found to be similar to the following isolates: (٣)isolates of *P. aeruginosa*,(٢)isolates of *Enterobacter* spp.,(٢)isolates of *Staph. aureus* and one isolate of *Enterococcus faecalis,* which are isolated from the skin of burn patients and at the same time, these isolates are found in the burn unit. This may indicate that there is a good relation between the skin and burn unit bacteria suggesting that the burn unit is an important origin for the affection of the skin of burn patients and vice versa. Table (٣-١١) illustrates eight isolates of skin and burn unit that have similar antibiogram profile. Also, there is a good relation between the bacteria isolated from the skin and blood of burn patients Table(٣-١٢) illustrates nine samples of blood and skin swab cultures for the same patient sharing the same bacterial isolates. Five of these isolates have the same antibiotic profile. This result supports the known idea that the

Staph. aureus	**skin**	+	+	+	+	-	+	-	+
	burn unit	+	+	+	+	-	+	-	+
Staph. aureus	**skin**	+	+	-	+	-	+	-	+
	burn unit	+	+	-	+	-	+	-	+
Enterococcus faecalis	**skin**	+	+	+	+	-	+	+	+
	burn unit	+	+	+	+	-	+	+	+
***Enterobacter* spp.**	**skin**	+	+	+	+	+	+	-	+
	burn unit	+	+	+	+	+	+	-	+
***Enterobacter* spp.**	**skin**	+	+	-	+	+	+	-	+
	burn unit	+	+	-	+	+	+	-	+
P.aeruginosa	**skin**	+	+	+	+	+	+	+	+
	burn unit	+	+	+	+	+	+	+	+
Pseudomonas aeruginosa	**skin**	+	+	+	+	-	+	-	+
	burn unit	+	+	+	+	-	+	-	+
P.aeruginosa	**skin**	+	+	-	+	-	+	-	+
	burn unit	+	+	-	+	-	+	-	+

Table(٣-١١) Bacterial Isolates Obtained From Skin and Burn Unit With Similar Antibiotic Profile

- Sensitive + Resistant * Bacterial isolates sharing the same antibiotic profileAX: Amoxicillin Py: carbenicillin AZM: azithromycin CTX: cefotaxim AK: amikacin CN: gentamicin CIP: ciprofloxacin SXT: trimethoprim-sulfamethoxazol

Table(٣-١٢) Antibiotic Profile for Bacterial Isolates Obtained

From Skin and Blood of the Same Patient

Bacterial Isolate	Source of Bacteria	AX	Py	AZM	CTX	AK	CN	CIP	SXT
Staph. aureus	Blood	+	+	+	+	-	+	-	+
	Skin	+	+	-	+	-	+	-	+
*Staph. aureus**	Blood	+	+	-	+	-	+	+	+
	Skin	+	+	-	+	-	+	+	+
*Staph. aureus**	Blood	+	+	+	+	+	-	-	+
	Skin	+	+	+	+	+	-	-	+
*Enterobacter** spp.	Blood	+	+	-	+	-	+	-	+
	Skin	+	+	-	+	-	+	-	+
*Enterobacter** spp.	Blood	+	+	-	+	+	+	-	+
	Skin	+	+	-	+	+	+	-	+
Enterobacter spp.	Blood	+	+	-	+	-	+	-	-
	Skin	+	+	+	+	-	+	-	+
Enterobacter spp.	Blood	+	+	+	+	+	+	-	+
	Skin	+	+	+	+	+	+	-	-
*P. aeruginosa**	Blood	+	+	+	+	+	+	+	+
	Skin	+	+	+	+	+	+	+	+
P.aeruginosa	Blood	+	+	-	+	-	+	-	+
	Skin	+	+	+	+	+	+	+	+

- Sensitive **+ Resistant** *** Bacterial isolates sharing the same antibiotic profile**

AX: Amoxicillin **Py: carbenicillin** **AZM: azithromycin** **CTX: cefotaxim** **AK: amikacin**

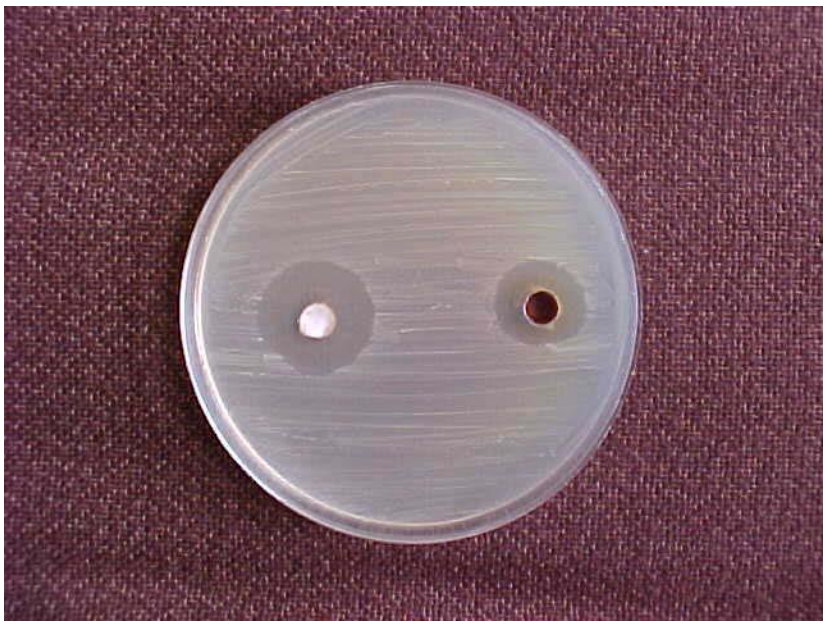
CN: gentamicin **CIP: ciprofloxacin** **SXT: trimethoprim-sulfamethoxazol**

burn wound is one of the sources of bacteria to invade the blood and finally to cause septicemia(Arslan *et al,*١٩٩٩).

٣.٦ Effect of Silver Sulphadiazin and Silver Nitrate on Bacterial Isolates of Skin.

The effects of silver sulphadiazin(SSD, ١٪) and silver nitrate(SN, ٠.٥٪) on the most frequent bacteria isolated from burned skin were tested in this study. These effects are shown in Figures(٣-٩ and ٣-١٠) and in Table (٣-١٣). There is no significant difference between the effect of SSD and SN on all the studied bacterial isolates (P>٠.٠٥). The most affected bacterial species by SSD is the *Staph. epidermidis* and the least susceptible is *Staph. aureus*, whereas the most affected bacterial species by SN is *P.aeruginosa* and the least affected are *Enterobacter* and *Staph. aureus*. The biological substances secreted by the damaged skin and the damaged or dead tissue itself may interfere with the action of SSD or SN on bacterial isolates colonizing the skin and may reduce their effectiveness. Drost *et al*.(١٩٩٣) pointed out that bacteria were protected by dead tissue in to which antibiotics diffuse poorly and they mentioned that local antibiotics are of little value in the treatment of infected burns as long as dead tissue is still present, and so they mentioned that the mainstay of treatment is the rapid removal of dead tissue by surgical excision in

the form of immediate burn wound excision with immediate skin graft.



١ ٢

Figure(٣-٩) Effect of Silver Sulphadiazin(١) and Silver Nitrate(٢) on *Staph. epidermidis*

Figure(٣-١٠) Effect of Silver Sulphadiazin(١) and Silver Nitrate(٢) on *Staph. aureus*

Table(٣-١٣) Effect of Silver Sulphadiazin and Silver Nitrate on Bacterial Isolates from Burned Skin

Bacterial	Diameter of Inhibition Zone(mm)	Significance

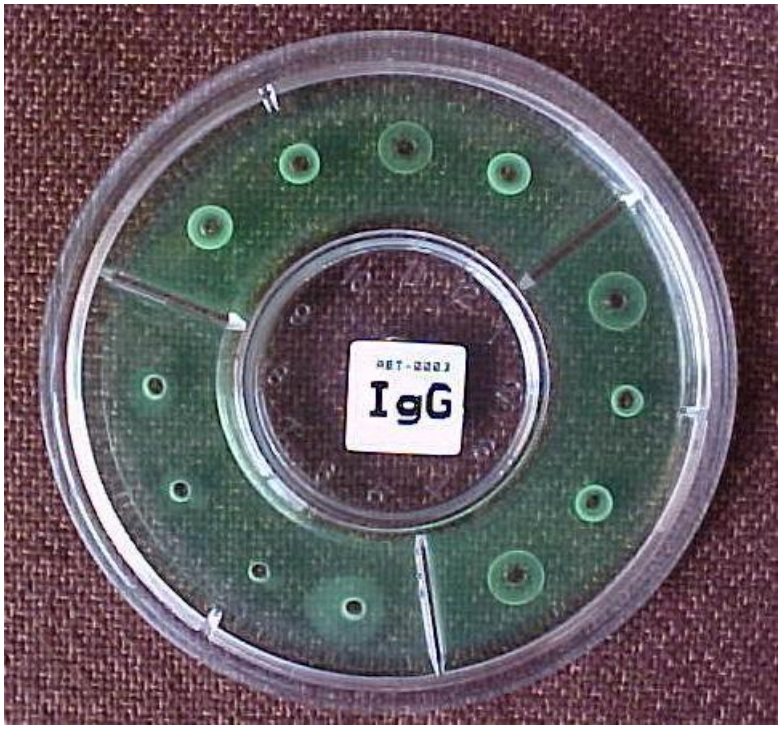
Isolates	SSD ١٪	SN ٠.٥٪	
Staph. aureus	١٣	١٣	Not Significant P>٠.٠٥
Staph. epidermidis	٢٣	١٨	Not Significant P>٠.٠٥
P. aeruginosa	١٧	١٩	Not Significant P>٠.٠٥
***Enterobacter* spp.**	١٥	١٣	Not Significant P>٠.٠٥
K. pneumoniae	١٨	١٤	Not Significant P>٠.٠٥
E.coli	٢٠	١٨	Not Significant P>٠.٠٥

Al-Akayleh(١٩٩٩) found that ٠.٥٪ SN and ١٪ SSD had an effect in the reduction of the incidence of burn wound sepsis. Besides, Noranha and Almeida(٢٠٠٠) pointed out that SSD was the drug of choice for prophylaxis of burn wound infection in most burn patients, besides, clinical trials have shown that SSD is efficacious in reducing bacterial numbers and delaying burn wound colonization with Gram negative bacteria(Parikh *et al*., ٢٠٠٥). SSD has a wide antibacterial range effective against both Gram positive and Gram negative bacteria. Although it is effective in prophylaxis of *Pseudomonas* infection, it is not as good as sulphamylon for the treatment of established infection(Muir *et al*., ١٩٨٧). Although various attempts have been made to develop more effective silver compounds, so far SSD remains the most widely used substance of this type(Klasen, ٢٠٠٠). Because of its minimal absorption, SN is an excellent prophylactic agent, but it is not indicated for established wound infection. However, SN solution must be used by soaking bulky wet dressings which must be kept wet every two hours to keep the concentration of the agent at less than ٢٪ which is caustic and cytotoxic (Press, ١٩٩٧).

٣.٧ Humoral Immune Response of Burn Patients

The results expressed in Table (٣-١٤) show that there is a significant decrease in the serum level of IgG of burned patients(١٤٢٢.٦) mg/dl (Figure٣-١١) in comparison with the ٠.٠٥). This agrees with Robins(١٩٩٠) <controls(٢٣٤٣.٣٩) mg/dl(P who pointed out that serum IgG levels were decreased after burn injury. Yurt(٢٠٠٠) stated that the serum IgG levels were decreased secondary to burn injury and he mentioned that persistently decreased levels of IgG had been related to mortality.

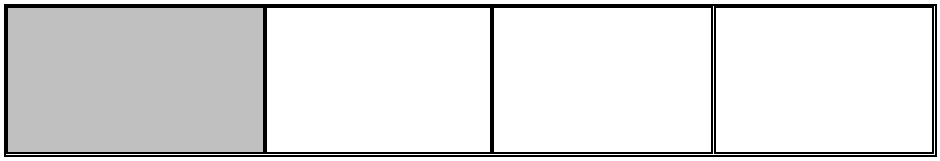
This low level of IgG may predispose burned patients to bacterial infection. It is known that the IgG is important in the mechanisms of bacterial antigen clearance in blood. These mechanisms involve the association of IgG with the antibody-dependent-cell-cytotoxicity (ADCC), association with complement-mediated bacterial cytolysis or by its opsonization role for phagocytic cell (Abbas *et al*., ٢٠٠٤).



Figure(٣-١١) Single Radial Immunodiffusion Test(SRID) for IgG

Table (٣-١٤) Concentrations of Immunoglobulins IgM, IgG and IgA(mg/dl) in Burn Patients and Controls

		IgM	IgG	IgA
Patient	M	١٥٢.١٧٨٩	١٤٢٢.٦٠٧٠	٩٠.٠٦٣٢
	SD	٣٩.١٥٦٧	٨٠٢.٢٨٩٨	٤٤.٧٦٢٩
Control	M	١٣٨.٠٢٠٠	٢٣٤٣.٣٩٦٠	٣٤٨.٩٠٠٠
	SD	٤٠.٢٠٩٤	٤٧٦.٤٤٤٥	٨٠.٠٠٤٧
Significance		Not Significant P>٠.٠٥	Significant P<٠.٠٥	Significant P<٠.٠٥



The mean serum levels of IgA (Figure ٣-١٢, Table ٣-١٤) are also significantly decreased in the burned victims (٩٠.٠٦)mg/dl in comparison with the control individuals(٣٤٨.٩) mg/dl (P<٠.٠٥). IgA in the circulation play relatively less important role when compared to the role of secretary IgA(Stites, ٢٠٠١). Thus, the low level of IgA in patients may reflect the decreasing level of secretary IgA in the epithelial surface(in skin) which may lead to the easy penetration of bacteria into the blood stream. This interpretation depends on the central role of S-IgA to act as a first line of defense against infectious agents in mucosal surfaces(Strober and James, ٢٠٠١).

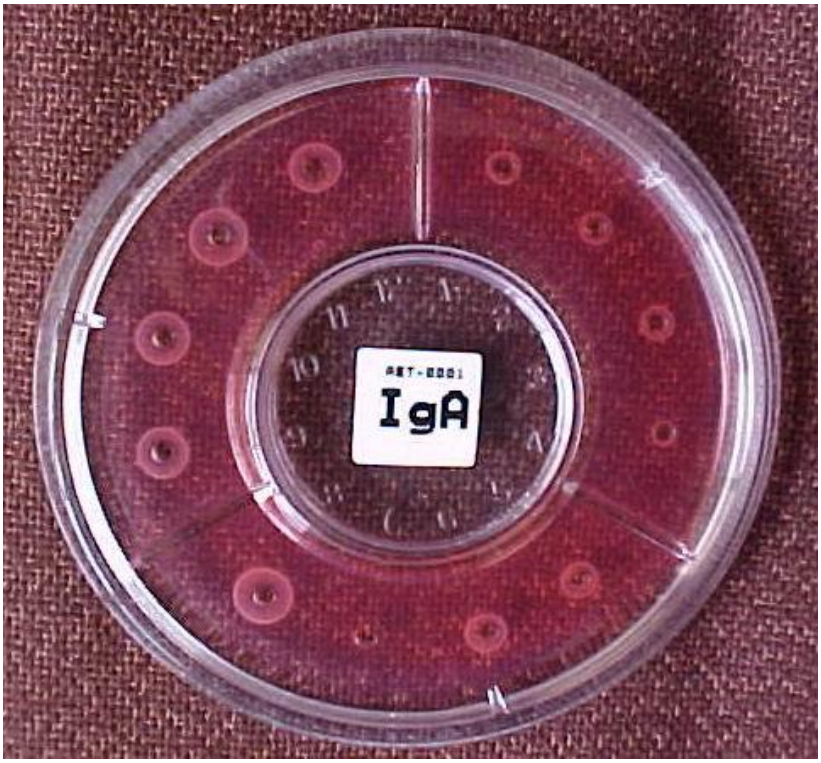
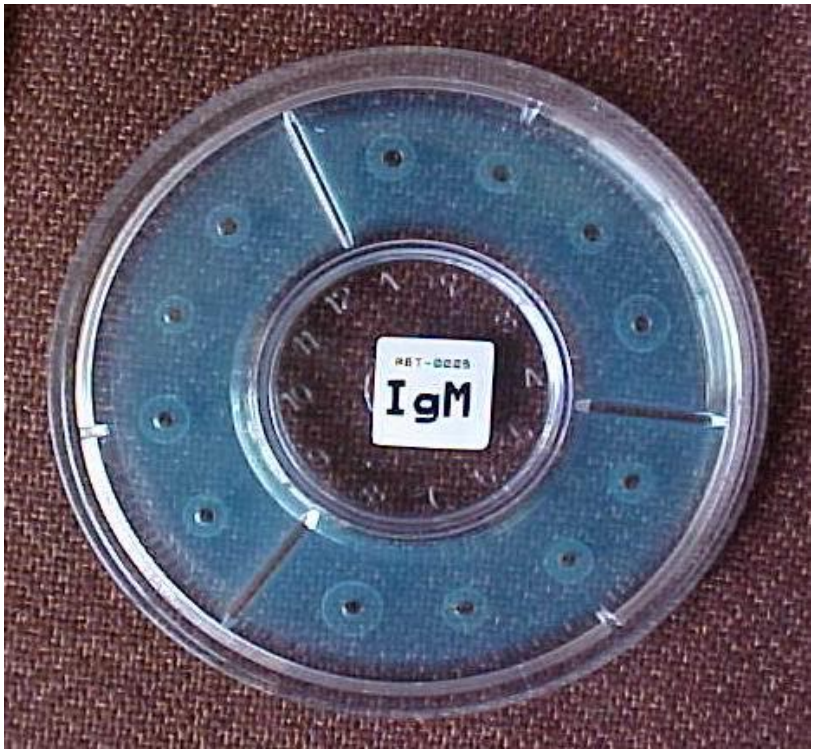


Figure (٣-١٢) Single Radial Immunodiffusion Test (SRID) for IgA

Regarding the mean serum levels of IgM(Figure ٣-١٣, Table ٣-١٤), there is no significant difference(P>٠.٠٥) between burned patients and normal control individuals which are (١٥٢.١٧, ١٣٨.٠٢) mg/dl respectively. These results agree with(Dominioni *et al*., ١٩٩١; and Rodgers,١٩٩٤) who pointed out that the serum level of IgM in burn patients might remain within the normal levels. During the acute bacterial infection, the serum IgM level increases several folds above the normal range(Paul, ٢٠٠٣), but in the present study, the IgM levels of patients with proved bacterial infections(bacterial-sensitized) were significantly similar to that of normal (bacterial-non-sensitized) subjects; this may be interpreted as there is failure of

IgM-B-cells to produce an increased level of IgM in these patients. This may indicate that the rate of IgM is not so enough to overcome the bacterial infection and this may be caused by the suppressor effect of burns on humoral immunity (Demling, ٢٠٠٥). The improvement of immune state of burn victims is now considered as one of the most important ways in the treatment of burn infection(Bowler *et al.,* ٢٠٠١).



Figure(٣-١٣) Single radial immunodiffusion test(SRID) for IgM

Barlow(١٩٩٤) pointed out that recognition of immunoglobulin deficiencies: IgM, IgG and IgA in burned patients may permit focused therapy, such as specific replacement of these proteins. It has been demonstrated that therapy by using *Pseudomonas* immunoglobulin(Ig) in burn wound infection may be more effective

on bacterial translocation than treatment using general immunoglobulin; however, the use of Ig treatment decreases the incidence of bacteraemia from burn wound infection, protects the intestinal ecological equilibrium by decreasing the bacterial overgrowth in the intestinal micro-flora, decreases the number of translocated bacteria and prevents bacterial translocation spread beyond the mesenteric lymph nodes; for these reasons the administration of Ig in burn wound infection effectively prevents sepsis due to bacterial translocation(Herek *et al.*٢٠٠٠).

Regarding the complement components: C٣ and C٤ levels (Figures ٣-١٤, and ٣-١٥), the results of this study revealed that there is no significant difference (P>٠.٠٥) between the mean level of serum C٣ in patients (١٣٣.١٦) mg/dl and in the controls (١٢٢.٣٢) mg/dl. The same results are obtained regarding serum C٤ estimation for both patients and control individuals (٣١.٠٢, ٤٣.٥٦) mg/dl respectively; this is illustrated in Table (٣-١٤). The unaffected levels of both C٣ and C٤ in the serum agree with Abbas *et al.*(٢٠٠٤). Functionally, Roitt *et al.*(١٩٩٨) revealed that harmful effects of complement may occur during tissue necrosis involving thermal burns, so the regulation of complement activation should be under control to avoid the negative effect of complement activation during thermal burns which is mediated by the release of anaphylatoxins(Gelfand *et al.,* ١٩٨٢).

Table (٣-١٥) Concentrations of Complement Components C٣ and C٤ (mg/dl) in Burn Patients and Controls

		C٣	C٤
Patient	M	١٣٣.١٦٨٤	٣١.٠٢١١
	SD	٥٤.٤٣٤٥	١٥.٥٣٥٨
Control	M	١٢٢.٣٢٠٠	٤٣.٥٦٠٠
	SD	٣٣.٠٣٤٩	١٤.٢٥٣٢
Significance		Not Significant P>٠.٠٥	Not Significant P>٠.٠٥

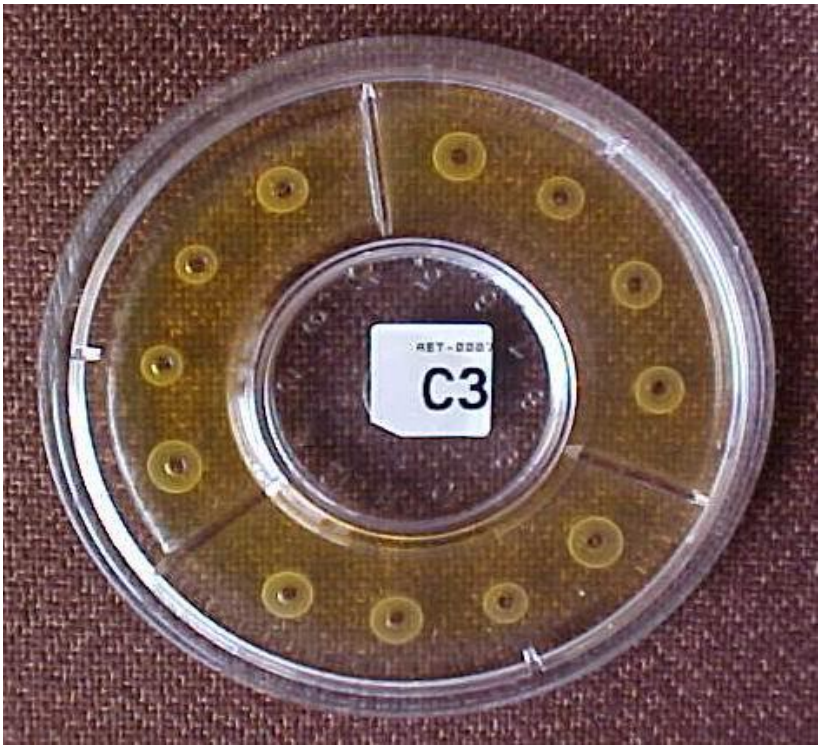


Figure (٣-١٤) Single Radial Immunodiffusion Test(SRID) for C٣

Figure (٣-١٥) Single Radial Immunodiffusion Test(SRID) for C٤

Conclusions

١. Burn injury is more frequent in female than male patients.

٢. Generally, burn injury because of flaming is more frequent than scald injury. The scald injury is more frequent in children (١–٥ years) than flame injury.

٣. Gram negative bacteria are more predominant as causative agents for burn infections than the Gram positive type.

٤. The burn unit is an important aetiology for burn wound sepsis.

٥. Mortality increases with the increase of burn percentage.

٦. In vitro, amikacin and ciprofloxacin are the most effective antibiotics.

٧. There is no significant difference between the effect of silver sulphadiazin and silver nitrate on bacterial isolates from infected burn wounds.

٨. The serum levels of IgG & IgA are decreased in burn patients, IgM shows no increased level and the levels of C٣ and C٤ complement component are not significantly differing from that of controls.

Recommendations

١. In the light of the conclusions, there is a need to recommend that immunotherapy will aid in the improvement of burn patients by reducing their susceptibility to bacterial infection.

٢. Early surgical management of burn wound, use of effective antibiotic and early enteral feeding may help in decreasing the morbidity and mortality in burn victims.

٣. Introduction of new molecular techniques instead of the conventional cultural techniques for the quantitation of bacterial isolates in burn wound and for biotyping of bacteria for more precise identification of the aetiology of burn wound infections.

٤. More attention should be directed to the burn unit as an aetiology for burn wound infections.

٥. The use of antibiotics should be highly selective in order to decrease the chance of emergence of bacterial drug resistance.

٦. Sepsis of burn wounds should be regarded as an additional factor in increasing the morbidity and mortality of burn patients.

٧. Further studies for the aetiology and management of burn wound infections in different Iraqi specialist centers.

References

Abbas, A.K.; Lichtman, A.H. and Pober, J.S.(٢٠٠٤). Cellular and molecular immunology.٥th ed. Suarders, an imprint of Elsevier Science.

Abston, S.; Blakeney, P. and Desai, M. (٢٠٠٠). Post-burn infection and sepsis. Resident Orientation Manual. Galveston Shriners Burn Hospital and University of Texas Medical Branch Blocker Burn Unit.

Ahmad, M.; Shahid, H.S.; Ibrahim, K.M. and Malik, S.A.(٢٠٠٦). Pattern of bacterial invasion in burn patients at the Pakistan institutes of medical sciences, Islamabactt. Annals of burns and fire Disasters. ١٩ (١).

Ahuja, R.B. and Bhattacharya, S.(٢٠٠٤). Burns in the developing world and burn disaster. Clinical review. ABC of burns. BMJ. ٣٢٩:٤٤٧-٩.

Al-Akayleh, A.T. (١٩٩٩). Invasive burn wound infection. Annals of burns and fire disaster. ١٢(٢).

Alden, N.E.; Bessey, P.Q. and Rabitts, A. (٢٠٠٥). Burns in the city: socioeconomic risk factors. Proceedings of the American Burn Association, ٣٧th annual meating, Chicago, USA.

Alexander, J.W.; Boyce, S.T. and Babcock, G.F. (١٩٩٠). The process of microbial translocation Ann. Surg. ٢١٢: ٤٩٦-٥١٠.

Alhambra, A.; Cuadros, J.A. and Cacho, J. (٢٠٠٤). In vitro susceptibility of recent antibiotic resistant urinary pathogens to ertapenem and ١٢ other antibiotics. Antimicrob. Chemother. ٥٣(٦):١٠٩٠-٤.

Al-Saedi, I. A. (٢٠٠٠). Isolation and Identification of *Klebsiella pneumoniea* from various infections in Hilla province and detection of some virulence factors associated in their pathogenicity. M.Sc. Thesis College of Science, Babylon University. Iraq.

Al-Saeed, M. S. (١٩٩٧). Upper respiratory tract infection in Babylon province. Ph.D Thesis. College of Science, Baghdad University. Iraq.

Al-Shukri, M. S. (٢٠٠٣). A study on some bacteriological and genetical aspects of *Acinetobacter* isolated from patients in Hilla city. M.Sc Thesis.. College of Science, Babylon University. Iraq.

Ansermino, M. and Hemsley, C. (٢٠٠٤). Intensive care management and control of infection. BMJ. **٣٢٩**: ٢٢٠-٣.

Arpin, C.; Coze, C.; Rogues, A. M.; Gachie, J. P.; Babear, C.; and Quentin, C. (١٩٩٦). Epidemiological study of an outbreak due to multidrug resistant *Enterobacter aerogenes* in a medical intensive care unit. J.Clin .Microbiol. **٣٤**: ٢١٦٣ -٩.

Arslan, E.; Dalay, C.; Yavuz, M.; Gocenler, L. and Acarturk, S. (١٩٩٩). Gram-negative bacterial surveillance in burn patients. Annals of burns and fire Disaster. **١٢** (٢).

Asenssio, A.; Guerrero, A.; Quereda, C.; and Lizan, M.(١٩٩٦). Colonization and infection with methicillin-resistant *Staphylococcus aureus*: associated factors and eradication. Infect. Control. Hosp. Epidemiol.**١٧**:٢٠-٨.

Atiyeh, B.S.; and Al-Amm, C.A. (٢٠٠١). Immunology of burn injury-an overview. Annals of Burns and fire Disasters. ١٤ (٢).

Attia, A.F.; Sherif, A.A.; Mandil, A.M.; Massound, M.N; Abou-Nazel, M.W. and Arafa, M.A.(١٩٩٧). Epidemiological and sociocultural study of burn patients in Alexandria, Egypt. Eastern Mediterranean Health Journal. ٣:٤٥٢-٦١.

Bagdonas R., Tamelis A. and Rimdeika R.(٢٠٠٣). Staphylococcus aureus infection in the surgery of burns. Medicina. ٣٩(١١).

Bagdonas, R.; Tamelis, A.; Rimdeika, R. and Kiudelis, M.(٢٠٠٤). Analysis of burn patients and the isolated pathogens. Lithuanian Surgery. ٢(٣):١٩٠-٣.

Barlow, Y.(١٩٩٤).T-lymphocytes and immunosuppression in the burned patient: A review Burns. ٢٠ (٦):٤٨٧-٩٠.

Baron E. J.,Peterson L.R., and Finegold S.M.(١٩٩٥). Bailey and Scott's Diagnostic microbiology. ٩th ed. C.V. Mosby company.

Benson, H.J. (١٩٩٨). Microbiological application: Laboratory manual in general microbiology. ١٧th ed. W.b. McGraw Hill. pp: ١١٢-٣٠.

Bollero, D.;Cortellini,M. and Stella, M.(٢٠٠٣).Pan-antibiotic resistance and nosocomial infection in burn patients: Therapeutic choices and medico-legal. Annals of Burns and fire Disasters. ١٦(٤).

Bollinger, R.R. and Delford, L.S.(١٩٩١). Transplantation. Textbook of Surgery,١٤th ed.Sabiston,D.C.,W.B.SaundersCo.Philadelphia.

Bonnet, R.K. (٢٠٠٤). *Enterobacter* infections. J. Mind. Med. ٤٨:١٤٢.

Bonten, M.J.; Willems, R. and Weinstein, R.A. (٢٠٠١). Vancomycin-resistant enterococci: Why are they here, and Where do they come from? Lancet Infect. Dis. **١**: ٣٢٥-٤١.

Bowers, D. (١٩٩٧). Statistics for health care professionals. John Wiley and Sons. New York.

Bowler, P.G.; Duerden, B.I.; and Armstrong, D.G. (٢٠٠١). Wound microbiology and associated approaches to wound management. Clin. Microbial. Rev. **١٤**: ٢٤٤-٦٩.

Brooks, G.F.; Butel, J.S. and Morse, S.A.. (٢٠٠٤). Jawetz, Melnick and Aldelberg's Medical Microbiology. ٢٣rd ed. Lange Medical Books, McGraw- Hill.

Capell, C. J.; Kirby, R.M.; and Moss, M.O.(١٩٩٥). A method and medium for electrical detection of *Listeria* spp. from food. Int. J. Food Microbiol. **٢٥**: ١٦٩-٧٧.

Carcin, H.;Wassermann, D. and Pannier,M.(٢٠٠٤).A silver sulphadiazine-impregnated lipido colloid wound dressing to treat second-degree burns. J. wound care. **١٣**(٤):١٤٥.

Cetinkaya, Y.; Falk, P. and Mayhall, C.G. (٢٠٠٠). Vancomycin-resistant *enterococ*ci. Clin. Microbiol. Rev.**١٣**: ٦٨٦-٧٠٧.

Christian, P.S.; Fraceps, A. and Maria, D. (٢٠٠٣). *Enterobacter* infection. J. Inst. Med. **٢٠**: ٤٢٠.

Church, D.; Elsayed, S.; Reid, O.; Winston,B. and Lindsay, R.(٢٠٠٦). Burn wound infections. Clinical Microbiology Reviews. **١٩**(٢): ٤٠٣-٣٤.

Cisneros, J.M.; Reyes, M.J. and Packon, J.(١٩٩٦). Bacteriemia due to

Acinetobacter baumannii: epidemiology, clinical finding, and prognostic features. Clin. Infect. Dis. **٢٢**: ١٠٢٦-٣٢.

Clark, N.M.; Patterson, J. and Lynch, J.P. (٢٠٠٣). Antimicrobial resistance among Gram negative organisms in the intensive care unit. Curr. Opin. Crit. Care. **٩**(٥): ٤١٣-٢٣.

Collee, J.; Fraser, A. G.; Marmian, B. P.; and Simmon, S. A. (١٩٩٦). Mackie and McCartney Practical Medical Microbiology. ٤th ed. Churchill and Livingstone, INC.

Collier, M.(٢٠٠٣). Understanding wound inflammation. Nurs. Times. **٩٩** (٢٥).

Cook, N. (١٩٩٨). MRSA versus the burn patient. Burns. **٢٤**: ٩١-٨.

Cruickshank, R.; Duguid, J.; Mamion, B. and Swain, R. (١٩٧٥). Medical Microbiology. Practice of Medical Microbiology. ٥th ed. Logram Grouplimitted Britain. pp: ١١٣٤.

Dasco, C.C.; Luterman, A. and Curreri, P.W. (١٩٨٧). Systemic antibiotic treatment in burned patients. Surg. Clin. North, Am. **٦٧** (١):٥٧-٦٨.

De Macedo, J.L.S.; Rosa, S.C. and Castro, C. (٢٠٠٣). Sepsis in burned patients. Revista da Sociedade Brasileria de Medicina Tropical. **٣٦**(٦):٦٤٧-٥٢.

Decker, D.; Schondorf, M. and Bidlingmarier, F. (١٩٩٦). Surgical stress induces a shift in the type-١/typ-٢ T-helper cell balance. Surgery. **١١٩**:٣١٦.

Demling, R.H. (٢٠٠٥). The burn edema process: current concepts. Journal of burn care and rehabilitation. **٢٦** (٣):٢٠٧-٢٧.

Deved, M.; Sengezer, M. and Kopal, C.(١٩٩٨). Use of mepitel on grafted areas in burn patients. Annals of Burns and fire Disasters. **١٢**(٢).

Devos, D.; Lim, A. Jr.; Pirnay, J.P.; Struelens, M. and Vandenvelde, C.(١٩٩٧). Direct detection of *Pseudomonas aeruginosa* in clinical samples such as skin biopsy specimens and expectorations by multiplex PCR based on two outer membranes lipoprotein genes, oprI and oprL.J.Clin. Microbiol. **٣٥**:١٢٩٥-١٢٩٩.

Di Piro, J.T.; Howdieshell, T.R. and Gooddard, J.K. (١٩٩٥). Association of interleukin-٤ plasma levels with traumatic injury and clinical course. Arch. Surg. **١٣٠**:١١٥٩.

Diekema, D.J.; and Pfaller, M.A. (٢٠٠٣). Infection control epidemiology and clinical microbiology. In Murry, P.R.; Baron, E.J.; and Pfaller, M.A.Manual of clinical microbiology. ٨th ed. washinyton, D.C.

Dimick, A.R. (١٩٨٨). Burns and cold injury and bites and stings. In Hardy, J.D.; Kukora, J.S.; and pass, H.I. Hardy's textbook of surgery. ٢nd ed. J.B. Lippincott Company. Philadelphia.

Doebbeling, B.N.; Stanley, G.L. and Sheetz, C.T. (١٩٩٢). Comparative efficacy of alternative band-washing agents in reducing nasocomial infections in intensive care units. N. Engl. J. Med. **٣٢٧**:٨٨-٩٣.

Dominioni, L., Dionigi, R.; and Zanello, M. (١٩٩١). Effects of high dose IgG on Survival of surgical patients with sepsis scores

of ٢٠ or Greator. Arch. Surg. ١٢٦:٢٣٦-٤٠.

Donnel, J.A. and Gelone, S.P. (٢٠٠٠). Antimicrobial therapy: Fluoroquinolones. J. Clin. Infect. Dis. N. Am. ٤: ٤٨٨-٥١٣.

Drost, A.; Burleson, D.; and Cioffi, W. (١٩٩٣). Plasma cytokines following Thermal injury and their relation-ship with patient mortality, burn size, and time post-burn. J. Trauma. ٣٥:٣٣٥.

Eggman, S.; Lofler, S. and L. Burmn. (١٩٩٧). An allelic variant of the chromosomal class A-β-lactamas K٢ spesific for *Klebsiella pneumoniae*. J. Antimicrob. Agents. Chem. ٤١(١٢): ٢٧٠٥-٩.

EI Morsi, H.A.R. (١٩٩٠). The diagnosis and treatment of infection in the burn patient. Annals of the MBC. ٣ (١).

El-Sonbaty, M.A. and El-Oteify, M.(١٩٩٠). Epidemiology of burns in Assiut province during the last two years. Assiut medical journal.

M.(١٩٩٤). Noocomial Staphylococcal outbreak. Emmerson, Scandinavian Journal of infectious diseases. Suppl.٩٣: ٤٧-٥٤.

Estahbanati, H.K.;Kashani, P.P.; and Ghanaatpisheh, F.(٢٠٠٢). Frequency of *Pseudomonas aeruginosa* in burn wound infections and their resistance to antibiotics. Burns.٢٨:٣٤٠-٤٨.

Fairuze-Ali, G. M. H.(٢٠٠٦). Isolation and identification of cases of bacteraemia in human and detection of some virulence factors associated in their pathogenicity in Najaf Governorate. M.Sc. Thesis. College of Education for

Women, University of Kufa. Iraq.

Fernandez-Cuenca, F.; Pascual, A. and Ribera, A.(٢٠٠٤). Clonal diversity and antimicrobial susceptibility of *Acinetobacter Baumauni* isolated in Spain. A nation wide multicenter study: GETH-Ab project (٢٠٠٠). Enferm. Infect. Microbiol. Clin. **٢٢**: ٢٦٧-٧١.

Fowler, V.G. Jr.; Olsen, M.K.; Corey, G.R and Woods, C.W.(٢٠٠٣). Clinical identifiers of complication *Staphylococcus aureus* bacteria. Arch. Intern. Med. **١٦٣**: ٢٠٦٦-٧٢.

Franz, C.M.; Holzapfel, W.H. and Stiles, M.E. (١٩٩٩). *Enterococci* at the cross roads of food safety. Int. J. Food. Microbiol. **٤٧**: ١-٢٤ .

Gang, R.K.; Bang, R.L.; Sanyal,S.C.; Mokaddas, E.and Lari,A.R. (١٩٩٩). *Pseudomonas aeruginosa* septicaemia in burns. Burns. **٢٥**:٦١١-١٦١.

Gelfand, J.A.; Donelan, M.; and Hawiger, A. (١٩٨٢). Alternative complement pathway activation increases mortality in a model of burn injury in mice J.Clin. Invest. **٧٠** (٦):١١٧٠-٧٦.

Gillespie, S. and Bamford, K. (٢٠٠٣). Medical microbiology and infection at a glance. Blackwel Publishing.

Glasheen, W.P.;Attinger, E.O.; and Anne, A.(١٩٨٣). Identification of the High risk population for serious burn injuries. Burns .Incl. Therm. Inj. **٩** (٣): ١٩٣-٢٠٠.

Gold, H.S. And Moellering, R.C. (١٩٩٦). Antimicrobial-drug

N.Engl. J. Med. ٣٣٥:١٤٤٣-٥. resistance.

Goldmann, D.A.; and Peir, G.B.(١٩٩٣). Pathogenesis of infections related to intravascular catheterization. Clin. Microbial. Rev. ٦(٢):١٧٦-٩٢.

Gregory, M.S.; Duffner, L.A.; faunce, D.E. and Kovacs, E.J.(٢٠٠٠). Estrogen mediates the sex difference in post-burn immunosuppression. Journal of Endocrinology.١٦٤:١٢٩-٣٨.

Hall, S.L. (١٩٩١). Coagulase-negative *Staphlococcal infections* in neonates. Pediatr. Infect. Dis. J. ١٠: ٥٠-٦٧.

Hatano, K.; Tateda, K. and Itirakata, Y.(١٩٩٦). Bacterial translocation of intestinal *Pseudomonas aeruginosa* in post burn infection of mice. Journal of infection and chemotherapy.١(٣):١٩٣-٦.

Hauser, A.R. and Sriram, P. (٢٠٠٥). Severe *Pseudomonas aeruginosa* infections postgraduate medicine. ١١٧(١).

Hazinski, M.F.; Francescutti, L.H. and Lapidus, G.D. (١٩٩٣). Pediatric injury prevention. Ann. Emerg. Med. ٢٢:٤٥٦-٦٧.

Herek, O.; Ozturkk, H.; Ozyurt, M.; Albay, A. and Cetinkursun, S.(٢٠٠٠). Effects of treatment with immunoglobulin on bacterial translocation in burn wound infection. Annals of Burns and Fire Disasters.١٣ (١).

Herendon, D.N.; and Spies, M.(٢٠٠١). Modern burn care. Semin. Pediatr. Surg. ١٠:٢٨

Hettiaratchy, S. and Dziewulski, P.(٢٠٠٤). Pathophysiology and types of burns. BMJ. ٣٢٨: ١٤٢٧-٩.

Hettiaratchy, S. and Papini, R. (٢٠٠٤). Initial management of a major burn: II-assessment and resuscitation. BMJ. ٣٢٩: ١٠١-٣.

Hodle, A.E.; Richter, K.P. And Thompson, R.M.(٢٠٠٦). Infection control practices in U.S. burn units. J. burn care Res. ٢٧(٢):١٤٢-٥١.

Honari, S. (٢٠٠٤). Topical therapies and antimicrobials in the management of burn wounds. Crit. Care Nurs. Clin. North Am. ١٦(١):١-١١.

Howland, R.D. and Mycek, M.J. (٢٠٠٦). Pharmacology. ٣rd ed. Lippincott Williams and Wilkins.

Hpa, S.V.(٢٠٠٣). *Acinetobacter s*pecies in bacterimia in North Ireland. J. Infect. Dis. ٢٣: ٤٥٦-٦٠.

Hsueh, P.; Teng, L.; Yong, P. and Chen, Y. (١٩٩٨). Persistence of a multi drug-resistant *Pseudomonas aeruginosa* clone in an intensive care burn unit. Journal of clinical microbiology. ٣٦(٥):١٣٤٧-١٣٥١.

Hsueh, P.R., Chen, M.L., Sun, C.C. and Chen, W.H. (٢٠٠٢). Emergence of Antimicrobial drug resistance of major pathogens causing nosocomial Infection at a university hospital in Taiwan, ١٩٨١ -١٩٩٩. Emerg. InfectDis. ٨: ٦٣-٨.

Huda, A.R.; Burkarie, M.D. and Ibrahiem, M.S.(٢٠٠١). Antimicrobial resistance among pathogens causing complicated UTIs. Saudi Arabia J. Infect. Med. ١٨ (٧): ٣٥٨-٦٢.

Humphreys, H.; Slack, R. and T. Pentherin, T. (٢٠٠٤). Medical microbiology, a guid to microbial infections. Churchill Livingstone. London. ٦thed. PP: ١٧٤-٨٨.

Husain, M.T.; Karim, QN. And Tajuri, S. (١٩٨٩). Analysis of infection in a burn ward. Burns. **١٥**(٥): ٢٩٩-٣٠٢.

Iskandar, S.B.; Guha B.; Krishnaswamy, G. and Roy I.M. (٢٠٠٣). *Acinetobacter baumannii pneumonia*: a case report and review of the literature. Tenn. Med. **٩٦**: ٤١٩-٢٢.

Jalal, S. and Wretlind, B. (١٩٩٨). Mechanisms of quinolone resistance in clinical strains of *Pseudomonas aerogenosa*. Microb. Drug Resist. **٤**:٢٥٧-٦١.

Japoni, A.; Hayati, M. and Alborzi, A.(٢٠٠٥). *In vitro* susceptibility of *Pseudomonas aeruginosa* isolated from a burn center to silver sulfadiazine and silver nitrate in Shiraz, South of Iran.IJMS.**٣٠**(٢).

Johnson, B.M. and Richard, R.(٢٠٠٣). Partial-thickness burns: identification and management. Adv. Skin wound Care.**١٦**:١٧٨-٨٧.

Jones, W.G.; Barie, P.S.; Yurt, R.W. and Goodwin, C.W. (١٩٨٦). Enterococcal burn sepsis. A highly lethal complication in severely burned patients. Jama and archives. Archives of surgery. **١٢١**(٦).

Kamel, A.H. and EL Megeed, E.A. (١٩٩٧). The role of aztreanam in the control of Gram-negative burn wound infection. Annals of burns and fire disasters. **١٠**(١).

Kanchannapoom, T. and Khardori, N. (٢٠٠٢). Management of infections in patients with severe burns: Impact of multi-resistant pathogens. J. Burns. ١(١):١-١٧.

Kao, C.C.; and Garner, W.L. (٢٠٠٠). Acute burns. Plast Reconstr. Surg. ١٠٥ (٧): ٢٤٨٢-٩٢.

Karlowsky, J. A., Jones M. E., Draghi D. C., Thomsberry C., Sahm D.F., and Volturo G. A. (٢٠٠٤). Prevalence and antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in ٢٠٠٢. Ann. Clin. Microbiol. Antimicrob. ٣: ٣-٧.

Karlowsky, J. A., Zelenitsky, S.A.; and Zhanel, G.C. (١٩٩٧). Aminoglycoside Adaptive resistance Pharmacotherapy. ١٧:٥٤٩-٥٥.

Kehinde, A.O.; Ademola, S.A. and Okesola, A.O. (٢٠٠٤). Pattern of bacterial pathogens in burn wound infections in Ibadan, Nijeria. Annals of burns and fire disasters. ١٧(١).

Kelly, J.L.; Lyons, A.; Sobery, C.C.; Mannick, J.A. and Lederer, J.A. (١٩٩٧). Anti-interleukin-١٠ antibody restores burn induced Defect in T cell function. Surgery. ١٢٢(٢): ١٤٦-٥٢.

Kharazmi, A. and Nielsen, H.(١٩٩١). Inhibition of human monocyte hemotaxis and chemiluminescence by *Pseudomonas aerugenosa* elastase. APMIS. ٩٩:٩٣-٥.

Kim, B.N.; Lee so.; Choi. SH.; Kim, NJ.; Woo, JH.; Ryu, J.; kim, Ys. (٢٠٠٣). Outcome of antibiotic therapy for third – generation

cephalosporin-resistant. Int. J. Antimicrob. Agent. ٢٢ (٢):١٠٦-١١.

Klasen, H.J. (٢٠٠٠). A historical review of the use of silver in the treatment of Burns. II. Renewed interest for silver. Burns. ٢٦:١٣١-٨.

Klein, M.B.; Heimbach, D.; and Gibran, N.(٢٠٠٤). Management of the burn wound: introduction. ACS Surgery online.

Kobayashi, M.; Takahashi, H. and Sanford A.P. (٢٠٠٢). An increase in the susceptibility of burned patients to infectious complications. The Journal of Immunology. ١٦٩: ٤٤٦٠-٦.

Komolafe, O.O.; James, T.; and Kalongolera, L.(٢٠٠٣). Bacteriology of burns at The Queen Elizabeth Central Hospital, Blantyre, Malaw: Burn. ٢٩: ٢٣٥-٨.

Koupil, J.; Brychta, P.; Rihova, H. and Kincova, S. (٢٠٠٠). Special features of burn injuries in olderly patients. Acta. ٤٣.

Kovacs, K.; Paterson, D.L. and Yu, V.L. (١٩٩٨). Antimicrobial therapy for *Pseudomonas aeruginosa*. Infect. Med. ١٥(٧):٤٦٤-٨.

Kucers, A.; Growe, S.; Grayson, M.L.; and Hoy, J. (١٩٩٧). The use of antibiotics: A Clinical Review of Antibacterial, Antifungal and Antiviral drugs. ٥th ed. Oxford: Butter-worth Heinemann.٤٥٢-٧.

Lenguas, F.; Herruzo, R.; Pintade, R.; Denia, R.; Mariscal, F. and Silva M.J. (١٩٩١). Evaluation of empirical antibiotic treatment in sepsis In the burn patient with cephtazidime-aminoglycoside association. Annals of the MBC. ٤(٤).

Lesseva, M. and Hadjiiski, O. (١٩٩٨). Treatment of serious infectious complications In burned patients with imipenem /cilastatin. Annals of burns and fire disasters. ١١(٢).

Lewis, S.M.; Bain, B.J., and Bates, I. (٢٠٠١). Dacie and Lewis. Practical Haematology. ٩th ed. Churchill Living stone, London.

Nosocomial Lortholary, O.; Fagon, J.Y. and Buutloi, A(١٩٩٥). risk *Acinetobacter baumannii*: multiresistant of aquision Clin.Infect. Dis. ٢٠:٧٩٠-٦. factors and prognosis.

Lowell, C.(٢٠٠١). Clinical laboratory detection of antigens and antibodies. In Stites, D. P.; Terr, A. I. and Parslow, T.G. Basic and Clinical Immunology. ١٠th ed. Appelton and Lange.

Lowy, F.D. (١٩٩٨). *Staphylococcus aureus* infections. N. Engl. J. Med. ٣٣٩: ٥٢٠ - ٣٢.

Lyczak, J.B.; Cannon, C.L.; and Peir, G.B. (٢٠٠٠). Establishment of *Pseudomonas aeruginosa* infection lessons from a versatile opportunist. Microbes Infect. ٢:١٠٥١-٦٠.

MacFaddin J.F.(٢٠٠٠). Biochemical test for identification of medical bacteria. ٣rd ed. Williams and Wilkins-Baltimor. New York.

Mack, V.E.; McCarter, M.D. and Naama, H.A. (١٩٩٦). Dominance of T-Helper ٢-typ cytokines after sever injury. Arch. Surg. ١٣١:١٣٠٣.

MacMicking, J.; Xie, Q.W. and Nathan, C. (١٩٩٧). Nitric oxide and macrophage function. Ann. Rev. Immunol. ١٥: ٣٢٣-٥٠.

Magliacani, G. and Stella, M. (١٩٩٠). the use of gamma- globulins and immuno-Modulators in the therapy of infections in serious

burn patients. annals of the MBC. ٣ (١).

Maitra, A. (٢٠٠٣). Environmental diseases, In Kumar, V.; Cotran, R.; and Robbins, S. Robins Basic Pathology, ٧th ed. Saunders, an imprint of Elsevier Science, London.

Mansour, A. and Enayat, K. (٢٠٠٤). Bacteriological monitoring of hospital borne septicemia in burn patients in Ahvas, Iran. J. Burns and Surg. Wound care. ٣ (١):٤.

Margulies, D.R.; Navarro, R.A. and Kahn, A. M. (١٩٩٨). Molten metal burns: Early treatment improves outcome. Am. Surg.٦٤: ٩٤٧.

Marks, R. (٢٠٠٣). Roxburgh's common skin diseases, ١٧th ed. International student's edition. London.

Massoud, M.N. and Mandil, A.M.A. (١٩٩٢). Towards a burns prevention programme for children and adolescents in Alexandria. Alexandria journal of pediatrics. ٦ (٣): ٦٤١-٥.

Mayhall, C.G.(١٩٩٦). Nosocomial burn wound infections. In G.C. Mayhall, Hospital epidemiology and infection control. The Williams and Wilkins Co., Baltimore Md.

Mayhall, C.G.(٢٠٠٣). The epidemitiology of burn wound infections: Then and now. Health care epidemiology. CID. ٣٧.

McGregor, J.C. (١٩٩٨). Profile of the first four years of the regional burn unit based at St. Johns hospital, West Lothian (١٩٩٢-١٩٩٦). J.R. coll. Surg. Edinb. ٤٣:٤٥-٨.

McIrvine, A.J.; O'Mahony, J.B.; and Saporoschetz, I.(١٩٨٢). Depressed immune Response in burn patients: Use of monoclonal

antibiotics and functional Assays to define the role of suppressor cells. Ann. Surg. ١٩٦:٢٩٧.

Meakins, J.L. (١٩٩٠). Etiology of multiple organ failure. J. Trauma. ٣٠:١٦٥-٨.

Mercier, C. and Blond, M.H. (١٩٩٦). Epidemiological survey of childhood burn injuries in france. Burns, ٢٢: ٢٩-٣٤.

Messingham, K.A.; Heinrich, S.A.; and Schilling, E.M.(٢٠٠٢). Interleukin-٤ Treatment restores cellular immunity after ethanol exposure and burn Injury. Alcohol Clin. EXP. Res. ٢٦(٤):٥١٩-٢٦.

Messingham, K.A.; Shirazi, M.; and Duffer, L.A. (٢٠٠١). Testosterone receptor Blockade restores cellular immunity in male mice after burn injury. Journal of Endocrinology. ١٦٩:٢٩٩-٣٠٨.

Mims, C.; Docknell, H.M.; Goering, R.V.; and Roitt, I.(٢٠٠٤). Medical microbiology. ٣rd ed, Elsevier Limited.

Mousa, H.A.(١٩٩٧).Aerobic, anaerobic and fungal burn wound infections. J. Hosp. Infect. ٣٧:٣١٧-٢٣.

Muir, I.F.K.; Barclay, T.L.; and Settle, J.A.D. (١٩٨٧). Burns and their treatment, ٣rded. Butter-worth. London, Boston.

Munster, A.M. (١٩٩٦). Burns of the world. J. Burn care Rehabil. ١٧:٤٧٧.

Murray, B.E.(٢٠٠٠). Vancomycin- resistant *Enterococcal* infections. N. Engl. J. Med. ٣٤٢:٧١٠ -٢١.

Murray, P.R.; Baron, E.J.; Jorgensen, J.H. and Pfaller, M.A. (٢٠٠٣). Mannual of clinical microbiology. ٨th ed. Washington, D.C.

Nega, K.E. and Lindtjorn, B.(٢٠٠٢). Epidemiology of burn injuries in Mekele Town, Northern Ethiopia: A community based study. Ethiop. J. Healtjh Dev. ١٦(١):١-٧.

Noronha, C. and Almeida, A. (٢٠٠٠). Local burn treatment-topical antimicrobial agents. Annals of burns and fire disasters. ٨(٤).

Ogunsola, F.T.; Oduyeebo, O.; Iregbu, K.C. and Coker, A.O.(١٩٩٨). A review of nosocomial infections at LUTH: problems and strategies for improvement J. Nigerian Infection Control Association. ١:١٤-٢٠.

Oteo J., Lazaro E., de Abajo F. J., Baquero F., and Campos J. (٢٠٠٥). Antimicrobial resistant invasive *Escherichia coli*, Emerg. Infect. Dis. ١١: ٥٤٠-٥٣ .

Palastanga, N.; Field, D.; and Soames, R. (٢٠٠٠). Anatomy and human movement, Structure and function. ٣rd ed. Butterworth Heinemann. Oxford.

Pandey, A., Kapil, A., Sood ,S. and Goel, V.(١٩٩٨). In vitro activities of ampicillin-sulbactam and amoxicillin-clavulanic acid against *Acinitobacter baumannii*.J.Clin. Microbiol. ٣٦:٣٤١٥-٦.

Parikh, D.V.; fink, T.; and Rajasek haran, K. (٢٠٠٥). Antimicrobial Silver/Sodium. Carboxymethyl cotton Dressings for burn wounds. Textile Research Journal.

Passador, L.C.; Cook, J.M.; Rust, L.S.; Lewiski, B.H. and M.J. Cambello, M.J. (١٩٩٣). Expression of *Pseudomonase aeruginosa* virulence genes requires cell-to-cell

communication. J. Bact. Infect. ٢٦٠(٤):١١٢٧-٣٠.

Paul, W.E. (٢٠٠٣). Fundemental immunology, ٥th ed. Lippincott Williams and Wilkins, London.

Pechere, J.C. and Kohler, T. (١٩٩٩). Patterns and modes of β-lactam resistance in *Pseudomonas aeruginosa*. Clin. Microbiol. Infect. ٥(suppl ١):S١٥-S١٨.

Pirnay, J.P.; DeVos, D. and Cochez, C. (٢٠٠٣). Molecular epidemiology of *Ps. Aeruginosa* colonization in a burn unit. Journal of Clinical Microbiology. ٤١ (٣): ١١٩٢.

Planas, M.; Wachtel, T. and frank, H. (١٩٨٢). Characterization of acute renal failure in the burned patient. Arch. Intern. Med. ١٤٢: ٢٠٨٩-٩١.

Podschum R., Penner I. and Ulmann U. (١٩٩٢). Interaction of Klibsiella capsule typ ٧ with human polymorpho nuclear leucocytes. Microbal. Pathog. ١٣:٣٧١-٩.

Podschum, R. and Ulmann, U.(١٩٩٨). *Klebsiella* spp. As nasocomial pathgens: epidemiology, taxonomy, typing methods and pathogencity factors. American society for microbiology. Clinical Microbiology Reviews. ١١(٤):٥٨٩-٦٠٣.

Press, B. (١٩٩٧). Treatment principles for out patient burn management. Recognition and management of infections in Aston, S.J.; Beasley,R.W.; And thorne, C.H.M. Grabb and Smith's plastic surgery, ٥th ed. Lippincoot-Reven.

Pruitt, B.A. and McManus, A.T. (١٩٩٢). The changing epidemiology of infection in burn patients. World J. Surg. ١٦:٥٧-٦٧.

Pruitt, B.A.; and Mason, A.D.(١٩٩٦). Epidemiological demographic and outcome characteristics of burn injury. In Hemdon, D.N. Total Burn care. London, W.E. Saunders.

Pruitt, B.A.; Goodwin, C.W.; and Pruitt, S.K. (١٩٩٧). Burns including Cold, chemical and electric injuries. In Sabiston, D.C. ;and Lyerly, H.K.Textbook of surgery.The biological basis of modern surgical practice ١٥th ed. Philadilphia.

Pruitt, B.A.; McManus, A.T.; Kim, S.H.; and Goodwin, C.W. (١٩٩٨). Burn wound Infections: current status. World . J. Surg. ٢٢:١٣٥-٤٥.

Rabson, M.C. (١٩٨٨). Burn sepsis. Crit. Care Clin. ٤(٢):٢٨١-٩٨.

Rachid, S.A.; Witte, V.M. and Hacker, J.Z. ٢٠٠٠. Effect of subinhbitory antibiotic concentration on Intracellular adhesion expression in biofilm-forming *Staphylococcus epidermidis*.J. Antmicrob. Agents. ٣٢: ٧٥٥-٦٠.

Rashmi S., Lal S. C., and Bhumneshwar K. (٢٠٠٥). Antibacterial resistance: current problem and possible solution. Indian J. Med. Sci. ٥٩: ١٢٠-٩ .

Rastegar Lari, A.R., Alaghehbandan, R. and AKhlaghi, L.(٢٠٠٥). Burn Wound infections and antimicrobial resistance in Tehran, Iran: an increasing problem. Annals of Burns and Fire Disasters. ١٨ (٢).

Reardon, C.M; Brown, TP., Stephensson, A.J. and Freed lander, E. (١٩٩٨). Methicillin-resistant *Staphylococcus aureus* in burns Patients-Why all the fuss? Burns. **٢٤**:٣٩٣-٧.

Reidy, J.J.; and Ramsy, G. (١٩٩٠). Clinical trails of selective decontamination of The digestive tract: review. Crit. Care. Med. **١٨**(٢): ١٤٤٩-٥٦.

Reig, A.; Tejerina, C.; Codina, J. and Mirabet, V. (١٩٩٢). Infections in burn patients. Annals of the MBC. **٥**(٢).

Revathi, G.; Puri, J. and Jain, B.K. (١٩٩٨). Bacteriology of burns. Burns. **٢٤**:٣٤٧-٩.

Reynoldes, R.; Porz, N.; Colman, M.; William, A.; Livermore, D.; MacGowan, A. (٢٠٠٤). Antimicrobial susceptibility of the pathogens of bacteremia in the UK and Irland ٢٠٠١-٢٠٠٢: the basic bacteremia resistance surveillance programme. J. Antimicrob. Chemother. **٣٥** (٦):١٠١٨-٣٢.

Richard, P.; floch, R.L.; Chamoux, C. and Pannier, M.(١٩٩٤). *Pseudomonas aerugenosa* outbreak in a burn unit: role of antimicrobials in the emergence of multiply resistant strains J. Infect. Dis. **١٧٠**:٣٧٧-٨٣.

Roberts, R.L.; and Steihm, E.R. (٢٠٠١). Antibody (B-cell) and immunodeficiency disorders. In Parslow, T. G.; Stites, D.P.; Terr, A. I.; and Imboden, J.B.A. Medical Immunology.١٠th ed. Lange Medical Book. NewYork.

Robin, D.(٢٠٠٥). Glycopeptide-resistant *Enterococci*: Clincal prespectives division of infections disease. J. Infect. Dis. **٤٥**:

١٢٣-٥.

Robins, E.V.(١٩٩٠). Immunosuppression of the burned patient. Crit. Car Nurs. Clin. North AM. ٢(١).

Rodgers, L.(١٩٩٤). Clinical laboratory detection of antigens and antibodies. In Stites, D. P.; Terr, A. I. and Parslow, T.G. Basic and Clinical Immunology. ٨th ed. Prentice-Hall international Inc.

Roitt, I.; Brostoff, J. and Male, D. (١٩٩٨). Immunology ٥th ed. Mosby, London.

Roitt, I.; Brostoff, J. and Male, D. (٢٠٠١). Immunology ٦th ed. Mosby, London.

Sanyal, S.C.; Mokaddas, E.M.; Gang, R.X. and Bang, R.L.(١٩٩٨). Microbiology of septicemia in burn patients. Annals of burns and fire disaster. ١١(١).

Schwacha, M.G. and Somers, S.D. (١٩٩٨). Thermal injury induces macrophage hyperactivity through pretussis toxin- sensitive and insensitive pathways. Shock. ٩:٢٤٩-٥٥.

Schwacha, M.G.; Chung, C.S. and Ayala, A. (٢٠٠٢). Cyclooxygenase-٢-mediated suppression of macrophage interleukin-١٢ production following thermal injury. Am.J. physiol cell physiol. ٢٨٢:٢٦٣-٧٠.

Sener, G.; Sehirli, A.O. and Satiroglu, H. (٢٠٠٢). Melatonin improves oxidative organ damage in a rat model of the mal injury. Burns. ٢٨: ٤١٩-٢٥.

Shahid, M. and Malik, A. (٢٠٠٥). Resistance to aminoglycosid

modifying enzymes in *Pseudomonas aeruginosa* isolates from burns patients. Indian J Med Res.١٢٢:٣٢٤-٩.

Shahin, A.; hadata, G.; Franka, M.R.; Abusetta, A.; Brogouski, A. and Ezzaidi, M.M.(١٩٩٨).Complications of burns in children-a Study of ٢٦٦ severely burns children admitted to a burns Center. Annals of burns and fire Disasters.١١(١).

Shankowsky, H.A., Callioux, L.S. and Tredget, E.E.(١٩٩٤). North America Survey Of hydrotherapy in modern burn care. J. Burn Care Rehabil.١٥:١٤٣-٦.

Silver, S.; Phungle, T.; and Silver, G.(٢٠٠٦). Silver as biocides in burn and wound Dressings and bacterial resistance to silver compounds. J.Ind. Microbiol Biotechnol. ٣٣(٧):٦٢٧-٣٤.

Smith, J.; Howell, J.M. and Scott, J.L. (١٩٩٨). Emergency Medicine. Philadelphia, W.B. Saunders, ١١٠٧-٩.

Song, W.; Lee, K.M.; Kang, H.J.; Shin, D.H. and Kim, D.K.(٢٠٠٠). Microbiologic aspects of predominant bacteria isolated from the burn patients in Korea. Burns. ٢٧:١٣٦-٩.

Starodub, M.E. and Trevors, J.T. (١٩٨٩). Silver resistance in Escherichia coli R١. J. Med. Microbiol. ٢٩: ١٠١-١٠.

Still, J.; Law, E. and Orlet, H.K. (١٩٩٧). An unusual mechanism of burn injury. Am. Surg. ٦٣:٢٥٢.

Stites, D. P.; Terr, A. I. and Parslow, T.G. (٢٠٠١). Basic and Clinical Immunology. ١٠th ed. Appelton and Lange.

Strober, W. and James, S.(٢٠٠١). The mucosal immune system. In Stites, D. P.; Terr, A. I. and Parslow, T.G. Basic and Clinical Immunology. ١٠th ed. Appelton and Lange.

Strong, V.E.; Mackrell, P.J. and Conannon, E.M. (٢٠٠٠). Blocking prostaglandin E٢ after trauma attenuates pro-inflammatory cytokines and improves survival. Shock. ١٤:٣٧٤-٩.

Subrahmmangam, M. (١٩٩١). Topical application of honey in treatment of burns. British journal of surgery. ٧٨:٤٩٧-٨.

Tabata, T.; de Serres S.; and Meyer, A.A. (١٩٩٦). Differences in IgM Synthesis to gut bacterial peptiedoglycan poly-saccharide after burn injury and gut Ischemia. J. Burn Care Rehab. ١٧(٣):٢٣١-٦.

Tarkkamen, A. M.; Allen, B. and Westerland, B(١٩٩٠). Type V collagen as target for type -٣ fimbriae, enterobacterial adherence organelles. Mol. Microbiol. ٤: ١٣٥٣-٦١.

Thomson, C.J. and S.G. Amyes. (١٩٩٣). Selection of variants of TEM-١ β-lactamase encoded by a plasmid of clinical origing with increased resistance to β-lactamase inhibitors. J. Antimicrob. Chem. ٣١: ٦٥٥-٦٤.

Tonkic M., Barisic I.G., and Punda – Polic V. (٢٠٠٥). Prevalence and antimicrobial resistance of extended-spectrum β-Lactamases producing *Escherichia coli* and *Klebsiells pneumoniae* strains isolated in a university hospital in Split, Croatia. Int. Microbiol. ٨: ١١٩-٢٤ .

Torregrossa, M.V.; Valentino L.; Cucchiara, P., Masellis, M. and

Sucameli M. (٢٠٠٠). Prevention of hospital-acquired infections in the Palermo burns center. Annals of Burns and Fire Disasters. ١٣ (٣).

Towner, K.J. (١٩٩٧). Clinical importance and antibiotic resistance of *Acinetobacter spp*. J. Med MIcrobiol. ١٩٩٧; ٤٦:٧٢١-٤٦.

Vindenes, H. and Bjerknes, R. (١٩٩٥). Microbial colonization of large wounds. Burns. ٢١:٥٧٥-٩.

Wassermann, D. (٢٠٠٢).Criteria for burn severity. Epidemiology. Prevention, organization of management. Pathol. Biol. (Paris). ٥٠(٢): ٦٥-٧٣.

Weinstein, J.T.; Roe, M.K. and J.A. Sanders, J.A. (١٩٩٦). Resistant *Enterococci* a prospective study of prevalence and factors association with colonization. J. Infect. Cont. Hosp. Epidem. ١٧(١): ٣٦-٤١.

Wibbenmeyer, L.; Danks, R. and faucher, L. (٢٠٠٦). Prospective analysis of nosocomial infection rates, antibiotic use, an patterns of resistance in a burn population. Burn care Res. ٢٧(٢):١٥٢-١٦٠.

Wiener, J.; Quinn, J.P. and Bradford, P.A.(١٩٩٩). Multiple antibiotic-resistant *Klebseilla* and *Escherichia coli* in nursing homes. JAMA. ٢٨١:٥١٧-٢٣.

Wilson, S.E. (١٩٩٥). Carbapenems: Monotherapy in intra abdominal sepsis. Scand. J. Infect. Dis. Suppl. ٩٦:٢٨-٣٣.

Yarbrough, D.R. (١٩٩٨). Burns due to aerosol can explosions. Burns. ٢٤: ٢٧٠.

Yeoh, C.; Nixon, J.W.; and Dicksone, W. (١٩٩٤). Patterns of scald injuries. Arch. Dis. Child. ٧١:١٥٦.

Yin, H.Q.; Langford, R. and Burrell, R.E. (١٩٩٩). Comparative evaluation of the antimicrobial activity of acticoat antimicrobial barrier dressing J. Burn care Rehabil. ٢٠(٣):١٩٥-٢٠٠.

Yotis, W. (٢٠٠٥). Appelton and lange outline review microbiology and immunology. International edition. McGraw-Hill, New York.

Youn, Y.K.; Landon, C. and Demling, R. (١٩٩٥). The role of mediators in the response to thermal injury. World J. Surg. ١٦: ٣٠-٦.

Yurt, R.W. (٢٠٠٠). Burns. In mandell, G.L.; Bennett, J.E.; and Dolin, R. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. ٥th ed. Churchill livingstone. Philadelphia.

Zahac, L.; Mady, U.; Lig,Y. and Yu, Y.(٢٠٠٣). The states of drug resistance and amp C gene expression in *Enterobacter cloacae*. J. Chine. Med. ١١٦: ١٢٤٤-٧.

Zhang, Y.; Six, R. and Yang D. (٢٠٠٣). Comparison of genomes between *Staphylococcus epidermidis* and *Staphylococcus aureus*. Chine. Clin. Hum. Gen. Cent. ٤٥: ٣٤-٤٠.

.