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UNIVERSITY OF BABYLON

COLLEGE OF MEDICINE

A CLINICAL AND BACTERIOLOGICAL STUDY OF BREAST ABSCESS IN FEMALE PATIENTS

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
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February ٢٠٠٦ **Muharram ١٤٢٧**

n

((وَوَصَّيْنَا الإِنْسَانَ بِوَالِدَيْهِ حَمَلَتْهُ أُمُّهُ وَهْناً عَلَى وَهْنٍ وَفِصَالُهُ فِي عَامَيْنِ أَنِ اشْكُرْ لِي وَلِوَالِدَيْكَ إِلَيَّ الْمَصِيرُ))

صَدَقَ اللهُ العَلِي العَظِيم

سورة لُقمان ((الآية: ١٤))

Certification

We certify that this thesis was prepared under our supervision at Department of Medical Microbiology, College of Medicine, University of Babylon and do hereby recommended that it be accepted in partial fulfillment of the requirement for the Degree of Master of Science in Medical Microbiology.

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Dedication

To . . .
The Memory of my mother and my father who illuminate my path for believe;
My Father – in law Professor, Salih Mahdi;
My support in life: my wife, Wafa';
and
My hope in this world: my daughter, Aya.

Hussein

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Last, but not least, I am really indebted to the staff members of the Clinical Laboratory of Hilla Teaching Hospital and the Breast Disease Center of Hilla Teaching Hospital for their sincere co-operation and honest help.

الخلاصة

تم وفق هذا البحث إخضاع إحدى وسبعين مريضة للدراسة ممن راجعن مركز أمراض الثدي في مستشفى الحلة التعليمي للفترة من أيلول ٢٠٠٤ إلى حزيران ٢٠٠٥ ، وقد تم تصنيف العينات إلى مجموعتين : الأولى تضم ثلاثة وخمسون مريضة من المرضعات اللاتي تتراوح أعمارهن بين ١٧ – ٤٥ سنة ، والثانية تضم ثمانية عشر مريضة من غير المرضعات تتراوح أعمارهن بين ٣ أشهر إلى ٥٧ سنة .

لقد أظهرت النتائج نسبة عالية (٧٤.٦ %) من الإصابة بخرّاج الثدي بين المريضات المرضعات ، وتضمنت (٥٤ %) من الإصابة بخرّاج الثدي بين المريضات اللواتي تتراوح أعمارهن بين ٢٠ إلى ٢٩ سنة ، بينما كان أعلى معدل للإصابة في المجموعة الثانية في الأعمار التي تتراوح بين ٤٠ إلى ٤٩ سنة ، حيث بلغت (٤٤.٤ %) . وقد ظهر كذلك أن معدل الإصابة بخرّاج تحت الجلد كان أعلى بين مريضات المجموعة الأولى (٤٥.٣ %) مقارنةً بالأنواع الأخرى لخرّاجات الثدي ، في حين كان خرّاج تحت الهالة في المجموعة الثانية أعلى من الأنواع الأخرى (٧٧.٨ %) . إضافة إلى ذلك ، لوحظ أن النسبة العالية لخرّاجات الثدي أصاب الثدي الأيسر (٦٠.٥٦ %) ، كذلك لوحظ أن الإصابات كانت أعلى بين المريضات في المناطق القروية (٦٤.٨ %) .

إن النسبة العالية لخرّاج الثدي للمجموعة الأولى حدثت على الغالب في الشهر الثالث بعد الولادة (٤٥.٣ %) . وظهر أيضاً بأن خرّاج الثدي كان أعلى معدل بين النساء متعددة الولادات (٦٦.٢ %) .

كان ثمّة ٥٦ عينة موجبة للزرع البكتيري موزعة بواقع ٤٥ عينة (٨٥ %) في المجموعة الأولى ، و ١١ عينة (٦١.١ %) في الثانية ، في حين كان هناك ١٥ عينة سالبة للنمو البكتيري الأمراضي موزعة بواقع ٨ عينات (١٥.١ %) في المجموعة الأولى ، و ٧ عينات (٣٨.٩ %) في الثانية .

أظهرت نتائج التشخيص البكتيري أن أنواع ونسب البكتريا المعزولة في المجموعة الأولى هي: *Staphylococcus aureus* (٧٣.٦ %) و *Moraxella catarrhalis* (٥.٧ %) و *Streptococcus pyogenes* (٣.٨ %) و *Staphylococcus epidermidis* (١.٩ %) ، أما في المجموعة الثانية فإن النمو البكتيري كان وفق الآتي : *S. aureus* (٢٧.٨ %) و *Arcanobacterium Haemolyticum* (٢٢.٢ %) و *M. catarrhalis* (١١.١ %) .

وقد تم اختبار قدرة جميع عزلات *S. aureus* على إنتاج كل من FAME ، lipase و Slime ، قد بينت النتائج بأن (٨١.٨ %) من عزلات *S. aureus* كانت منتجة لإنزيم (FAME) ، بينما (٨٦.٤ %) من العزلات كانت منتجة لإنزيم (lipase) ، أما العزلات التي أنتجت (slime) فقد كانت (٤٠.٩ %)

لقد تم إجراء اختبار الحساسية للمضاد الحيوي لجميع العزلات البكتيرية ولـ (١١) مضاداً حيوياً . وقد بينت النتائج اختلافاً بيناً لاختبار الحساسية للمضاد الحيوي بين العزلات البكتيرية المختلفة ، لكن على العموم ، ظهر أن مضاد ciprofloxacin حقق التأثير الأكبر على العزلات البكتيرية الأمراضية ، حيث بينت النتائج إن عزلات بكتيرية قليلة مقاومة لهذا النوع من المضادات ، كما عُـدّت مضادات ampicillin و amoxicillin و cloxacillin الأدنى تأثير موجه ضد العزلات البكتيرية .

ABSTRACT

In this study samples from seventy-one female patients admitted to the Breast Diseases Center, Hilla Teaching Hospital from September ٢٠٠٤ to June ٢٠٠٥ have been investigated. The samples have been classified into two groups: the first one included ٥٣ lactating patients, ranging from ١٧ to ٤٥ years, and the second did ١٨ non-lactating patients, where age ranged from ٣ months to ٥٧ years.

The results have revealed a remarkably high incidence of breast abscess among lactating patients (٧٤.٦٪). The high infection rate (٥٤.٧٪) in the first group has been among those who were ٢٠-٢٩ years old, whereas in the second group, the high infection rate (٤٤.٤٪) has been among those who were ٤٠-٤٩ years old. It has also been found that the breast abscess is higher among multiparty women (٦٦.٢٪). As for the first group, the high incidence of breast abscess occurs mostly at the third month postpartum (٤٥.٣٪). It has also been found that the incidence of subcutaneous abscess is higher than other types of breast abscess among the first group patients (٥٢.٨٪), whereas in the second group, the subareolar abscess is higher than other types (٧٧.٨٪). Furthermore, it has been found that the high incidence of breast abscess occurs in the left breast (٦٠.٥٦٪). Meantime, infection is higher among women of rural area (٦٤.٨٪).

There have been ٥٦ positive samples for bacteriological culture divided into ٤٥ samples (٨٥٪) in the first group, and ١١

samples (٦١.١٪) in the second one. But ١٥ samples have been negative for pathogenic bacterial growth; they have been divided into ٨ samples (١٥٪) in the first group, and ٧ samples (٣٨.٩٪) in the second.

The results of the bacteriological diagnosis have revealed that the species and percentage of bacteria isolated from the first group are *Staphylococcus aureus* (٧٣.٦%), *Moraxella catarrhalis* (٥.٧٪), *Streptococcus pyogenes* (٣.٨%) and *Staphylococcus epedermidis* (١.٩%). Whereas in the second group, the bacterial growth has been *S. aureus* (٢٧.٨٪), *Arcanobacterium haemolyticum* (٢٢.٢٪) and *M. catarrhalis* (١١.١٪).

All the isolates of *S. aureus* (٤٤ isolates) have been assayed for the production of fatty acid modifying enzyme (FAME), lipase, and slime. The results revealed that (٨١.٨٪) of *S. aureus* isolates have been positive for FAME activity, (٨٦.٤٪) positive for lipase, and (٤٠.٩٪) have produced slime.

Antibiotic sensitivity of all pathogenic bacterial isolates has been made with ١١ antibiotics, and the results have revealed a clear variation in the antibiotics sensitivity test among different bacterial isolates. In general, ciprofloxacin has shown the greatest effect on bacterial isolates, since only few bacterial isolates have revealed resistance to such antibiotic. Ampicillin, amoxicillin and cloxacillin have been considered the least effective antibiotics against most bacterial isolates.

Appendix (١)

Data Sheet

١. Name:

٢. Age:

٣. Date of Admission:

٤. Address:

٥. Marital State:

٦. Lactating or Not:

٧. If Lactating; Time after Starting Lactation:

٨. Presentation: pain ☐ mass ☐ fever ☐ nipple discharge ☐

٩. Type of Abscess: subcut ☐ Subaerolar ☐ intralobular ☐
retromammary ☐

١٠. Recurrent Breast Abscess: Ni ☐ second ☐ third ☐

١١. Affected Breast: right ☐ left ☐

١٢. Nipple State: normal cracked ☐ retracted ☐

١٣. Other Diseases Associated with Breast Abscess:

١٤. U / S:

١٥. Mammography:

١٦. Bacteriological Results:

Table (٤.١٤): Sensitivity of bacterial isolates to ١١ antibiotics

Type of bacteria	No. of isolates	No. (%) of isolates sensitive to antibiotics										
		AMX	AMC	AM	GM	LN	E	OB	CIP	CE	RA	VA
S. aureus	٤٤	٢٠ (٤٥.٥)	٣٢ (٧٢)	٥ (١١.٣)	٣٧ (٨٤)	٢٠ (٤٥.٤)	٣١ (٧٠.٤)	١٤ (٣١.٨)	٣٦ (٨١.٨)	٣٦ (٨١.٨)	٣٨ (٨٦.٣)	٤٤ (١٠٠)
M. catarrhalis	٥	٣ (٦٠)	٤ (٨٠)	١ (٢٠)	٥ (١٠٠)	٥ (١٠٠)	٥ (١٠٠)	١ (٢٠)	٥ (١٠٠)	٣ (٦٠)	٣ (٦٠)	٢ (٤٠)
A.haemolyticum	٤	٢ (٥٠)	٣ (٧٥)	٠ (٠)	٣ (٧٥)	٣ (٧٥)	٤ (١٠٠)	٠ (٠)	٣ (٧٥)	٢ (٥٠)	٣ (٧٥)	٣ (٧٥)
S. pyogens	٢	١ (٥٠)	٢ (١٠٠)	٠ (٠)	١ (٥٠)	٢ (١٠٠)	١ (٥٠)	٠ (٠)	٢ (١٠٠)	٢ (١٠٠)	٢ (١٠٠)	٢ (١٠٠)
S. epidermidis	١	١ (١٠٠)	١ (١٠٠)	٠ (٠)	١ (١٠٠)	١ (١٠٠)	١ (١٠٠)	١ (١٠٠)	١ (١٠٠)	١ (١٠٠)	١ (١٠٠)	١ (١٠٠)

AMX: Amoxycillin, AMC: Amoxyclave, AM: Ampicillin, GM: Gentamycin, LN: Lincomycin, E: Erythromycin, OB: Cloxacillin, CIP: Ciprofloxacillin, CE: Cefotaxime, RA: Rifampicin, VA: Vancomycin.

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List of Abbreviations

SEAG	Enterotoxin A, B, C, D and G
TSST	Toxic Shock Syndrome toxin
ET	Exfoliation Toxin
FAME	Fatty Acid Modifying Enzyme
FNA	Fine Needle Aspiration
Amx	Amoxycillin
Amc	Amoxyclave
Am	Ampicillin
Gm	Gentamycin
Ln	Lincomycin
E	Erythromycin
Ob	Cloxacillin
Cp	Ciprofloxacillin
Ct	Cefotaxime
Ra	Rfampicin
Va	Vancomycin

CHAPTER ONE

١.١ Introduction

An abscess can be defined as an infected area of tissue that contains pus. The collection of pus is usually localized. The breast appears structurally and functionally to be relatively uncomplicated, but it is the site of a surprising broad array of pathological alterations; the breast abscess is one of these important and critical pathological alterations (Peter, ١٩٩٨).

Breast abscess is more likely to develop in women when breast feeding a new baby especially if they have cracked nipples. This occurs as a sequel of mastitis. When a patients do not immediately seek medical attention for treatment of puerperal mastitis (Hale, ٢٠٠٠). The breast abscess also occurs in non-lactating women but it is uncommon. This type of breast abscess must be differentiated from a rare form of breast cancer (Surani *et al*., ١٩٩٣).

Patients with duct ectasia or periductal mastitis may be at higher risk for the development of breast abscess. It remains unclear whether the presence of periductal mastitis or that of obstructed, secretion-filled ducts is more important in the development of breast abscess (Mcfarlane, ٢٠٠١).

Breast abscess occurs mostly in women with infectious mastitis (Denar and Inan, ٢٠٠٣). Marshall *et al*. (١٩٧٥) have reported that the incidence of the mastitis among lactating

women is ٢.٥٪; from those patients, only ١١.١٪ have developed breast abscess.

There are many species of pathogenic bacteria that can cause breast abscess, like *S. aureus, S. epedermidis, S. pyogens* and *Bacteroides* (Razeq *et al*., ٢٠٠٠). Most of the studies conducted on breast abscess have concluded that *S. aureus* is the most common cause of breast abscess. Amir (٢٠٠٢) has mentioned that *S. aureus* is the predominant cause of the breast abscess in both lactating and non-lactating patients. Furthermore, Efem (١٩٩٥) has said that the commonest cause of breast abscess in lactating patients is *S. aureus*, whereas in non-lactating patients the commonest cause is *S. aureus* and *bacteroides*.

The increasing number of methicillin resistant *S. aureus* (MRSA) cases occur in hospitals, places where women have recently delivered or hospitalized at risk. An infection with MRSA may require vancomycin therapy, the only antibiotic effective against it (Howard, ٢٠٠٥).

Breast abscess can be divided into four types according to the site of the pus: subcutaneous, subareolar, reteromammary, and interlobular (Russell *et al*., ٢٠٠٤).

Patients with breast abscess may be present with fever, tachycardia and leuckocytosis. The breast may be engorged, red and tender with painful local swelling (Lawrence, ١٩٩٣). Breast abscess has critical complications like septicemia and

toxic shock syndrome (Demey *et al.*, ١٩٨٩). Besides, it may lead to both an indirect effect on infant health, and to wean the child too early (Fetherston, ١٩٩٧).

Neonatal breast abscess occurs infrequently. It occurs in full term infants one to five weeks of age, and in as many females and males, usually unilaterally. It is unrelated to maternal mastitis and usually occurs in bottle-fed infants. In recent years, the rare cases that occur are seen in conjunction with manipulation of the neonatal breast to express the natural secretion when the breast is engorged (Ruth and Robert, ١٩٩٩).

١.٢ Aims of Study

١. Assessment of the type, site, and phase of presentation of breast abscess.

٢. Isolation and characterization of aerobic bacteria associated with breast abscess and its relation to the clinical presentation.

٣. Detection and characterization of some virulence factors regarded as important causes of abscess formation.

٤. Evaluation the susceptibility of bacterial isolates to antibiotics.

CHAPTER TWO

٢.١ Anatomy

The breast lies embedded in the superficial fascia of the chest wall. It extends vertically between the second and the sixth costal cartilage and horizontally from the edge of the sternum nearly to the midaxillary line (Russell *et al.*, ٢٠٠٤). A process of the breast, known as the axillary tail, extends upward and laterally along the lower border of the pectorals major into the axilla, where it reaches as high as the third rib. This process, unlike the breast, lies below the deep fascia (Anbazhagan *et al*., ١٩٩١). The breast lies upon three muscles: pectorals major, saratus anterior and extensor oblique, but is separated from these by deep fascia (Russell *et al.*, ٢٠٠٤).

The axillary tail of the breast is of considerable surgical importance. In some normal cases it is palpable, and in a few it can be seen premenstrully or during lactation. A well- developed axillary tail is sometimes mistaken for a mass of enlarged lymph nodes or a lipoma (Lawrence, ١٩٩٣).

The lobules are the basic structural unit of the mammary gland. The number and the size of the lobules are very enormously; they are most numerous in young women. From ١٠ to ١٠٠ lobules empty via ductules into lactiferous duct of which there are ١٥ to ٢٠. Each lactiferous duct is lined by a spiral arrangement of contractile myoepithelial cells and is provided

with a terminal ampulla –a reservoir for milk or abnormal discharges (Egan, ١٩٨٨).

Lymphatic of the breast drains predominantly into the axillary and internal mammary lymph nodes. The axillary nodes receive approximately ٧٥٪ of the drainage (Russell *et al.*, ٢٠٠٤).

٢.٢ Physiology of the Mammary Gland

Mammary gland development and function are initiated by neural stimuli and a variety of hormonal stimuli, including: estrogen, progesterone, prolactine, oxytocin, thyroid hormone, cortisol, and growth hormone. The estrogen initiates ductal development, whereas the progesterone is responsible for lobular development and prolactin for lactogensis in late pregnancy and post partum period (Guyton and Hall, ١٩٩٦).

٢.٢.١ Changes during Menstrual Cycle

There is an increase in the size of the breast in the second half of the cycle. Moreover, in the pre-menstrual period, there is an increase in the size, nodularty, density and sensitivity of the breast (Edwaerds *et al.*, ١٩٩٥).

٢.٢.٢ Changes during Pregnancy

There is an increase in the circulatory, ovarian and placental estrogen and progesterone which leads to an increase in the glandular tissue. The areola becomes darker, and the

areolar gland prominent due to duct and lobules proliferation (Guyton and Hall, ١٩٩٦).

٢.٢.٣ Changes during Post Partum Lactation

There is a sudden decrease in the level of estrogen and progesterone after delivery with full expression of the lactogenic action of prolactine. During lactation, oxytocin causes myoepithelial contraction around the duct, leading the milk to appear (Ruth and Robert, ١٩٩٩).

٢.٢.٤ Changes during Postmenopausal Period

There is a decrease in estrogen and progesterone leading to a progressive involution of ductal and glandular component. Fibrous tissue and parenchyma are replaced by adipose and stroma tissue (Guyton and Hall, ١٩٩٦).

٢.٣ Bacteriology

٢.٣.١ Non Pathogenic Species

Bacterial colonization in the mammary gland is a natural process that occurs directly after delivery. A number of microorganisms similar to the normal one found on the skin are colonized in the lactiferous ducts. Some of these microorganisms might be pathogenic (such as *S. aureus*); but their presence does not necessarily imply that they are the cause infection (WHO, ٢٠٠٠).

A study conducted by Marshall *et al*. (١٩٧٥) on samples of milk taken from healthy lactating women has shown that the non-pathogenic microorganism isolated from those women are; *S. epidermidis*, non- hemolytic *Streptococci,* and diphtheroid species. Thomsen (١٩٨٢) has managed to differentiate between pathogenic microorganisms and non pathogenic ones by means of immune response, depending on the fact that the pathogenic microorganisms are covered by IgA, and IgG indicating an immunological response to infection.

٢.٣.٢ Pathogenic Species

٢.٣.٢.١. *Staphylococcus* species

It is a Gram-positive bacteria found either individually or in group like grape clusters; it is nonmotile and non-spore forming (Brooks *et al*., ٢٠٠٤).

٢.٣.٢.٢.١ *Staphylococcus aureus*

In spite of the fact that *S. aureus* is regarded as normal flora in the mucosal membrane and the skin of the healthy persons, sometimes it becomes an important pathogenic agent causing opportunistic infection (Brooks *et al*., ٢٠٠٤).

S. aureus expresses many potential virulence factors such as:

a. Surface proteins that promote the colonization of host tissues (Todar, ٢٠٠١).

b. Invasions that promote bacterial spread in tissues like; leukocidin, kinases and hyaluronidase (Brooks *et al*., ٢٠٠٤).
c. Surface factors that inhibit phagocytic engulfment which includes capsule and protein A (Katherin and Jean, ٢٠٠٤).
d. Biochemical properties that enhance their survival in phagocytes, like carotenoids and catalase production (Brooks *et al*., ٢٠٠٤).
e. Immunological disguises which include protein A, coagulase and clotting factor (Brooks *et al*., ٢٠٠٤).
f. Membrane-damaging toxins that lyse eukaryotic cell membranes and this include hemolysins, leukotoxin and leukocidin (Brooks *et al*., ٢٠٠٤).
g. Exotoxins that damage host tissues or otherwise provoke symptoms of disease. These exotoxins include, SEA-G, Toxic shock syndrome toxin – ١ (Toder, ٢٠٠٢).
h. Inherent and acquired resistance to antimicrobial agents (Brooks *et al*., ٢٠٠٤).
i. Other extracellular enzymes: *S. aureus* expresses a lipase, a deoxyribonuclease (DNase), protease, and a fatty acid modifying enzyme (FAME). The first three enzymes (lipase, DNAse, and protease) probably provide nutrients for the bacteria, and it is unlikely that they have anything but a minor role in pathogenesis. However, the FAME enzyme may be important in abscess, where it could

modify anti- bacterial lipids and prolong bacterial survival (Todar, ٢٠٠١).

S. aureus is the most pathogenic as it is responsible for a variety of pyogenic infections. This bacteria is the main cause of acute mastitis and breast abscess (Osterman and Rahm, ٢٠٠٠). It has the ability to opportunistically cause mastitis and breast abscess despite the fact that it often normally lies on lactating women bodies. It is commonly found in hospitals and causes infection in term of prevalence especially those strains of very virulence and resistance to penicillin (Fetherston, ٢٠٠١).

٢.٣.٢.١.٢ *Staphylococcus epidermidis*:

It is a coagulase negative *Staphylococci* and a common member of the normal flora of the skin and mucous membranes. The first appearance of *S. epidermidis* in clinical materials could be dismissed as contamination; it is now one of the most important agents of hospital-acquired infection, and immunosuppressed patients are particularly at risk (Baron *et al*., ١٩٩٦). Some strain produce a viscous extracellular polysaccharide slime or boifilm, which appears as an important virulence factor (Brooks *et al*., ٢٠٠٤). The slime layer is enclosed around the bacterial wall, it is mechanical barrier ti antimicrobial agents and host defense mechanism (Christensen *et al*., ١٩٨٢). *S. epidermidis* may also express a FAME; this extracellular enzyme inactivates bactericidal fatty acid by esterifying them to cholesterol. FAME may provide protection

for *S. epidermidis* by inactivating these lipids (Chamberlain and Brueggemann, ١٩٩٧).

It is the other *Staphylococcus* species causing breast abscess; it does not contaminate the milk but is considered an opportunistic microbe that becomes pathogenic when body resistant is low (Thomsen, ١٩٨٢). In an experimental study, these bacteria have been isolated from lactating women who suffer from mastitis. They have been injected in the mammary gland of rats, and it has been found that (٧٨-٩٣ %) of the mammary glands of the rats are infected. The abscess occurs in few cases, which may indicate that these bacteria are possible cause of breast abscess (Thomsen *et al*., ١٩٨٥).

٢.٣.٢.٢ *Streptococcus* species

It is a Gram positive bacteria arranged in the form of short or long chains; it is nonmotile and non-spore forming. The most important pathogenic specie is the *S. pyogenes*. They can be considered the main human pathogen associated with local or systemic invasive and post-streptococcal immunological disorder (Brooks *et al*., ٢٠٠٤). It has many virulence factors that include: the hyaluronic capsule which is regarded as a weakly antigen; M proteins are clearly virulence factors associated with resistance to phagocytosis; lipoteichoic fimbria appears to mediate bacterial attachment to host epithelial cells; and extra cellular substances like streptolysin, hyaluronidase, streptokinase, and pyrogenic exotoxins (Bisno and Stevvens,

١٩٩٦). These bacteria are the main cause of mastitis and they share with *S. aureus* causing breast abscess (Fetherston, ٢٠٠١). Viridanse group of Streptococcus and *Enterococcus faecalis* are a very rare cause of breast abscess, which is found living normally in the oral and nasopharyngeal cavities. Although it has palpable roles in causing mastitis, it is considered opportunistic and becomes pathogenic when body immunity is low (Thomsen *et al*., ١٩٨٣). An usual *S. pneumonia* breast abscess has been reported in a ٣٨- years old woman who has been partially breast feeding her nine-months old infant (Wast, ١٩٩٥).

٢.٣.٢.٣. Other Pathogenic Species

Pseudomonas aeruginosa is a Gram negative bacteria; it is rarely involved in breast abscess, even in patients with AIDS (Roca *et al*., ١٩٩٦). Although Buchwald and Blaser (١٩٨٤) suggested that the isolation of the pure *Salmonella typhi* from breast abscess is impossible, Jayakumur *et al*. (٢٠٠٣) refer that *S. typhi* has been a rare cause of breast abscess. Furthermore, Peter (١٩٩٨) has mentioned that another patient, a ٤٣ years old woman, has been presented with fever and a painful breast mass. Needle biopsy has revealed acute inflammation in the breast, and *S. typhi* has been isolated from the wound. Occasionally, other *Salmonella* spp. are associated with breast abscess like *S. enterica* serotype *landweisser* and *S.* sero-group B has implicated in breast abscess respectively (Razeq *et al*., ٢٠٠٠).

Memish *et al*., (٢٠٠١) have reported breast abscess in a breast implant as an unusual complication of laboratory-acquired brucellosis. An anaerobic bacteria are regarded as an important cause of breast abscess, especially in non-puerperal breast abscess (Semba and Neville, ١٩٩٩).

٢.٤ Predisposing Factors of Breast Abscess

There are many factors that help in causing breast abscess:

٢.٤.١. Milk Stasis

Milk stasis is considered the main predisposing factor of breast abscess. It has been noticed that infection occurs in many lactating women often having had milk stasis. This might be attributed to the fact that stasis milk provides an appropriate media for bacterial growth and infection (WHO, ٢٠٠٠). Gunther (١٩٥٨) has indicated that mastitis might not be a primary manifestation; it could be secondary, due to milk stasis. Removing the stasis milk greatly helps in avoiding infection.

Milk stasis results from not fully voiding milk from the mammary gland. This case occurs due to the gland engorgement developing after delivery, and may be attributed to blockage of the lactiferous ducts due to the damage of epithelial debris (Evans *et al*., ١٩٩٥).

Engorgement may also occur when the lactiferous ducts are blocked due to small soft peddles (about ١ μm) accumulating on the nipple peak. This may be due to the

excessive growth of the epithelial cells. They may cause blockage of the poles, which results in the blockage of the lactiferous ducts causing the engorgement of the gland (WHO, ٢٠٠٠). Fetherston (١٩٩٨) has said that the long sleeping of the baby and the sudden weaning may also cause engorgement.

There is a relation between thrush (Candidiasis) of the nipple and the recurrence of the mastitis; candidiasis of the nipple might lead to sore nipple causing milk stasis inside the gland due to difficulty of breast feeding, and this helps in producing a rout to the microorganisms to enter to the gland and multiply there (Amir *et al.*, ١٩٩٦).

٢.٤.٢. Lowered Immune Status

Reduced immune defense may increase the risk of breast abscess. In a study conducted in Cambia, it has been shown that the decrease of IgA and C٣ in lactating women milk might make them more susceptible to have mastitis and breast abscess (Prentice *et al.*, ١٩٨٥). Women affecting from low immune disease (i.e. diabetes) are more susceptible to have breast abscess (Kaufmann and Foxman, ١٩٩١).

٢.٤.٣. Previous Attack of Mastitis

Lactating women suffering from previous mastitis are more susceptible to have mastitis three times than normal women (Jonsson and Pulkkinen, ١٩٩٤). The scars formed due to previous infection might cause blockage of the lactiferous ducts

resulting in engorgement that might lead to bacterial invasion (Niebyl *et al.*, ١٩٧٨).

٢.٤.٤. Age

Age plays a significant role in the occurrence of breast abscess. It occurs among lactating women of ٢١ – ٣٤ years old (Jonsson and Pulkkinen, ١٩٩٤). Another study has indicated that the breast abscess spread among women more than ٣٠ years old (Foxman *et al.*, ١٩٩٤).

٢.٤.٥. Parity

In one study, reported that lactating women with single parity are more susceptible to have breast abscess than other women (Kaufmann and Foxman, ١٩٩١). Another study showed that breast abscess in single para and multipara is the same (Jonsson and Pulkkinen, ١٩٩٤). In contrast, Evan *et al*. (١٩٩٥), show that multiparas is more susceptible to have breast abscess.

٢.٤.٦. Other Factors

There are many other factors that may increase the risk of breast abscess such as; cigarette smoking, fibrocystic changes in the breast, tight clothing such as underwear bras and past injury to the breast (Niebyl *et al.*, ١٩٧٨).

٢.٥ Routes of Infection

There are three routes for mammary gland infection:

٢.٥.١. Retrograde Route

The bacteria are carried directly to the mammary gland through the lactiferous ducts or indirectly through the nipple

when the bacteria enter to the gland if cracks or abrasions occur in the nipple (WHO, ٢٠٠٠). The infant might catch the bacteria from the carriers "attendants "like the medical staff at hospitals. The bacteria are found in the nasopharynx region and are carried while milking directly to the mammary gland through the lactiferous ducts. The bacteria attack these ducts and multiply in lacteal cavities. The infection may occur as epidemic acute mammary adenitis (Devereux, ١٩٧٠). It is also likely that the infant catches the bacteria through the mother's skin; they are carried to the nipple where they enter via the cracks and abrasions of the nipple. This infection attacks the connective tissues which lie between the gland lobules; it occurs during various lactating intervals and is called sporadic acute mammary cellulitis (Niebyl *et al*., ١٩٧٨; Benson, ١٩٨٢).

٢.٥.٢. Haematogenous Route

Bacterimia may lead to mastitis and breast abscess (Rench and Baker, ١٩٨٩). In one study mentioned that septicemia due to *S. typhi* may cause breast abscess (Jayakumer, ٢٠٠٣).

٢.٥.٣. Lymphatogenous Route

The bacteria are carried through the lymphatic vessels to the mammary gland. Some studies refer that in case of endemic pulmonary tuberculosis, the bacteria causing tuberculosis may be carried from the lung through the lactiferous ducts to the mammary gland causing tubercular abscess (Gupta *et al*., ١٩٨٢).

٢.٦ Pathogenesis of the Abscess

Breast abscess is caused by invasive bacteria which produce potent exotoxins affecting all types of cells and promote considerable emigration of neutrophil polymorphs, resulting in formation of pus, either in a body cavity or in spaces formed by toxic necrosis of tissue and digestion of the dead tissue by the neutrophil polymorphs. The causal bacteria are termed pyogenic (pus forming) like *S. aureus, Strept. Pyogens* (Editorial, ١٩٧٦).

Suppuration indicates that infection has progressed to a stage where the defense mechanisms are rendered largely ineffective; because influx of exudates into an abscess gradually diminishes as the pressure rises and the beneficial effect of a continuous flow of exudates are thus lost. As a result, bacteria continue to multiply in the pus and their toxins cause necrosis of the surrounding tissue, with increase in size of the abscess (Fetherston, ٢٠٠١). Suppurating infection tends to persist and, even if the infection is eventually overcome, maturation of the large amount of granulation tissue formed may result in considerable scaring (May, ١٩٩٤).

Sometime a hard mass results from the calcification of the inspissated pus (Fetherston, ٢٠٠١). More often, the abscess eventually ruptures on to a surface and the pus is discharged. When this happens, the pressure is relieved and flow of exudates recommences with either elimination of the infection and

healing with scarring, or a persistent sinus discharging pus (Vercellotti *et al*., ١٩٨٥).

٢.٧ Clinical Features

The manifestations of the breast abscess are redness, swelling and pain of the breast (Ruth and Robert, ١٩٩٩). At the early stage when the abscess has not yet been formed, there is a lump in the breast accompanied by swelling, pain, difficult lactation, chills, fever, headache, nausea and dire thirst. Growing of the lump with local bright redness and intermittent throbbing pain indicate suppuration (Russell *et al.*, ٢٠٠٤).

Rarely may breast abscess be present as the initial presentation of sequamas cell carcinoma of the breast (Tan *et al*., ٢٠٠٢).

٢.٨ Diagnosis

The diagnosis of the breast abscess is easily confirmed by:

a. Presence of the signs and symptoms of the breast abscess (Russell *et al.*, ٢٠٠٤).
b. Ultrasound examination of the breast is diagnostic of the breast abscess (Tiu *et al*., ٢٠٠١).
c. The breast abscess is then confirmed by fine needle aspiration (FNA) to be sent for cultural study (Jay and Marce, ١٩٩٨).

٢.٩ Treatment

٢.٩.١ Treatment of Cellulitic Stage

During the cellulitic stage, the patient should be treated with an appropriate antibiotic, and the breast rested, with feeding on the apposite side. The infected breast should be emptied of milk using a breast pump. Support of the breast, local heat and analgesia will help to relieve the pain. Because the breast abscess is usually caused by *S. aureus*, it is recommended to use antibiotics with activity against multidrug resistant bacteria such as vancomycin and cefazolin. Delayed and / or an inappropriate treatment of cellulitic stage of breast infection can lead to abscess formation (Denar and Inan, ٢٠٠٣).

٢.٩.٢ Treatment of Localized Abscess

In a case of localized abscess, the antibiotics have little to offer and this is due to the fact that the bacteria present inside the abscess cavity beside acute inflammatory cells, protein exudate and necrotic tissue, result in surrounding the abscess cavity with granulation tissue (a vascular layer); the antibiotics could therefore, not reach the bacteria (Michie *et al*., ٢٠٠٣).

The presence of pus can be confirmed with a needle aspiration. In this situation, the breast should be incised and drained (Russell *et al.*, ٢٠٠٤).

Because subareolar abscess has an extraordinary high frequency of recurrence, the cause of the subareolar abscess is

plugging of lactiferous duct within the nipple by keratin. To prevent recurrence the abscess, ampoule with its plugged duct needs excision (Meguid *et al*., ١٩٩٥). An aspiration under local anesthesia combined with ultra sound imaging is an effective alternative to incision and drainage (O'Hara *et al*., ١٩٩٦). The patients should be treated by antibiotics for about ٣-٧ days after surgical drainage or aspiration. The antibiotics should be used according to culture and sensitivity (Gateley and Mansel, ١٩٩١).

After abscess drainage, the patients are advised not to nurse their babies from the breasts with abscess for about two weeks. They may continue to nurse from the other breasts. At the same time, a breast pump can be used to remove milk from the infected breasts (Dixon, ١٩٩٤).

CHAPTER THREE

٣.١ Materials

Besides the biological materials, many types of instruments and chemical materials were used in this study. These materials were taken from different sources and companies all listed in tables (٣.١) and (٣.٢). The stain and the reagent solutions are listed in table (٣.٣). The culture media used in this study are listed in table (٣.٤) and the potency of antibiotics disks in table (٣.٥).

Table (٣.١): Instruments

No	Instrument	Company
١	Autoclave	Stermite, Japan
٢	Benson's burner	Germany
٣	Biconcave slide	GFL, Germany
٤	Centrifuge	Hermle, Japan
٥	Distillator	GFL
٦	Hot air oven	Memmert, Germany
٧	Incubator	Memmert
٨	Inoculating Loop	Japan
٩	Inoculating needle	Japan
١٠	Light microscope	Olympus, Japan
١١	Micropipette	Oxford, USA
١٢	Millipore Centrifuge	Hermle
١٣	Millipore filter	Satorius membrane filters Gm, W. Germany
١٤	pH meter	Hoelese and Cheluis, KG, Germany
١٥	Refrigerator	Concord, Italy
١٦	Sensitive electronic balance	A and D, Japan
١٧	Water bath	Memmert

Table (٣.٢): Biological and chemical materials

Material	Company
٩٩٪ Ethanol	Fluka, Germany
Acetone	BDH, England
Albert stain	Difco, USA
Alpha-naphthol amine	BDH
Brimothymol blue agar	Difco
Fraizer reagent	BDH
Fructose powder	Fluka
Glucose powder	Fluka
Glycerol	BDH
Gram stain solutions (crystal violet, iodine, ethanol, safranine)	Difco
H_2O_2 (٣٪)	Fluka
Lactose powder	Fluka
Maltose powder	Fluka
Mannitol powder	Fluka
Methyl red reagent	Fluka
Na_2HPO_4, KH_2PO_4, KOH, NaCl, NaOH, $CaCl_2$, HCl, $HgCl_2$, K_2HCO_4, KNO_3	Fluka
Oil immersion	BDH
Peptone	BDH
Phenol red	Difco
Salicin powder	Fluka

Material	Company
Sucrose powder	Fluka
Sulfanilic acid solution	BDH
Tetra methyl-p-pheneylen diamine dihydrochloride (oxidase reagent)	Fluka
Urea copper sulphate	Difco
Xylene	Ajax, Australia

Table (٣.٣): Culture media

Medium	Company
Blood agar base	Oxoid, England
Brain-heart infusion broth	Mast Diagnostic, England
Crystal violet agar	Mast Diagnostic
DNase agar	BDH, England
Gelatin agar	BDH
Lipolytic agar	Oxoid
MacConkey agar	Difco, USA
Mannitol salt agar	Difco
Motility medium	BDH
Muller-Hinton agar	BDH
Nutrient agar	Oxoid
Tryptic soy broth	Mast diagnostic
Urea agar	Fluka, Germany

Table (٣.٤): Antibiotics disk potency

Antibiotic	Potency (mg/disk)	Company
Amoxicyllin (AMX)	١٠	Oxoid, England
Amoxyclave (AMC)	٣٠	Oxoid
Ampicillin (AM)	١٠	Oxoid
Cefotaxime (CE)	٣٠	Oxoid
Cephalexin (CI)	٣٠	Troge, Germany
Ciproflaxacin (CF)	٣٠	Troge
Cloxacillin (OB)	٥	Troge
Erythromycin (E)	١٥	Troge
Gentamicin (GM)	١٠	Troge
Lincomycin (LN)	٢	Troge
Rifampicin (R)	٥	Troge
Vancomycin (VA)	٣٠	Troge

٣.٢ Methodology

٣.٢.١ Preparation of Reagents and Solutions

٣.٢.١.١ Oxidase reagent

It was prepared by dissolving ٠.١ gm of Tetramethyl-P-phenylenediamine dihydrochloride in ١٠ ml of distilled water and stored in a dark container (Baron *et al*., ١٩٩٦).

٣.٢.١.٢ Fraizer reagent

It was prepared by dissolving ٥ gm of $HgCl_2$ in ٢٠ ml of HCl, and then added to ١٠٠ ml of distilled water (Collee *et al*., ١٩٩٦). It was used for the detection of gelatin breakdown.

٣.٢.١.٣ Nitrate reduction reagent

The reagents consist of:

i. Sulfanilic acid solution: It was prepared by dissolving ٨ gm of sulfanilic acid in one litter of ٣٠٪ acetic acid.

ii. Alpha-naphthyl amine: It was prepared by dissolving ٥ gm of alpha-naphthyl amine in one litter of ٣٠٪ acetic acid. Nitrate reduction reagent was prepared immediately before use by mixing equal volumes of solutions (i) and (ii) (MacFaddin, ٢٠٠٠).

٣.٢.١.٤ Phosphate buffer saline

٨ gm of NaCl, ٠.٣٤ gm of KH_2PO_4, ١.١٢ gm of K_2HPO_4; all of them were dissolved in ١٠٠٠ ml of distill water. The pH was adjusted to ٦.٠. The mixture was then sterilized in an autoclave at ١٢١^{o}C (Baron et. al., ١٩٩٦).

٣.٢.٢ Preparation of culture and diagnostic media

٣.٢.٢.١ Ready – prepared media

Media used in this study (list in table ٣.٤) was prepared in accordance with the manufactur's fixed on their containers. All the above media were sterilized in the autoclave at ١٢١^{o}C for ١٥ min. After sterilization, blood agar base was supplemented with ٥٪ defibrinated human blood and crystal violet blood agar was supplemented with ١٠٪ human blood, and urea agar base was supplemented with ٢٠٪ sterile urea solution. All media were used for isolation and diagnosis of bacteria. Tryptic soy broth

was used to detect the ability of *S. aureus* to express FAME. Lipolytic agar was supplemented with ٢.٥ ml of oleic acid.

٣.٢.٢.٢ Laboratory prepared media

٣.٢.٢.٢.١ Nitrate reduction broth

It was prepared by dissolving ٠.٢ gm of KNO_3 and ٥ gm of pepton in one litter of distilled water. The mixture was distributed in test tubes (٥ ml amounts) and sterilized by autoclaving at ١٢١^{o}C for ١٥ minutes (Collee *et al*., ١٩٩٦).

٣.٢.٢.٢.٢ Oxidation and fermentation medium

It was prepared by dissolving ٢ gm of pepton, ٥ gm of NaCl, ٠.٣ gm of dipotassium hydrogen phosphate (K_2HPO_4), ٠.٠٨ gm of bromthymol blue and ٢.٥ gm of agar in one letter of distilled water. The glucose to be added was sterilized separately by Millipore filter (٠.٢٢ μm) and added to give a final concentration of ١٪. The medium was then tubed to a depth of ٤ cm. Duplicate tubes were used in this test. One of them was covered with a layer of sterile paraffin oil. The medium was used to identification of aerobic / facultative aerobic bacteria (Sancho *et al*., ١٩٩٩).

٣.٢.٢.٢.٣. Carbohydrate fermentation broth

This medium was consisted of:

a. Basal medium: It was prepared by dissolving ١٠ gm of peptone, ١ gm meet extract, ٥ gm NaCl and ٠.٠١٨ gm phenol red in one litter of distilled water. Then pH was then adjusted to ٧.٤. The medium was distributed on test

tubes and Durham tubes were added to each test tube, then sterilized by an autoclave (MacFaddin, ٢٠٠٠).

b. Carbohydrate sources: the following carbohydrate sources were used; sucrose, glucose, fructose, lactose, maltose, salicin and manitol (MacFaddin, ٢٠٠٠).

c. After carbohydrate solutions were sterilized by Millipore filters (٠.٢٢ μm), they were separately added to the basal medium in sterile tubes (٥ ml portions) to give a final concentration of ١ %. The medium was used to test the ability of bacterial isolates to ferment a specific carbohydrate incorporated in a basal medium.

٣.٢.٢.٢.٤ Maintenance medium

This medium was prepared by adding ٢٠٪ of Glycerol to brain heart broth and the sterilized by autoclave at ١٢١^{o}C for ١٥ min. and then distributed into sterile plastic tubes (Felthham *et al*., ١٩٧٨).

٣.٢.٣ Patients

From September ٢٠٠٤ to June ٢٠٠٥, seventy-one patients of breast abscess visited Breast Center at Hilla Teaching Hospital. Their ages ranged from ٣ months to ٥٧ years. All patients underwent the following investigations: ultrasound of the affected breast, fine needle aspiration (FNA) sent for cultural study. Out of the total number of patients twenty were on antibiotics, two were diabetic and one was suffering from sickle cell anemia.

٣.٢.٤ Collection of Pus

Fine needle aspiration was done for all patients to obtain the pus from localized abscess: the pus was collected in sterilized plastic tubes containing ٥ ml of brain heart broth and incubated at ٣٧^{o}C for ١٨ – ٢٤ hr. before being plated on a screening media. Before FNA, the females breast were disinfected with ٢% of iodine solution, then disinfected after area dryness with ٧٠% ethanol (Thomesen *et al*., ١٩٨٤).

٣.٢.٥ Identification of Bacteria

A single colony was taken from each primary positive culture (blood agar, nutrient agar and MacConkey agar), identified by depending on its morphology (shape, size, borders and texture), and then examined under microscope after staining it with Gram stain or other specific stains (such as Albert stain). After staining, the biochemical tests were done for each isolate to reach the final identification according to Bergy's Manual for Determinative Bacteriology (Holt *et al*., ١٩٩٤).

٣.٢.٦ Biochemical tests

٣.٢.٦.١ Catalase test

A colony of the organism was transferred to a drop of ٣٪ $H_٢O_٢$ on a microscope slide. The presence of catalase meant that the formation of gas bubbles had occurred indicating a positive result (Collee *et al*., ١٩٩٦).

٣.٢.٦.٢. Oxidase test

A piece of filter paper was saturated in a petri dish with oxidase reagent, then a colony of organism was spread onto the filter paper. When the color around the smear turned from deep purple, the oxidase test was positive (Collee, *et al*., ١٩٩٦).

٣.٢.٦.٣. Coagulase test

Several colonies of bacteria were transferred with a loop to a tube containing ٠.٥ ml of human plasma. The tube was covered to prevent evaporation and incubated at ٣٧^{o}C overnight. The test was read by tilting the tube and observing clot formation in the plasma (Collee, *et el*., ١٩٩٦).

٣.٢.٦.٤. Growth onto mannitol salt agar

This medium was streaked with bacterial culture. A colour conversion to yellow in the medium refers to positive result and mannitol fermentation. This medium was selected for diagnosis of *S. aureus* (MacFaddin, ٢٠٠٠).

٣.٢.٦.٥. Growth onto crystal violet blood agar

This medium was streaked with bacterial culture and incubated at ٣٧^{o}C for ٢٤-٤٨ hours. The growth on this medium indicated that the bacteria were *S. pyogenes* (Collee *et al*., ١٩٩٦).

٣.٢.٦.٦. Urease test

The urea agar base was inoculated by bacterial cultures and incubated for ٢٤-٤٨ hours at ٣٧^{o}C. The positive result was a deep pink colour (Benson, ١٩٩٨).

٣.٢.٦.٧. Bacitracin sensitivity test

The blood agar was streaked with bacterial culture and the bacitracin disk put in the center of the peter dish. The diameter of inhibition zone was equal to or more than ١٢ mm, indicating a positive result. This test was used to diagnosis of *S. pyogenes* (MacFaddin, ٢٠٠٠).

٣.٢.٦.٨. Optochin sensitivity test:

The blood agar was streaked with bacterial culture and the optochin disk put in the center of the peter dish. If the diameter of the inhibition zone was equal to or more than ٥ mm from the margin of the disk, it would indicate a positive result. This test was used to diagnosis of S. *pneumoniae* (MacFaddin, ٢٠٠٠).

٣.٢.٦.٩: Motility test

This test was studied as mentioned in MacFaddin (٢٠٠٠).

٣.٢.٦.١٠. Gelatin breakdown test

This test was studied as mentioned in Collee *et al*., (١٩٩٦).

٣.٢.٦.١١. Nitrate reduction test

The test tubes were inoculated with pure culture and incubated at ٣٧^{o}C for ٢٤-٩٦ hours, then ١ ml of nitrate reduction reagent was added. The appearance of a red colour during ٣٠

seconds would indicate a positive result, proving the reduction of nitrate to nitrite (Collee *et al*., ١٩٩٩).

٣.٢.٦.١٢. Oxidation and fermentation test

The bacteria under study were inoculated in two long narrow tubes containing oxidation and fermentation medium. One tube was covered with paraffin layer to incite an aerobic environment that would force the isolate to ferment, and the two tubes were then incubated at ٣٧^{o}C for ٢٤-٩٦ hours. Fermentation bacteria would give a copious production of acid in both tubes, seen by the yellow turning of the indicator. Oxidative bacteria would suffer this reaction only in the tube without paraffin. (MacFaddin, ٢٠٠٠).

٣.٢.٦.١٣. Carbohydrate fermentation test:

Carbohydrate fermentation medium was inoculated with bacterial colonies and incubated at ٣٧^{o}C for ٢٤-٩٦ hours. The colour change of the medium from red to yellow with or without gas formation in the Durham tube indicated a positive result (MacFaddin, ٢٠٠٠).

٣.٢.٦.١٤. Growth on DNase agar:

This medium was used to study the microorganism DNase production. The medium was inoculated with a single bacterial colony and incubated at ٣٧^{o}C for ٢٤ hours. The positive reaction was observed by the appearance of a clear zone surrounding the growth, with the rest of the plate remaining turbid (MacFaddin, ٢٠٠٠).

٣.٢.٧. The virulence factors

٣.٢.٧.١. Production of hemolysin

Hemolysis production was shown on blood agar media. The organism was inoculated onto blood agar plates and incubated for ٢٤-٤٨ hours at ٣٧^{o}C to detect any hemolysis around the colonies (either α- or β- hemolysis) (De Boy *et al*., ١٩٨٠).

٣.٢.٧.٢ Slime production

a. A heavy smear of organism was prepared and allowed to dry in the air. ١٪ crystal violet was applied to non-heated fixed smear, waiting for ٥-٧ minuets.
b. The smear was washed with ٢٠٪ copper sulfate solution, then was gently blot dry and was later examined under oil immersion that the slime would appear blue in contrast to the deep purple of the cell (Chamberlain and Brueggemann, ١٩٩٧).

٣.٢.٧.٣. Lipase production

It was qualitatively prepared by incubating the isolates for ٢٤-٤٨ hours at ٣٧oC on lipolytic agar. Opaque zone of the medium around the colonies would indicate an isolate producing lipase (MacFaddin, ٢٠٠٠).

٣.٢.٧.٤ Fatty Acid Modifying Enzyme (FAME) Production

٣.٢.٧.٤.١ FAME assay

a. One ml of frozen *S. aureus* was added to ١٠٠ ml of tryptic soy broth (TSB), pre-warmed to ٣٧^{o}C, and incubated at ٣٧^{o}C with agitation for ١٦ hours.

b. One ml of this culture was then added to ١٠٠ ml of pre-warmed TSB and incubated as above.

c. Culture filtrates were then obtained by passing the culture through Millipore filter (٢٢ μm). The culture filtrates were stored as frozen divided volumes until required.

d. One ml of culture filtrate added to ٩ ml of buffer saline (pH ٦), then added ٠.٢ ml of oleic acid and this incubated for ٣٠ min. at ٣٧^{o}C. ١٠ micron of this solution added to ١ ml of colour reagent (cholesterol esterase), waiting for ١٠ min. at room temperature or ٥ min. at ٣٧^{o}C, then assay for cholesterol level according to the absorbance of test and standard tube (Tietz, ١٩٩٩).

e. Isolates were considered positive for FAME activity if the cholesterol ester / $OD_{٦٠٠}$ was > ٢٠٠ (Chamberlain and Brueggemann, ١٩٩٧).

٣.٢.٧.٤.٢. FAME production during growth cycle

a. *S. aureus* isolate (No. ٣٢) was incubated in TSB for ١٦ hours at ٣٧^{o}C with agitation; ١ ml of this culture was

centrifuged (١٢٠٠ rpm) for ٥ minuets, and the pellet was resuspended in ١ ml of sterile TSB.

b. This was repeated three times, after which ٠.٧٥ ml of the washed cells were added to ١٠٠ ml of pre-warmed TSB (٣٧^{o}C) and the culture was incubated at ٣٧^{o}C for ١٢ hours with agitation.

c. Samples (١ ml) were taken every hour for ١٢ hours and $OD_{٦٠٠}$ and FAME activity was determined as the same as in item ٣.٢.٧.٤.١.d (Chamberlain and Brueggemann, ١٩٩٧).

٣.٢.٨. The antibiotics sensitivity tests

Antibiotic sensitivity test of bacterial isolates was studied against the antibiotics shown in table (٣.٤) by the disk diffusion technique on Muller-Hinton agar, using inhibition zone size criteria recommended by the disk manufacturer and based one method of Baur and Kerby, (١٩٦٦). The selection of antibiotics disks was performed according to recommendation of the National Committee for Clinical Laboratory Standards (NCCLS, ٢٠٠٣).

٣.٢.٩. Preservation of Isolates

The bacterial isolates were preserved by inoculating ٨٥٪ of bacterial growth at age of ١٨-٢٤ hours to the maintenance medium, mixed very well, and then stored by freezing at – ٢٠^{o}C (Feltham *et al.*, ١٩٧٨).

CHAPTER FOUR

٤.١ Clinical Aspects

٤.١.١ Patients

In this study, seventy one patients who have been suffering from breast abscess visited were investigated. Most of the patients were presented with classical features of the breast abscess (Table ٤.١). These included mass (١٠٠٪), pain (٩٨.٦٪), fever (٩٧.٢٪), and bright reddens (٩١.٥٪). This result agreed with all studies, dealing with clinical aspect of the breast abscess. Marchant (٢٠٠٢) referred that the infection might take place in the parenchyma tissue of the breast and cause swelling of the parenchymal tissue outside the milk duct. This swelling would compress on the milk duct, resulting in pain, redness and tender swelling of the infected breast. Russell *et al*. (٢٠٠٤) also said that the affected breast might be present with the classical signs of acute inflammation including fever, throbbing pain, tender mass and redness. Furthermore, Foxman *et al.* (٢٠٠٢) mentioned that if the lesion progressed to localized abscess, the patient would present local and systemic signs of infection like, pain, tenderness, mass, fever, malaise and leuckocytosis.

A sonographic diagnosis of abscess was made when a round, oval, or irregularly shaped hypoechoic lesion (homogeneous or inhomogeneous) was present and showed at least some acoustic enhancement within which no vessels were observed at colour

Doppler US. This finding is similar to those obtained by Ryan *et al.* (٢٠٠٤).

Table (٤.١): Clinical Features among ٧١ Patients of Breast Abscess

Clinical feature	Number	%
Mass	٧١	١٠٠
Pain	٧٠	٩٨.٦
Fever	٦٩	٩٧.٢
Reddens	٦٥	٩١.٥

٤.١.٢ Lactation

This study showed that the high incidence of breast abscesses occurred among lactating women who represented ٧٤.٦٪ of the patients. In non-lactating women, the rate of breast abscess was ٢٥.٤% (Table ٤.٢). This result agreed with many studies like (Karstrup *et al.*, ١٩٩٣) and (Tropy *et al.*, ٢٠٠٣) who said that the breast abscess was a disease of lactating women and it was very rare among non-lactating women. This might be due to the fact that lactation might lead to milk stasis in ducts and lobules, which might create a microenvironment fastering bacterial growth (WHO, ٢٠٠٠). Surani *et al.* (١٩٩٣) also mentioned that the breast abscess was infrequent among non lactating women, Efem, (١٩٩٥) showed that breast abscess among lactating women represented ٩٥٪ of the patients whereas

in non lactating women it was ٥٪. He explained that the low incidence of breast abscess in non lactating women correspond to low incidence of cigarette smoking and mammary duct ectasia, whereas the incidence of lactation breast abscess would corresponded to high rate of breast feeding and low level of personal hygiene. The results of this study did not agree with (Jay, ١٩٩٨) who said that nonpuerperal breast abscess were far more common than puerperal breast abscess. In this study, ٨.٥٪ of breast abscess were in the puerperium, and ٣٪ of those were lactating at the time of the presentation.

Table (٤.٢): Incidence of breast abscess among lactating and non-lactating women

Lactation	No. of cases	%
Lactating women	٥٣	٧٤.٦
Non lactating women	١٨	٢٥.٤
Total	٧١	١٠٠

٤.١.٣ Age

In lactating women, the high incidence of the breast abscess was noticed in the age group of the ٢٠-٢٩ years which constituted ٥٤.٧٪ of the patients and in the age group of ٣٠ – ٣٩ years which constituted ٣٧.٧٪ whereas the incidence rate decreased when the ages were less than ١٩ years and above ٤٠ years (Table ٤.٣). Such finding was confirmed by Vogel *et al.*

(١٩٩٩) who found that ٦٨.٧٪ of the breast abscess would occur in the age group of ٢٥-٣٥ years, and the finding agreed with Jonsson and Pulkinen (١٩٩٤) who referred to the reduction of the breast abscess rate at the ages less than ٢١ years and above ٣٤ years. This result did not match with Kaufmann and Foxman (١٩٩١) who showed the high breast abscess rate in the advanced woman ages. These findings could most probably be due to the fact that lactation was common at these reproductive periods, and that the lactation might lead to milk stasis considered a very important risk factor of breast abscess. This explains why the breast abscess is widely spread in these age groups (WHO, ٢٠٠٠).

On the other hand, in non lactating women, the high incidence rate was noticed in the age group of ٤٠ – ٤٩ years which constituted ٤٤.٤٪ of the patients (Table ٤.٣), which agreed with results obtained by Howard (٢٠٠٥) who observed that non-lactating breast abscess would encompass the third to the eight decades of life. These results likewise would agree with those obtained by Surani *et al*. (١٩٩٣) who mentioned that breast abscess in non-lactating women were very uncommon and it usually affected women older than ٤٠ years. In addition, Thomsen *et al*. (١٩٨٤) referred that non lactation breast abscess often affected older women, when the super added bacterial infection in periductal mastitis could present as abscess.

However, the incidence of breast abscesses in non-lactating women between ٢٠ – ٢٩ years would be zero when compared to the lactated women.

Table (٤.٣): The relation between the age and the occurrence of breast abscess in lactating and non-lactating patients

Age/years	Lactating patients		Non- lactating patients	
	No.	%	No.	%
< ١	٠	٠	٢	١١.١
١-٩	٠	٠	٠	٠
١٠-١٩	٣	٥.٧	٣	١٦.٧
٢٠-٢٩	٢٩	٥٤.٧	٠	٠
٣٠-٣٩	٢٠	٣٧.٧	٢	١١.١
٤٠-٤٩	٠	٠	٨	٤٤.٤
٥٠-٥٩	١	١.٩	٣	١٦.٧
Total	٥٣	١٠٠	١٨	١٠٠

٤.١.٤ Parity

This study showed that the high incidence rate occurred in women that had more than one child, representing ٦٦.٢٪ (Table ٤.٤). This finding might most likely be ascribed to recurrent mastitis which is a high risk factor of breast abscess. The recurrent mastitis is higher in multipara than primipara. So the breast abscess occurs in multipara more than primipara does. Mohrbacher and Stock (٢٠٠٣) also found that the mothers who had previous breast feeding became engorged sooner and more severely than first time breast feeding mothers, and this sever engorgement might be predisposed to mastitis and breast

abscess. This result also agreed with Vogel *et al*. (١٩٩٩) who referred that ٦٥.١٪ of breast abscess occurred in women having two children and more, but it did not agree with that of Kaufmanns and Foxman (١٩٩١) who showed that the high incidence occurred in those having one child. They ascribed this result to the fact that mothers having one child would lack the experience of lactation, which might lead to pain, stress, and mental anguish which would interfere with let-down reflex. Milk stasis and engorgement would soon occur and lead to mastitis and breast abscess.

Table (٤.٤): The relation between the parity and occurrence of breast abscess in married patients with breast abscess.

Parity	No. of cases	%
Nili para	٣	٤.٦
Primipara	١٩	٢٩.٢
Multipara	٤٣	٦٦.٢
Total	٦٥	١٠٠

٤.١٠ Months of Postpartum

It has been shown that the incidence of breast abscess is high during the first three months after delivery, representing ٤٥.٣% of the patients (Table ٤.٥). This result agreed with Vogel *et al*. (١٩٩٩) who found that ٦٢.٨٪ of breast abscess might occur

in the first three months after delivery. Ruth and Robert (١٩٩٩) referred those mothers might contract mastitis and breast abscess during the first few weeks, through exposure to virulent bacteria at the hospital after having given birth. This was once a major problem when hospital stay was longer. Another possible cause of increasing the rate of breast abscess during the first few weeks was that normal breast fullness might develop engorgement between the third and six days after birth; the engorgement would increase when the baby was given supplements (Mohrbacher and Stock, ٢٠٠٣).

Table (٤.٥): The relation between the age of infants and the occurrence of the breast abscess in lactating women

Months of postpartum	No. of cases	%
٠-٣	٢٤	٤٥.٣
٤-٦	٥	٩.٤
٧-٩	٥	٩.٤
١٠-١٢	٨	١٥.١
> ١٢	١١	٢٠.٨
Total	٥٣	١٠٠

Another study done by Devereux (١٩٧٠) described that the highest incidence of breast abscess was in the second and third weeks postpartum, attributing that to disturbed attitude and

emotion in the early weeks postpartum. This would have effect on the human maternal–infant bond leading to engorgement which would predispose to mastitis and breast abscess. Jay and Marce (١٩٩٨) explained that the occurrence of breast abscess might occur in a high rate in the first three months after delivery as a complication of puerperal mastitis typically occurring within two to three weeks of the lactation.

٤.١.٦ Type of Abscess

The study has shown that the common type of breast abscess in lactating women is subcutaneous abscess which represents ٤٥.٣% of the patients, whereas in non lactating ladies the most common type is supaereolar abscess which constitutes ٧٧.٨٪ of the patients (Table ٤.٦). This result agreed with Tiu *et al*. (٢٠٠١) and Livingstone (١٩٩٦) who said that the superficial breast abscess were the commonest type in puerperal abscess and that was due to blocked lactiferous ducts and stasis of the duct. Jay and Marce (١٩٩٨) mentioned that the subareolar breast abscess was more common in non lactating women and it appeared to be a distinct clinical entity having more aggressive natural history than peripheral breast abscess. He explained that the high occurrence of the subareollar breast abscess in non lactating women was due to sequamous epithelial neoplasm with keratin plug or ductal extension with associated inflammation.

Table (٤.٦): Types of the breast abscess in lactating and non-lactating women.

Type of abscess	Lactating		Non-lactating	
	No.	%	No.	%
Subcutaneous	٢٤	٤٥.٣	٠	٠
Subareolar	١٧	٣٢.١	١٤	٧٧.٨
Interlobular	١١	٢٠.٧	٤	٢٢.٢
Retromammary	١	١.٩	٠	٠
Total	٥٣	١٠٠	١٨	١٠٠

٤.١.٧ Affected Breast

It has been shown that the incidence of affected left breast is higher (٦٠.٦%) than that of the right breast (٣٩.٤%) (Table ٤.٧). This result agreed with that of WHO (٢٠٠٠), where ٣٧%-٥٢٪ of the cases involved the right breast and ٣٨%-٥٧٪ of the cases did the left breast. However, no significant differences were observed as to which of the two sides was affected more. A breast has been more affected as a result of milk stasis. It has been observed that many mothers found it easier to attach their infant to the breast on one side than on the other. It has been found that poor attachment would lead to milk stasis and mastitis might occur on the side that was more difficult (WHO, ٢٠٠٠). Mohrbacher and Stock (٢٠٠٣) referred that the breast abscess very rarely affected both breasts at the same time.

Table (٤.٧): Occurrence of abscess in left and right breast

Affected breast	No. of cases	%
Left	٤٣	٦٠.٦
Right	٢٨	٣٩.٤
Total	٧١	١٠٠

٤.١.٨

Address

This study has shown the incidence of the breast abscess is higher in rural areas (٦٤.٨%) than it is in urban ones (٣٥.٢%) (Table ٤.٨). This is due to the absence of health care and low educational level among the families of the rural communities, as well as low economic levels which might result in increasing the number of cases of malnourished and reduced immunity. Woolridge (١٩٩٠) said that the economic recession would also reduce patronage of artificial feeds, thus intensifying breast feeding and consequently breast abscess. Another study conducted by Ruth and Robert (١٩٩٩) showed that in rural areas, the incidence of the breast abscess was high due to poor hygiene and contaminated environment; besides, the duration of the average of breast feeding would be much longer than in urban areas.

Table (٤.٨): Distribution of breast abscess according to addresses

Address	No. of cases	%
Rural	٤٦	٦٤.٨
Urban	٢٥	٣٥.٢
Total	٧١	١٠٠

٤.١.٩ Nipple State

The study has shown that most patients have normal nipples (٧١.٨٪) whereas the cracked nipples represent ٢٥.٤٪ of the patients and the retracted nipples constitute only ٢.٨٪ of the patients (Table ٤.٩).

This result is not different from other studies, like Jay and Marce (١٩٩٨) who said that an irritated or cracked nipple was frequently associated with this infection and might provide a portal of entry for the bacteria. Ekland and Zeigler (١٩٧٣) mentioned that the nipple inversion might provide a portal of entry for pathogenic bacteria, making the breast more difficult for the baby to latch on well especially during the early weeks, and leading to engorgement and milk stasis which would facilitate the occurrence of breast abscess. They found nipple inversion in only ٩٪ of the patients with breast abscess. Sore nipple might usually occur in the early weeks (postpartum) due to poor lactation positioning, and pain from nipple damage might cause mothers to postpone feeding, resulting in an overly full breast, which might contribute to mastitis and breast abscess (Amir *et* al., ١٩٩٦).

Table (٤.٩): The state of the nipple associated with the breast Abscess patients

Nipple state	No.	%
Normal	٥١	٧١.٨
Cracked	١٨	٢٥.٤
Inverted	٢	٢.٨
Total	٧١	١٠٠

٤.١.١٠ Recurrence of the Breast Abscess

It has been shown that the second recurrence of breast abscess constitutes ١٢.٧٪ of the patients, whereas the study proves no third recurrence of breast abscess (Table ٤.١٠). All the patients with recurrent abscess have only the subareolar type of breast abscess: three of those patients are known cases of non insulin dependent of diabetes mellitus, two have history of prolonged use of steroid therapy due to bronchial asthma, and one has sickle cell anemia with history of frequent blood transfusion. This result agreed with Ruth and Robert (١٩٩٩) who said that the subareolar breast abscess had an extra ordinary high frequency of recurrence. In addition, Jay and Marce (١٩٩٨) referred that there were conditions such as diabetes, steroid therapy, or other infected skin lesions that would predispose to breast abscess.

Table (٤.١٠): The ratio of recurrence cases of breast abscess

Recurrence	No. of cases	%
Nil	٦٢	٨٧.٣
Second	٩	١٢.٧
Third	٠	٠
Total	٧١	١٠٠

٤.٢. Isolation of Bacteria Associated with Breast Abscess

٤.٢.١ In lactating patients

Associated pathogens were divided into two groups; the first group was for single infecting microbes (Table ٤.١١). It included *S. aureus*, *M. catarrhalis*, *S. pyogenes* and *S. epidermidis*. The high incidence (٧٣.٦٪) was of *S. aureus*, whereas the low incidence (١.٩٪) was of *S. epidermidis*.

This study has shown that the breast abscess is due to monobacterial infection only, whereas the dibacterial infection has not be detected. This result agreed with that obtained by Howard (٢٠٠٥), who mentioned that breast abscess mostly occurred due to single infection.

Table (٤.١١): Incidence of bacterial isolates in lactating and non-lactating patients

Bacterial isolate	Lactating		Non-lactating	
	No.	%	No.	%
S. aureus	٣٩	٧٣.٦	٥	٢٧.٨
M. catarrhalis	٣	٥.٧	٢	١١.١
S. pyogenes	٢	٣.٨	٠	٠
S. epidermidis	١	١.٩	٠	٠
A. haemolyticum	٠	٠	٤	٢٢.٢
No growth	٨	١٥	٧	٣٨.٩
Total	٥٣	١٠٠	١٨	١٠٠

Michie *et al*. (٢٠٠٣) also indicated that the breast infection would usually occur due to single infecting microbe, and *S. aureus* was regarded as the most common pathogen isolated from breast infections. Thomsen (١٩٨٢) suggested that the mammary gland infection would be commonly caused by single infecting bacteria. This result disagreed with that of Niebyl *et al*. (١٩٧٨), who found that multimicrobic growth in mammary infection constituted ٤٢٪. However, *S. pyogenes* and *M. catarrhalis* have also been isolated in this study from lactating women.

In the second group, however the associated pathogens could not be recovered. This group constituted ١٥٪ of the patients. The reasons of non-recoverable infection may be due to the following:

a. Infection was nonbacterial. It was termed nonbacterial infection and caused by *Cryptococcus* or viruses (WHO, ٢٠٠٠).
b. Mastitis and breast abscess might be caused by *Chlamydia*, *Mycoplasma*, or *Mycobacterium* (Thomsen *et al*. ١٩٨٣). that did not grow in culture conditions used in this study.
c. Antibioma which resulted from uses of antibiotics in treatment of un-drained pus. Uses of broad spectrum antibiotics would kill the bacteria and leave it as sterile pus (Thomsen, ١٩٨٢).

٤.٢.٢ In Non-Lactating Patients

As in lactating patients, associated pathogens were divided into two groups: monomicrobic representing ٦١.١٪, and unrecoverable pathogen constituting ٣٨.٩٪. The infecting bacteria included *S. aureus* (٢٧.٨٪), *A. haemolyticum* (٢٢.٢٪) and *M. catarrhalis* (١١.١٪) (Table ٤.١١).

٤.٣. Characteristics of Isolated Bacteria

٤.٣.١ *Stapylococcus aureus*

In this study, they were appeared as Gram positive *cocci* arranged in clusters. *S. aureus* produced large hemolytic golden yellow colonies in appearance. Its biochemical tests were catalase positive, oxidase negative, coagulase positive, mannitol fermentation positive and motility negative. These results were

agreed with (MacFaddin ٢٠٠٠). This study has revealed that *S. aureus* is the most frequent isolate of the breast abscess in both lactating and non-lactating women, where it represents (٧٣.٦٪) and (٢٧.٨٪) respectively (Table ٤.١١). These results are consistent with Blumstein *et al*. (٢٠٠٣), who suggested that *S. aureus* was the most common cause of breast abscess and typically originated from the nursing child. They also explained that this result was due to the ability of *S. aureus* to produce many virulence factors like capsule, surface protein, hemolysin, lipase, and DNase.

Moreover, Dufour *et al*. (٢٠٠٢), mentioned that the breast infection in lactating and non-lactating patients were usually caused by *S. aureus* being the common bacteria found on the normal skin. These bacteria might enter through a break or a crack in the skin, usually the nipple, although *S. aureus* is very sensitive to the bactericidal effects of several fatty acids of the skin. The bactericidal effect of fatty acids on *S. aureus* is thought to result from the incorporation of these lipophilic agents into the bacterial membranes, causing increased membrane fluidity and a decrease in vital membrane-associated functions. Production of carotenoids may help *S. aureus* stabilize its cell membrane and thereby prevent potentially lethal fatty acid-induced changes in the fluidity of its membrane (Chamberlain *et al*., ١٩٩١). For this reason, *S. aureus* has been regarded as the most common cause responsible for the majority

of breast abscess also causing many pyogenic skin infections like cellulitis, boils, carbuncles, impetigo and paronychia (Kinlay *et al.* ٢٠٠١). These results have been supported by Semba and Neville (١٩٩٩), who referred that *S. aureus* was the most common cause of breast abscess in both lactating and non-lactating women.

Furthermore, Moran and Mount (٢٠٠٣) concluded that *S. aureus* was the major cause of breast abscess in lactating and non-lactating ladies, and they reasoned that due to the prevalence of antibiotics-resistant strains and the recent emergence of clinical isolates resistant to vancomycin.

Newton and Newton (١٩٥٠) have mentioned that ٨٧٪ of breast abscess occur due to *S. aureus*. Marshall *et al.*, (١٩٧٥) have found that *S. aureus* constitutes ٤٨٪ of breast abscess. Additionally, Aabo *et al.* (١٩٩٠) have also shown that *S. aureus* is the offending pathogen in the vast majority of breast abscess, where it represents ٤٢.٥٪.

٤.٣.٢ *Moraxella catarrhalis*

This study have shown the detection of three isolates of *M. catarrhalis* which represent ٥.٧% of lactating patients, and two isolates which represent ١١.١% of non-lactating patients.

There are no previous studies on breast abscess being caused by these bacteria, but O'Riodan and Lee (٢٠٠٤) have mentioned that *M. catarrhalis* might lead sometimes to

suppurative infection in upper respiratory tract infection such as otitis media and sinusitis.

In this study, all isolates of *M. catarrhalis* were Gram negative, grow on nutrient agar, after incubation for ٢٤, hours producing small white colonies. After ٤٨ hours colonies are larger and more elevated. All isolates do not grow on media selective for pathogenic *Neisseriae*, but they grow on blood agar without hemolysis and also grow on DNase agar media.

The bacteria were catalase positive, oxidase positive, but additional tests were needed for further identification. Positive reaction for DNase production, reduces nitrate to nitrite; not producing acid from glucose, maltose, sucrose, lactose or fructose.

According to Flear (٢٠٠١) the identify of *M. catarrhalis* is best confirmed by positive reaction in at least three of these differentiating tests, since none of these is ١٠٠٪ sensitive or specific by itself.

This bacterium is a human respiratory pathogen that is currently the third leading cause of otitis media along with *S. pneumonia* and *H. influenzae* (Flear ٢٠٠١). It is present as a normal flora of the upper respiratory tract and its carriage rate in children is high (up to ٧٥٪); in contrast, the carriage rate in healthy adults is low (about ١٥٪) (Amyes *et al*., ٢٠٠٢).

M. catarrhalis also causes septicemia, meningitis, pericarditis (Hagr *et al.*, ١٩٩٨). This bacterium is now established as nosocomial pathogen. Because *M. catarrhalis* has long been considered a harmless commensal, relatively little is known about its pathogenic characteristics and virulence factors (Verduin *et al.*, ٢٠٠٢).

Flear (٢٠٠١) have suggested that the lipooligosaccharide of the bacterial outer membrane may be an important factor in the resistance of *M. catarrhalis* to the complement-mediated bactericidal effect of normal human serum, and its implicated as a potential virulence factor. Omar and Braun (٢٠٠٣) has mentioned that *M. catarrhalis* has unusual type ٤ pilli that are involved in the adherence of the bacteria to the epithelial cells.

٤.٣.٣ *Arcanobacterium haemolyticum*

In this study, all the isolates of *A. haemolyticum* were Gram-positive bacilli, non-spore forming. Gram-stain from cultures incubated for ٢٤ hours appears as club-shaped curved rode (Coryneform). As the culture ages, the bacilli are fragmented, giving the appearance of Gram positive cocci in chain. They have been found to be non-acid fast and non motile.

The organism grows at aerobic conditions. At ٢٤ hour's incubation on nutrient agar, the colonies are circular and white.

However, at ٤٨ hours, they exhibit a characteristic central black dot. Overnight aerobic culture on ٥٪ human blood agar has yielded pure growth of tiny, smooth, circular, and B- hemolytic colonies. On prolonged incubation for ٤٨-٧٢ hours, the colonies become bigger with widened area of haemolysis. Its biochemical tests are catalase negative; oxidase negative; not producing gas from glucose; they produce acid from glucose and lactose; it ferment maltose, galactose, and sucrose; but does not ferments mannitol; its urease is negative; gelatin liquefaction; and nitrate reduction negative. These results were agreed with that of Carlson (١٩٩٥) and MacFaddin (٢٠٠٠).

In this study, three isolates in non-lactation patients were detected, which constitute ٢٢.٢٪ of the patients; whereas it has not detected these bacteria in lactating patients. There are no previous studies dealing with breast abscess detecting these bacteria as causative agents of breast abscess.

A coryneform bacilli is likely to be encountered as a commensal of human skin or mucous membranes (Esteban *et al*. ١٩٩٤). *A. haemolyticum*, previously known as *Corynebacterium haemolyticum*, is a human pathogen most commonly implicated with nonstreptococcal pharyngitis. The organism has also been shown to cause cellulitis, wound infection, peritonsillar abscess, and sepsis. Less commonly, *A. haemolyticum* has been shown to cause brain abscess,

endocarditis, and osteomylitis (Wagner, ١٩٩١). Three extracellular toxins have been isolated from the filtrates of *A. haemolyticum*: hemolysin, neuramidase, and phospholipase D (Mann, ١٩٩٨).

٤.٣.٤ *Streptococcus pyogenes*

In this study, the bacteria are Gram positive, and occur mostly in chains. They produce large zones of β-hemolysis around colonies on blood agar. These colonies appear as white to gray and opaque colonies. Its biochemical tests were oxidase negative, catalase negative, growth on crystal violet agar, ferment (glucose and lactose), but not ferment (sucrose and mannitol). These bacteria were sensitive for bacitracin. This agrees with the typical characteristics of *S.pyogenes* (Holt *et al*., ١٩٩٤; Baron *et al*., ١٩٩٦; MacFaddin, ٢٠٠٠).

There have been two isolates detected in lactating patients (constitute ٣.٨%), and in non-lactating patients no isolates have been detected.

The isolation of these bacteria indicates its direct role in causing the breast abscess, and this is due to the fact that it is rarely present as normal flora on the skin, and on the nasopharynx; therefore, it most probably occurs as community acquired infection. Thomsen *et al*. (١٩٨٣) have referred that these bacteria are regarded as a causative agent of breast abscess

and constitute ٧.٣٪ of cases. Marshal *et al.* (١٩٧٥) have detected one isolate of these bacteria from patients of breast abscess (٢٪ of the cases).

٤.٣.٥ *Staphylococcus epidermidis*

In this study, all isolates of *S. epidermidis* produces small non-hemolytic white colonies in appearance. Its biochemical tests are catalase positive, oxidase negative, urease negative, coagulase negative, mannitol fermentation negative and motility negative. These results were agreed with those of MacFaddin (٢٠٠٠).

The study has shown only one isolate of *S. epidermidis* (١.٩٪) in lactating patients, with no isolate detected in non-lactating patients (Table ٤.١١). Although *S. epidermidis* has been isolated from the mammary gland infection, some study has revealed that it has no important role in causing this infection, because it is regarded as normal flora of the skin and it is non pathogenic bacteria (Matheson *et al.*, ١٩٨٨).

Another study has proved that *S. epidermidis* has an important role as a causative agent of mastitis, Thomsen (١٩٨٢), who referred that *S. epidermidis* might cause ١٧.٣٪ of mastitis cases. He attributed this result to the bacteria being present on the skin as normal flora, and under certain circumstances, it transformed into opportunistic pathogen, and this agrees with

Thomsen *et al*. (١٩٨٤), have mentioned that *S. epidermidis* cause ٢٥٪ of mastitis cases.

Table (٤.١٢): The diagnostic and biochemical tests of bacterial isolates

Test	*S. aureus*	*S. epidermidis*	*S. pyogens*	*A.haemolyticum*	*M. catarrhalis*
Oxidase	-	-	-	-	+
Catalase	+	+	-	-	+
Coagulase	+	-	-	-	-
Type of hemolysis	β	-	β	β	-
Growth on mannitol salt agar	+	+	/	/	/
Growth on crystal violet agar	/	/	+	/	/
Nitrate reduction	-	-	-	-	-
Gelatin breakdown	+	-	-	+	-
Motility	-	-	-	-	-
Oxidative and fermentation test	F	F	F	/	/
Ferment of: glucose	+	+	+	+	-
Lactose	+	+	+	+	-
Sucrose	+	+	-	+	-
Mannitol	+	-	-	-	-
Salicin	-	-	+	/	/
Sensitivity for bacitracin	/	/	+	/	/
Sensitivity for optachin	/	/	-	/	/
Urease	/	+	/	/	-
Growth on DNase	+	/	/	/	+

β: beta hemolysis.
F: ferment.
/: the test not done.

٤.٤ Detection of Fatty Acid Modifying Enzyme, Lipase and Slim in *Staphylococcus aureus*

Staphylococcus aureus can survive on the skin and occasionally cause suppurative localized lesions that result in abscess formation (Schaechter *et al*., ١٩٨٩). Studies using a murine intraperitoneal abscess model have shown that one way the host controls the growth and survival of *S. aureus* within abscesses is by the production of bactericidal fatty acids and monoglycerides (Dye and Kapral, ١٩٨١).

Fatty acid modifying enzyme (FAME) is an extracellular enzyme that inactivates bactericidal fatty acids by esterifying them to cholesterol. Inactivation of these fatty acids may allow *S. aureus* to live for long periods of time (Chamberlain, ١٩٩٩). Previous studies have shown a positive correlation between the ability of a strain to produce FAME and its ability to cause invasive disease (Mortensen, *et al*., ١٩٩٢).

This study describes the identification of FAME production, and also examines any association between the production of FAME and that of lipase and slime.

Forty four isolates have been assayed for the production of FAME, lipase and slime: ٣٦ isolates (٨١.٨٪) have been positive for FAME, ٣٨ isolates (٨٦.٤٪) positive for lipase activity, and ١٨ isolates (٤٠.٩%) have produced slime. All the slime-positive isolates have been lipase and FAME-positive. All the FAME-positive have had lipase-positive, whereas ٤.٥٪ of the lipase-positive isolates have had FAME-negative; therefore, there is no association between FAME activity and that of lipase activity. All slime-producing isolates have also produced lipase and FAME; therefore, there is an association between the lipase, FAME activity and the slime production.

Table (٤.١٣): Production of fatty acid modifying enzyme (FAME), lipase and slime among ٤٤ isolates of *S. aureus*

Virulence factor	No. of *S. aureus* isolates	%
Lipase	٣٨	٨٦.٤
FAME	٣٦	٨١.٨
Slime	١٨	٤٠.٩

A previous study has used larger sample (٥١) and demonstrated that ٨٨.٢٪ of *S. aureus* have produced FAME. Furthermore, ٩٢.٢٪ of isolates investigated in that study have produced lipase enzyme, and ١٣.٢٪ produced slime (Long *et al*., ١٩٩٢). It has also been shown that there is no correlation between lipase activity and FAME activity, but there is a

correlation between FAME and lipase activity and the slime production. No correlation indicates that although the enzymatic function of the two proteins is similar, they are probably not expressed by the same genetic locus (Farrell *et al*., ١٩٩٣).

Another study done by Long *et al*. (١٩٩٢) has mentioned that ٨٠٪ of *S. aureus* have produced both enzymes (lipase and FAME). They refer that there is a strong correlation between FAME and lipase production. Moreover, Votava and Woznicova (٢٠٠٠) have shown that ٥٦.٢% of *S. aureus* are able to produce slime. The temperature optimum for FAME has been ٣٧^{o}C and the pH optimum ٦. The optimum temperature and optimum pH for FAME activity have been in keeping with the environment of the skin (Chamberlain, ١٩٩٩). This optimum temperature and pH may be useful to the bacterium, which when causing infection, is at or near ٣٧^{o}C (Mortensen *et al*., ١٩٩٢). *S. aureus* FAME activity has been inactivated by boiling and decreased by extended incubation at room temperature (Chamberlain and Brueggemann, ١٩٩٧).

٤.٥ Production of FAME during the Growth Cycle

Detectable levels of FAME activity have not been obtained until ٣ hours of growth (Figure١). Activity has then increased steadily, peaking at the commencement of the stationary phase (٥-٦ hours), and the activity has then decreased

steadily during the stationary phase (٦-١٢ hours). This study shows that FAME production is not detectable in culture filtrates until the early logarithmic phase. Determination of when FAME is produced during the growth of *S. aureus* has revealed some interesting findings. These results are very similar to those obtained by Chamberlain and Brueggmann (١٩٩٧) and Chamberlain and Imanoel (١٩٩٦). These finding are also very similar to those observed for lipase produced by *S. aureus* (Farrell *et al*., ١٩٩٣). They have found that lipase is not detectable until early stationary phase and that there is a peak activity around ٦ hours of growth, followed by decreasing lipase activity.

Other studies on *S. aureus* have demonstrated that several extracellular virulence factors (e.g., toxic shock syndrome toxin, α-toxin, lipase, and β-toxin) are not produced until early stationary phase (Cheung and Ying, ١٩٩٤). This ability to delay production of extracellular factors may be beneficial to *S. aureus.* When organisms are few in numbers, few nutrients are needed. However, as organism numbers increase, other and more diverse sources of nutrients are needed. Lipase can break down triglycerides from sebaceous gland secretions to glycerol and fatty acids (Chamberlain, ١٩٩٩).

The bacteria could utilize the fatty acids obtained from the breakdown of triglycerides. Unfortunately, if the fatty acid

concentration gets too high, it can kill the *Staphylococci*. The ability of FAME to inactivate some of these fatty acids by the esterification of the fatty acids to cholesterol may help to lower fatty acid concentrations and possibly protect the organisms from killing themselves in search of other sources of nutrients (Chamberlain and Imanoel, ١٩٩٦).

Delaying expression of lipase and FAME until later in the growth cycle may conserve energy, and the coordinated expression of these two factors may help the organisms survive for longer periods of time (Chamberlain, ١٩٩٩).

Smeltzer *et al.* (١٩٩٣) have also said that the production of exo-and surface proteins is coordinately regulated in a growth phase dependant manner, occurring preferentially in the post-exponential and long phase of growth.

A previous study has shown that a marked decrease in FAME activity observed late in the stationary phase is probably caused by extracellular protease. This is probably due to the fact that extracellular proteases are more abundant at these times in the growth cycle destroying some of the FAME activity (Chamberlain and Brueggemann, ١٩٩٧).

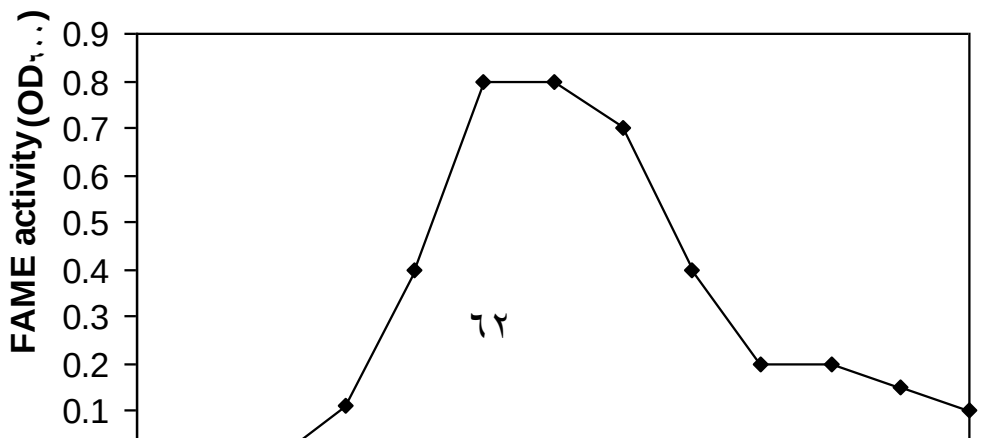

Figure (١): Fatty acid modifying enzyme activity during growth cycle

٤.٦ Effect of Antibiotics on Bacterial Isolates

The result of antibiotic sensitivity test that was done on the bacteria isolated from patients with breast abscess showed that some of these bacteria had variable degrees of resistance to some of the antibiotics used, as shown in table (٤.١٤).

As for vancomycin, ١٠٠٪ of *S. aureus, S. epidermidis*, and *S. pyogenes* were sensitive. These results correlated with those obtained by Coleman *et al.* (١٩٨٥), Seifent *et al.* (١٩٩٣) and Garrett *et al.* (١٩٩٩) who observed that these bacteria were highly sensitive to vancomycin. In addition, ٧٥٪ of *A. haemolyticum* and ٤٠٪ of *M. catarrhalis* were sensitive to vancomycin which agreed with the results obtained by Asiffa (٢٠٠٢) and Biswas *et al.* (٢٠٠٣) who found that ٨٠٪ of *A. haemolyticum* and ٢٥٪ of *M. catarrhalis* were sensitive to vancomycin.

Regarding the ampicillin, all four isolates of *A. haemolyticum*, two isolates of *S. pyogenes* and one isolate of *S. epidermidis* were resistant. These results agreed with those obtained by Deguchi *et al*. (١٩٩٠), Carlson *et al*. (١٩٩٩) and Al-Jebori (٢٠٠٣), who found that these bacteria were highly resistant to ampicillin. In addition, ٢٠٪ of *M. catarrhalis* and ١١.٤% of *S. aureus* were sensitive to ampicillin which agreed with the results obtained by Asiffa (٢٠٠٢) and Al-Jebori (٢٠٠٣), who found that ٢٠٪ of *M. catarrhalis* and ١٤.٣٪ of *S. aureus* were resistant to ampcillin. Neu (١٩٨٥) found that these bacteria were resistant to ampicillin and other penicillin drugs due to the production of β-lactamase enzyme. Furthermore, Tally and Bary (١٩٩٨) mentioned that the high resistance of *S. aureus* to penicillin and cephalosporin groups was due to alternative in penicillin binding protein (PBP).

With regard to amoxicillin, ٦٠٪ of *M. catarrhalis*, ٥٠٪ of *S. pyogenes*, ٥٠٪ of *A. haemolyticum*, and ٤٥.٥٪ of *S. aureus* were sensitive. In addition, one isolate of *S. epidermidis* was sensitive to amoxicillin. These results agreed with those obtained by Neu (١٩٨٥), Deguchi *et al*. (١٩٩٠), Asiffa (٢٠٠٢) and Biswas *et al.* (٢٠٠٣) who stated that these bacteria were highly resistant to amoxycillin due to the production of β-lactamase enzyme. Tally and Bary (١٩٩٨), however, mentioned that the high resistance of these bacteria to penicillin group was due to alternative in penicillin binding protein (PBP).

Eighty percent of *M. catarrhalis*, ٧٥٪ of *A. haemolyticum* and ٧٢.٧% of *S. aureus* were sensitive to amoxiclav; besides ١٠٠% of *S.pyogenes* and *S. epidermidis*. These results correlated with those obtained by Babay (٢٠٠٠), Corranglia and Fontana (٢٠٠٠), and Asiffa (٢٠٠٢), who showed that these bacteria were much more sensitive to amoxiclav than to amoxycillin and other penicillin drugs, due to the action of the calvulenic acid preventing the activity of β-lactamase enzyme.

It was also found that ١٠٠٪ of *M. catarrhalis* and *S. epedermidis* were sensitive to gentamicin. This result agreed with that obtained by Garcia *et al*. (١٩٩٥) and Al-Jebori (٢٠٠٣), who found that ١٠٠٪ of *M. catarrhalis* and *S. epedermidis* were sensitive to gentamicin. In addition, ٨٤.١% of *S. aureus* and ٧٥٪ of *A. heamolyticum* and one out of two isolates (٥٠٪) of *S. pyogenes* were sensitive to gentamicin, which correlated with results obtained by Al-Jebori (٢٠٠٣) and Biswas *et al*. (٢٠٠٣), who showed that ٨١.٦٪ of *S. aureus*, ٩٠٪ of *A. heamolyticum* and ٨٠٪ of *S. pyogenes* were sensitive to gentamicin.

Besides, ١٠٠% of *M. catarrhalis, S. pyogenes* and *S. epidermidis* were sensitive to lincomycin. This result was similar to those obtained by Seifert *et al*. (١٩٩٣), Tally and Bary (١٩٩٨), and Asiffa (٢٠٠٢), who found that most of these bacteria were sensitive to lincomycin. In addition, ٧٥٪ of *A. haemolyticum* was sensitive to lincomycin and it agreed with the results of Biswas *et al.* (٢٠٠٣), who showed that *A.*

haemolyticum had high sensitivity to lincomycin. Furthermore, in this study showed that ٤٥.٥% of *S. aureus* was sensitive to lincomycin and it agreed with Neu (١٩٨٥), who showed that most of *S. aureus* was resistant to lincomicin.

As for erythromycin, ١٠٠٪ of *M. catarrhalis*, *A. haemolyticum* and *S. epidermidis* were sensitive. This result was correlated with those obtained by Corranglia and Fontana (٢٠٠٠), Brook *et al.* (٢٠٠٢), and Al-Jebori (٢٠٠٣), who found that most of these bacteria were sensitive to erythromycin. In addition, ٧٠.٥% of *S. aureus* were sensitive to this antibiotic, and that did not agree with Al-Jebori (٢٠٠٣), who found that only ٥٧.١٪ of *S. aureus* were resistant erythromycin. Furthermore, in this study, showed that ٥٠٪ of *S. pyogenes* was sensitive to erythromycin and this was in accordance with that obtained by Al-Jebori (٢٠٠٣), who showed that ٦٠٪ of *S. pyogenes* was sensitive to erythromycin.

One hundred percent of *S. pyogenes* and *A. haemolyticum* were resistant to cloxacillin, ١٠٠٪ of *S. epidermidis*, ٣١.٨٪ of *S. aureus* and ٢٠٪ of *M. catarrhalis*, were sensitive to cloxacillin. These results agreed with those obtained by Al-Jebori (٢٠٠٣), Brook *et al.* (٢٠٠٠), and Asiffa (٢٠٠٢), who found that most of these bacteria were resistant to this antibiotic.

The study showed that ١٠٠٪ of *M. catarrhalis*, *S. pyogenes* and *S. epidermidis* were sensitive to ciprofloxacin. These results agreed with those obtained by Dipersio *et al.*

(١٩٩٨), Asiffa (٢٠٠٢), and Al-Jebori (٢٠٠٣). In addition, ٨١.٨٪ of *S. aureus* and ٧٥٪ of *A. haemolyticum* were sensitive to ciprofloxacin and it agreed with those obtained by Kuriyama *et al*. (٢٠٠٢), and Madhusudhan *et al*. (٢٠٠٣), who indicated that most of these bacteria were sensitive to this antibiotic. Neu (١٩٨٥) found that *S. aureus* was highly sensitive to ciprofloxacin even those resistant to methecillin (MRSA). These results did not agree with those obtained by George *et al*. (١٩٩٠) who found that *S. aureus* was highly resistant to ciprofloxacin.

With regard to cefotaxime, ١٠٠٪ of *S. pyogenes* and *S. epidermidis* were sensitive. This result agreed with those obtained by Thomson *et al*. (١٩٩١), Leblebicioglu and Esen (٢٠٠٣), who stated that these bacteria were susceptible to cefotaxime. In addition, ٨١.٨٪ of *S. aureus* were sensitive to cefotaxime and these results agreed with those obtained by Deguchi *et al*., (١٩٩٠), Sedlock *et al*. (١٩٩٠) and Iregbu *et al*. (٢٠٠٢). Furthermore, ٦٠٪ of *M. catarrhalis* and ٥٠٪ of *A. haemolyticum* were sensitive to cefotaxime which agreed with those obtained by Asiffa (٢٠٠٢) and Aspiroz-Sancho *et al*., (٢٠٠٢).

In respect of rifampicin, ١٠٠٪ of *S. pyogenes*, *S.epidermidis* and ٨٦.٤% of *S. aureus*, ٧٥٪ of *A. haemolyticum*, and ٦٠٪ of *M. catarrhalis* were sensitive. This result was in accordance with those obtained by Garcia *et al*. (١٩٩٥), Ibrahim (٢٠٠٢), Al-Jebori (٢٠٠٣) and Biswas *et al*. (٢٠٠٢), who showed that ١٠٠٪ of *S. Pyogenes* and *S. epidermidis*, ٨٨٪ of *S. aureus*,

٨٠٪ of *A. haemolyticum*, and ٦٥٪ of *M. catarrhalis* were sensitive to rifampicin.

CHAPTER FIVE

٥.١ Conclusions

١. The breast abscess mostly occurs in lactating women.

٢. Multipara women are more risky to get breast abscess.

٣. The high infection rate occurs in ٢٠-٢٩ years age group for the lactating patients, whereas in non-lactating patients, the infection rate is high in ٤٠-٤٩ years age group.

٤. In the lactating patients, the breast abscess mostly occurs in the first three months post partum.

٥. Breast abscess occurs in rural areas higher than it is in urban areas.

٦. The most common type of the breast abscess in lactating patients is subcutaneous abscess, whereas in non-lactating patients is subareolar abscess.

٧. Most of the cases of the breast abscess are due to monobacterial affection, whereas in the other cases the causative agent is not detected.

٨. *S. aureus* is the most common causative agent in both lactating and non-lactating patients.

٩. FAME is detected in ٨١.٨٪ of *S. aureus* isolates and it is regarded as an important virulence factor related to abscess formation.

١٠. Ciprofloxacin is the most effective antibiotic against most bacterial isolates, whereas the ampicillin and cloxacillin are the least effective antibiotics.

٥.٢ Recommendations

١. From the results of this work, it may recommend that further studies be conducted on breast abscess taking large samples and study both aerobic and anaerobic pathogenic bacteria associated with breast abscess

٢. Because there are more than one species of pathogenic bacteria that cause breast abscess, doctors are recommended to use antibiotics according to the results of culture and sensitivity.

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