A Bacteriological and Clinical Study of Gallbladder Stones and Biles After Cholecystectomy

A Thesis

Submitted to the Department of Microbiology,College of Medicine, University of Babylon, in Partial fulfillment of the Requirements for the Degree of Master in Medical Microbiology

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَقُل رَّبِّ أَدْخِلْنِي مُدْخَلَ صِدْقٍ وَأَخْرِجْنِي مُخْرَجَ صِدْقٍ وَاجْعَل لِّي مِن لَّدُنكَ سُلْطَانًا نَّصِيرًا.

(سورة الاسراء /٨٠)

صدق الله العظيم

Dedication

To my father and my mother, to all members of my family, to my husband, and to my son Ali.

Acknowledgement

I would like to express my thanks and indebtedness to my supervisor Dr. Muhammad Sabri Abdul-Razak for his continuous guidance, encouragement, advice, and extensive help throughout the whole working period.

My deep gratitude is to Dr. Ali Khair-Allah for his efforts in collecting gallbladder bile and stones samples in whole performing cholecystectomies and to his wisdom and suggestions in critical times.

Also, I wish to thank Dr.Mohammed A.K. Al-Saadi for his invaluable help in my work. Additionally, I wish to thank Dr. Zainab A. H. Al-Khafaji and Dr. Majid M. Al-khafaji for their cooperation in sample collection. Moreover my thanks go to the operation medical staff in Hilla Teaching Surgical Hospital especially Muhammad Hashim for helping me in collecting all the samples.

My appreciation is also extended to the staff of the microbiology department in Hilla Surgical Hospital for helping me in the laboratory work.

Farah

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List of Abbreviations

Abbreviation	Meaning
CT	Computerised Tomography
MRI	Magnetic Resonance Imaging
ERCP	Endoscopic Retrograde Cholangiopancreatography
EUS	Endoscopic Ultrasonography
HDL	High Density Lipoproteins
MR	Methyl Red Test
VP	Vogas-Proskaur Test
KIA	Kliglar Iron Agar
H_2S	Hydrogen sulphide
L F	Lactose Fermentation
EMB	Eosin Methylene Blue
CFU	Colony Forming Unit
H_2O_2	Hydrogen Peroxide

Abstract

The subjects of this study were ٨٠ patients with symptomatic gallstone disease who had undergone elective cholecystectomy.They were admitted to Hilla Teaching Hospital-department of Surgery during the period from October/ ٢٠٠٤ to June / ٢٠٠٥. Their ages ranged between (١٨-٨٧) years; (٨٠٪) of them were females and (٢٠٪) were males.

The most affected age group in females was between ٤٠-٤٩ years (٣٥.٩٪) while in males it was between ٥٠-٥٩ years (٤٣.٧٥٪). Gallstone disease was rare under ١٠ years, and increased significantly with age, thereafter declined gradually, the patients over ٦٠ were less affected.

Clinically, chronic cholecystitis was the commonest presentation (٥٢.٥٪) followed by acute exacerbation of chronic cholecystitis (٣١.٢٥٪), and then acute cholecystitis (١٦.٢٥٪).
Patients with symptomatic gallstone disease were presented with upper abdominal pain (٩٨.٧٥٪), the pain while most frequent in the right hypochndrial area (٨١.٢٥٪) was sometimes noted in the epigastrium (١٧.٥٪), the pain was associated with or without fever (٤٠٪), jaundice (٢٣.٧٥٪), nausea (٦٥٪),vomiting (٦١.٢٥%), abdominal distention (١.٢٥٪).

Those patients were analyzed for bile and gallstone

bacterial aerobic culture and the percentages of cholesterol and bilirubin in gallstones were measured to determine the relative frequency of occurance of cholesterol and pigment stones.

٣٤ patients (٤٢.٥٪) had either bile , gallstone, or both, positive bacterial cultures. The most common organism culture of bile was *E.coli* (٣٠٪) followed by *Klebsiella pneumoniae* (٢٠%), *Enterococcus fecalis* (١٥٪), *Enterobacter aerogenes* (١٥٪), *Acinetobacter* (١٠٪), and one isolate of *Staphylococcus aureus* (٥٪) and one Viridans streptococci (٥٪).

The commonest organisms in gallstone cultures was *Enterobacter aerogenes* (٣٥.٧٪), followed by *Klebsiella pneumoniae* (٢٨.٦%), *E.coli* (٢١.٤٪), and *Enterococcus faecalis* (١٤.٣%).

The commonest type of gallstone was cholesterol stones (٤٠٪), followed by pigment stones (٣٥٪), and mixed stones (٢٥٪).

Besides, the most mixed and pigment types of gallstones were culture positive (٤٠٪, ٢١.٤٪ respectively), whereas none of pure cholesterol stones were culture positive.

As Regards the effect of deoxycholate on the growth of bacterial isolates, *Acinetobacter* was more resistant to this reagent followed by *E.coli* , *Klebsiella pneumoniae* , *Enteobacter* , *Enterococcus faecalis* , Viridans streptococci , and *Staphylococcus aureus.*

As for the effect of cholesterol on the growth of bacterial isolates (gram negative and gram positive), no growth was detected on the plate cultivated with gram negative when treated with cholesterol (٢٥٠ mg/dl), whereas no effect was noted on gram positive bacteria .

In vitro, the effect of some antibiotics on bacterial isolates showed that ٧٠.٦٪ were resistant to ampicillin, ٦٤.٧٪ were resistant to doxycycline, ٤٤.١٪ to cephalothin, ٤٤٪ to aztreonam, ٢٩.٢٪ to ciprofloxacin and trimethoprim, ٢٠.٨٪ to gentamycin, and ١٧.٦٪ to cefotaxime.

الخلاصة

تضمنت هذه الدراسة ٨٠ مريضا كانوا يعانون من الام حصاة المرارة والذين اجريت لهم عملية استئصال المرارة في مستشفى الحلة التعليمي – قسم الجراحة للفترة من تشرين الثاني ٢٠٠٤ الى حزيران ٢٠٠٥ والذين تتراوح اعمارهم بين ١٨-٨٧ سنة حيث ان ٨٠% منهم اناث و ٢٠% منهم ذكور.

ان الفئة العمرية الاكثر عرضة للاصابة بين الاناث كانت بين ٤٠-٤٩ سنة (٣٥,٩%) والذكور بين ٥٠-٥٩ سنة (٤٣.٧٥%) .

ان امراض حصاة المرارة تكاد تكون نادرة تحت عمر ١٠ سنوات وتزداد مع تقدم العمر وتنخفض تدريجيا بعد عمر ٦٠ سنة .

ومن الناحية السريرية ان التهاب المرارة المزمن هو الاكثر شيوعا (٥٢.٥%) يأتي بعده الاعراض الحادة لالتهاب المرارة المزمن (٣١,٢٥%) ثم التهاب المرارة الحاد(١٦,٢٥%) .

ان المرضى المصابين بالتهاب حصاة المرارة يعانون من الام الجهة العليا من البطن (٧٥ ,٩٨ %) وغالبا مايكون في الجهة العليا اليمنى تحت الاضلاع (٢٥ ,٨١%) وفي بعض الاحيان يكون الالم في منطقة المعدة (١٧,٥ %) , حيث يكون الالم مصحوبا او غير مصحوبا بأرتفاع درجة الحرارة (٤٠%), اليرقان (٢٣,٧٥%), الرغبة في التقئ (٦٥%), التقئ (٦١,٢٥%) وانتفاخ البطن (١,٢٥%).

تم اخذ عينات للمرضى من حصى المرارة ومادة الصفراء للزرع البكتيري وتم قياس نسبة مادة البلروبين ومادة الكوليسترول كيميائيا وذلك لتحديد نوع حصاة المرارة ونسبة حدوثها .

كانت نتائج الزرع البكتيري لـ (٣٤) مريضا موجبة سواء كانت لحصى المرارة او مادة الصفراء او كليهما.وكانت انواع البكتريا الظاهرة في زرع المادة

الصفراء كالأتي : (٣٠%) *E.coli* , (٢٠%) *Klebsiella pneumoniae* , (١٥%) *Enterobacter aerogenes* , (١٥%) *Enterococcus faecalis* , (١٠%) *Acinetobacter* , وعزلة واحدة (٥%) *Staph. aureus* و (٥%) Viridans streptococci .

اما انواع البكتريا الظاهرة في زرع حصى المرارة كانت كالأتي (٣٥,٧%) *Enterobacter aerogenes* , (٢٨,٥%) *Klebsiella pneumonia* , (٢١,٤%) *E.coli* , (١٩,٢%) *Enterococcus faecalis* .

ان اكثر انواع الحصى التي عزلت كانت الحصى الكوليسترولية(٤٠%) (cholesterol stone), ثم الحصى الصبغية (٣٥%) (Pigment stone) ثم الحصى الكوليسترولية المختلطة (٢٥%) (mixed stone) .حيث كانت نتيجة الزرع موجبة للحصى الصباغية (٢١,٤%) والحصى الكوليسترولية المختلطة (٤٠%) بينما كانت نتائج الزرع سالبة للحصى الكوليسترولية.

تمت دراسة تأثير مادة ديؤكسيكوليت(Deoxycholate) على نمو البكتريا. حيث كانت *Acinetobacter* اكثر مقاومة لهذه المادة ويأتي بعدها *E.coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis , Enterobacter*, Viridans streptococci , و *Staph.aureus*.

وشمل هذا البحث دراسة تأثير مادة الكوليسترول بتركيز(٢٥٠ ملغم\١٠٠ مل) على نمو كل من البكتريا السالبة الصبغة والبكتريا الموجبة الصبغة حيث كانت البكتريا السالبة الصبغة حساسة للكوليسترول بينما البكتريا الموجبة الصبغة مقاومة له.

مختبريا تمت دراسة تأثير بعض المضادات الحيوية على العزلات البكتيرية حيث كانت (٧٠,٦%) مقاومة للامبسلين , (٦٤,٧%) مقاومة للدوكسيسايكلين , (٤٤,١%) مقاومة للسيفالوثين , (٤٤%) مقاومة للازتريونام , (٢٩,٢%) مقاومة للسبروفلوكساسين و المثبريم , (٢٠,٨%) مقاومة للجنتامايسين , (١٧,٦%) مقاومة للسيفوتاكسيم .

جامعة بابل

كلية الطب

دراسة بكتيرية وسريرية لحصاة المرارة و مادة الصفراء بعد اجراء عملية استئصال المرارة

اطروحة

مقدمة الى فرع الاحياء المجهرية-كلية الطب- جامعة بابل كجزء من متطلبات نيل درجة الماجستير في الاحياء المجهرية الطبية

من قبل الطالبة

فرح عبد الحسين كاظم الخفاجي

بكالوريوس في الطب و الجراحة العامة

٢٠٠٦ م ١٤٢٦ هـ

١.١. INTRODUCTION

Although disorders of the biliary tract do not garner the level of attention given to other conditions,theyare extremely common over ٩٥٪ of biliary tract diseases are directly attributable to cholelithiasis (the presence of gallstones within the gallbladder) or the closely related cholecystitis (gallbladder inflammation)(Kumar *et.al.*,٢٠٠٣).

Cholelithiasis is a major health problem in many countries of the world, more so in developed countries (Everhart *et.al.*, ١٩٩٩) .

Gallstones are more common in adults than in children or elderly persons ; in females than in males, and the cause for these age and sex related variations are now wellknown (Hulley *et.al.*,١٩٩٨) .

Gallstones are hard pieces of stone like material, round, oval faceted commonly occurring in the gallbladder or the bile duct.Most gallstones are about the size of a pea ,but in some cases there can be many of very small stones , like fine gravel, or a single stone so large that it nearly fills the gallbladder . They are more common in women than in men and about ١ in ١٠ older adults have gallstones. Most gallstones are made up of cholesterol, calcium carbonate, calcium bilirubinate, or a mixture of these, they are more likely to occur if the composition of bile is abnormal, if the outlet from the gallbladder is blocked or has a local infection, or if there is a family history of gallstones (Beckingham, ٢٠٠١).

The clinical presentation of gallstones diseases is diverse , and treatment options are varied . Gallstones are classified into three types according to their chemical composition (Carey,١٩٩٣)

١- Cholesterol stones.

٢-pigment stones.

٣-mixed stones .

Bacteria commonly isolated from bile in all disease states of the biliary tract were primarily gram negative enteric bacteria with *E.coli* alone accounting for more than ٥٠٪ of cases; others, such as *Klebsiella*, *Enterobactor* and *Proteus* were less common . *Enterococcus faecalis* was also common , anaerobes such as *Clostridium perfringens* were rare organisms from bile culture . No bacteria were found in healthy individuals , but in patients with gallstones there was increasing percentage of positive cultures corresponding to the severity of the disease and age , with a step rise in individuals older than ٦٠ (Csendes *et.al.*, ١٩٩٦a) .

Antibiotics should be used prophylactically in all patients undergoing elective biliary tract surgery. The risk of postoperative infectious complications corresponds to the presence of bactibilia, which occurs in ١١ to ٣٠% of patients with gallstones , but is difficult to determine before surgery in patient undergoing laproscopic cholecystectomy for chronic cholecystitis , the risks associated with single dose of the first generation cephalosporin are minimal , this agent provides good

coverage against the gram negative aerobes commonly isolated from bile and skin flora (Townsend *et.al.*, ٢٠٠١).

Therapeutic antibiotics are used in patients with acute cholecystitis and acute cholangitis. In both diseases, gram negative aerobes play a major role and are well covered by the second or third generation cephalosporins, aminoglycosides, uriedopenicillins, and flouroquinolone. Uriedopenecillins, such as pipercillin, offer the advantage of gram positive coverage . Most fluoroquinolone such as ciprofloxacin do not cover the anaerobes and should be used in combination with an agent that provides anaerobic coverage example metronidazole (Lipsett and Pitt,١٩٩٠).

The aims of this study are as follows:

١-Primarily,to identify the commonest types of gallstones and to determine the occurance of gallbladder stones in patients admitted to Al- Hilla Teaching Hospital which has not been performed previously.

٢-To determine the percentage of cholesterol and bilirubin in each type of gallstone.

٣-To determine the most common types of bacteria associated with gallstones and gallbladder bile with study of antibiotics sensitivity for isolated organisms.

٤-To study the effect of deoxycholate bile salts and cholesterol on the growth of isolated bacteria .

٥-Gallstones diseases can now be managed effectively through surgical treatment and probably with medications but preventive interventions still remain the challenge with our priorities for research in proper order we can move hopefully towards the preventions of gallstones and a better future for our patients .

١.٢. LITERATURE REVIEW

١.٢.١. Anatomy and Physiology of the Gallbladder

١.٢.١.١.Anatomy of the Gallbladder

Gallbladder is a pear – shaped reservior of bile situated in the gallbladder fossa on the inferior surface of the right lobe of the liver (Chaurasia ,١٩٩٥). Gallbladder is divided into the fundus , body and neck . The fundus is rounded and usually projected below the inferior margin of the liver .The body lies in contact with the visceral surface of the liver.The neck becomes continuous with the cystic duct , which turns into the lesser omentum to join the right side of the common hepatic duct (Snell,١٩٩٣).

١.٢.١.٢.Physiology of the Gallbladder

The main function of gallbladder is to concentrate and store hepatic bile during the fasting state and to deliver bile into the doudenum in response to a meal. The usual capacity of the human gallbladder is only about ٤٠ to ٥٠ ml (Townsend *et.al.,* ٢٠٠١).

The gallbladder epithelial cells secrete at least two important products into the gallbladder lumen : glycoproteins and hydrogen ions. Secretion of mucus glycoproteins occurs primarily from the glands of the gallbladder neck and the cystic duct. The resultant mucus

barrier may be very important in protecting the gallbladder epithelium from the strong detergent effect of the highly concentrated bile salts found in the gallbladder. The mucin glycoproteins play a role as a pronucleating agent for cholestrol crystalisation. The transport of H^+ ions by the gallbladder epithelium leads to decrease in the gallbladder bile pH through a sodium exchange mechanism. Acidification of bile promotes calcium solubility by preventing precipitations as calcium salts (Klein *et.al.*,١٩٩٦) .

١.٢.٢.Classification and Nomenclature of the Gallstones

An optimal classification system for gallstone is needed because the etiology , pathogenesis , clinical features and treatment can differ according to the classes (Kim *et.al.*, ٢٠٠٣) .

١.٢.٢.١.Classification of Gallstones by Composition:

Most investigators have agreed to classify gallstones into two groups , cholesterol and pigment stones , on the basis of their major composition. Gallstones containing cholesterol as the main constituent are classified as cholesterol stones ,whereas those predominantly composed of bile pigments are regarded as pigment stones (Carey, ١٩٩٣). Cholesterol stones are defined as stones with cholesterol comprising more than ٦٠٪ of the stone dry weight (Mukihara, ١٩٨١). Comparatively, the cholesterol contents in pigment stone is less than

٢٥٪ to ٣٠٪ (Takagi and Toda , ١٩٩٣) .However , the problem is that there is an intermediate group of stones containing ٣٠٪ to ٦٠٪ cholesterol called as mixed stones (Donovan and Carey , ١٩٩١).

The principle consitituent of pigment stone is calcium bilirubinate which forms an average of ٤٠٪ to ٦٠٪ of dry weight (Trotman and Soloway, ١٩٨٢). There are two types of pigment stones; black and brown pigment stones.The composition of black pigment stone differs from that of brown pigment stone. Calcium bilirubinate is the major component of both stones but there are minor components other than calcium bilirubinate and cholesterol such as calcium carbonate and calcium phosphate are contained in black pigment stone but are rarely found in brown stones .Calcium fatty acids are found only in brown stone . Therefore, measuring these minor components in addition to calcium bilirubinate would be helpful for differentiating between brown and black pigment stones (Takagi and Toda, ١٩٩٣).

١.٢.٢.٢.Classification by Morphology :

The indices that can be used for the morphological classification of gallstones are the external appearance (color-shape)and the internal structure (cross sectional shape) (Freilich *et.al.*,١٩٨٦).According to this classification, cholesterol stones can

be divided into pure and mixed stones (Kim *et.al.*, ٢٠٠٣).

Pure cholesterol stones have a radial structure from the center to

the periphery on cross section (Kaufman *et.al.*,١٩٩٤). The external appearance is usually oval to round , and the color range from white to yellow .The typical case has ambullary shape (Sato,١٩٨٣).

The cut surface of a mixed stone is with a blend of a concentric and radial shape because the main components of the stone ,cholesterol and pigment , are mixed throughout the layers (Ohto ,١٩٩٠) . The surface of the mixed stone exhibits various shapes ranging from round to faceted.The color of the surface varies; yellow white , yellow brown , greenish brown , or black brown . Pigment stones are divided into two groups , brown stones and black stones. On cross section brown stones have a concentric layer, while black stones have amorphous appearance (Sato , ١٩٨٣). Black pigment stones almost always occur in multiples and are irregular in shape having shiny or dull black color with powdery or hard consistency. They are formed predominantly in the gallbladder in sterile bile and are a major stone type found in patients with chronic haemolysis and cirrhosis (Soloway *et.al*., ١٩٧٧). Brown stones are usually large and friable , soft with earthy brown to yellow orange color (Leuschner and Baumgartel, ١٩٨٤).

١.٢.٣. Pathogenesis of the Gallstones

The information about the pathogenesis of gallstones formation remains incomplete although there has been considerable progress on the mechanisms involved during the past two decades.

Undoubtelly the pathogenesis of cholesterol stones is different from that of black pigment stones and brown pigment stones formation differs from both (Cuschieni *et.al.*,٢٠٠١).

١.٢.٣.١. Pathogenesis of the Cholesterol Gallstones

The pathogenesis of cholesterol gallstones is clearly multi-factorial but essentially involves ٣ stages :

I- Cholesterol supersaturation :

Bile facilitates the intestinal absorption of lipids and fat soluble vitamins and represents the excretion of certain organic solids, such as bilirubin, bile salts, phospholipids and cholesterol. Bilirubin is the breakdown product of spent red blood cells and is conjugated with glucuronic acid before being excreted .

Phosphlipids are synthesized in the liver in conjunction with bile salts synthesis. The final major solute of bile is cholesterol (Townsend *et.al.*,٢٠٠١) .

Cholestrol is highly nonpolar and insoluble in water , and thus in bile. The key to maintaining cholesterol in solution is the formation of both micelles(a bile salts–phospholipids –cholestrol complex),and cholesterol–phospholipids vesicles. A relative excess in cholesterol secretion can surpass the ability of these carriers to maintain cholesterol in solution , resulting in cholesterol supersaturation (Klien *et.al.*,١٩٩٦) .

II- Crystal nucleation :

Nucleation referes to the process in which solid cholesterol monohydrate crystal form and conglomerate. As bile is concentrated in the gallbladder ,a net transfer of phospholipids and cholesterol from vesicles to micelles occurs . The phospholipids are transferred more efficiently than cholesterol, leading to cholesterol enrichment of the remaining vesicles. These cholesterol rich vesicles aggregate to form large liquid vesicles that then precipitate cholesterol monohydrate crystals (Townsend *et.al.*, ٢٠٠١). Accelerated nucleation of cholesterol in the gallbladder could result from either an excess of a pronucleating factor or deficiency of antinucleating factor .When there is disturbance in the critical balance between pronucleating and antinucleating factors in the bile , the result is cholesterol crystal formation. Antinucleating proteins tend to stabilize vesicles formation and retard crystal formation, whereas pronucleating proteins destabilization resides and accelerate crystal formation (Afdhad and LaMont, ١٩٩٤). Several pronucleating factors, including mucin glycoproteins,immunoglobulins and transferrin, accelerate the precipitation of cholesterol in bile (Townsend *et.al.*, ٢٠٠١).

III-Stone Growth :

Once nucleation occurs, crystal growth follows. The rate at which crystals grow depends on a number of factors such as surface area , the diffusion rate, the degree of supersaturation , temperature, and total lipid concentration (Small, ١٩٨٠) .

The cholesterol crystals are so small when they are first formed in bile, they would normally be emptied by the gallbladder when it contracts after meal, but these crystals must be retained in some patients. The rate of crystal growth is related to the degree of supersaturation ; the higher the saturation is, the faster the growth is. Crystal growth can occur either by net transfer of cholesterol from bile to the growing crystals or by intercrystal transfer of cholesterol from small high energy crystals to larger more thermodynamically stable crystals, so aggregation of crystals results in the formation of mature cholesterol gallstone (Afdhad and LaMont,١٩٩٤).

١.٢.٣.٢. Pathogenesis of the Pigment Stones

Most physicians and investigators consider black stones as a disorder primarily associated with chronic hemolysis , sickle cell anaemia, thalassaemia, hereditary spherocytosis and cardiac valve prosthesis (Trotman ,١٩٧٩). Cirrhotic patients were found to increase secretion of total bilirubin twice more than normal , propably due to decreased red cell life span in liver diseases ending in hemolysis (Schwesinger *et .al.*, ١٩٨٥). In ileal resection , there is alteration in bilirubin metabolism that leads to increase concentration of bilirubin and calcium in gallbladder bile with reduction in the percent of bile acids (Pitt *et.al.*,١٩٨٤) . Supersaturation of bile with calcium bilirubinate is inhibited by bile salts , when supersaturation occurs,

usually due to increased concentration of bilirubinate anions, nucleation may be initiated by binding of calcium bilirubinate to mucin glycoproteins in bile.

In earthy brown stones, which are formed mainly in the bile duct, bacterial infection and deconjugation of bile by bacterial β-glucouronidase have been implicated as aetiological factors in the formation of brown pigment stones (Lam and Chan , ١٩٨٥) . As proposed by Maki , (١٩٦٦) , bacterial β – glucouronidase produced by bacteria is an important enzyme which deconjugates bilirubin diglucuronide resulting in the release of free bilirubin and glucouronic acid , the former precipitates with calcium ion to form calcium bilirubinate.The calcium bilirubinate combined with calcium palmitate, these components are formed due to hydrolysis ,by enzymes in infecting bacteria, of conjugated bilirubin and lecithin respectively (Ostrow, ١٩٨٤).

١.٢.٤.Epidemiology of the Gallstones

Gallstones occur in all societies and races ,in young and old people of both sexes , and in all states of health .They are however, increasingly prevalent with age and three times more common in females (Heaton *et.al.*, ١٩٩١).Though gallstone disease is already a major problem in western society, its major growth will be seen in indigenous groups within those societies and in developing countries

as dietary practices changed toward western patterns (Grimaldi *et. al.*, ١٩٩٣). The type of diet seems to play a part in the incidence of gallstones . Increased intake of simple sugar and dietary energy was associated with increased risk of gallstones , more commonly cholesterol stones. Moderate alcohol consumption was associated with marked decrease in the risk of gallstones probably due to reduced cholesterol saturation of the bile (McMicheal and Watts , ١٩٨٤) .The true prevalence of gallstones in any given population is unknown since estimates are based on limited survey or on autopsy data which probably do not give an accurate representation of the population in any particular country (Bateson,١٩٨٦). In fact, gallstone disease needs an epidemiological research in order to achieve three basic scientific purposes :

i- Definition of the size of the problem (prevalence, incidence and mortality).

ii-The search for cases or risk factors .

iii-The demonstration of the feasibility of primary prevention through preventive trials (Menotti, ١٩٨٤). The results indicated that most gallstones in the United States are of cholesterol stones and are found mostly in the gallbladder, while in Hong Kong Chinese population, bile pigment stones predominate and are mostly located in bile duct . In Japan the proportion of patients with pigment stone approaches ٧٠ % in rural localities , but is decreasing in urban areas where cholesterol stones predominate (Soloway *et.al.*,١٩٧٧) .

١.٢.٥. Risk Factors of the Gallstones

The major risk factors of cholesterol and pigment stones are listed in Table (١-١) .

Table (١-١) Risk Factors of the Gallstones(Kumar *et.al.*,٢٠٠٣).

- Cholesterol Stones	
Unmodifiable (constitutional) risk factors include	**Modifiable (environmental) risk factors include**
-Demography:North Europe, North and South America. -Advancing age . -Female sex hormones: - Female gender. -Oral contraceptive. -Pregnancy	-Obesity. -Rapid weight loss . -Hypertriglyceridaemia . -Drugs lowering serum cholesterol . -Slow intestinal transit . -Gallbladder transit . -High calorie diet . -Low fiber diet . -Alcohol abstinence . -Smoking . -Sedentary behaviour .
- Pigment Stones	
-Demography :Asian more than Western, rural more than urban. -Chronic hemolytic syndrome . -Biliary infection . -Gastrointestinal disorders:Ileal disease (e.g.Crohn's disease), Ileal resection or bypass, Cystic fibrosis .	

١.٢.٥.١. Gender ,Parity and Oral Contraceptives

In all populations of the world, women are almost twice as likely as men to experience cholelithiasis. Gender is one of the most powerful influences on gallstones , they are more common in females during their fertile years as in males. This preponderance persists to a lesser extent into the post menopausal period , but the sex differences narrows with increasing age (Everhart *et.al.*,١٩٩٩).

The influence of the female sex hormones have been studied in normal female, during pregnancy,and in women using oral contraceptives (Barbara *et.al.*,١٩٨٧).

Pregnancy favour gallstone formation through the hormonal influence on bile composition (increased biliary cholesterol secretion , decreased and unbalance bile acid pool). Decreased gallbladder motility during the third trimester of pregnancy and altered function of gallbladder mucosa may favour nucleation and growth of stones . Most gallstones will disappear spontaneously within few weeks after delivery (Valdivieso *et.al.*,١٩٩٣). Several studies have confirmed an association between gallstones and use of exogenous oestrogen replacement, or oestrogen administration in men (Scragg *et.al.*,١٩٨٤).

١.٢.٥.٢.Age

Gallstones are generally uncommon in infants and children (Waldhausen and Benjamin ,١٩٩٩). All epidemiological studies showed that increasing age was associated with an increased

prevalence of gallstone. Gallstones are four to ten times more frequent in older than young subjects . Biliary cholesterol saturation increases with age, due to a decline in the activity of cholesterol ٧-α hydroxylase ,the rate limiting enzyme for bile acid synthesis (Bertolotti *et.al.*,١٩٨٩). In the elderly, bile acid synthesis is reduced, biliary cholesterol output is increased and cholesterol saturation of bile increase both in women and men (Einarrson *et.al.*, ١٩٨٥).

١.٢.٥.٣. Genetic Factors

Cholestrol gallstones prevalence varies widely ,from extremely low (< ٥٪) in Asian and African populations , to intermediate (١٠ – ٣٠ %) in European and Northern American populations, and to extremely high (٣٠-٧٠٪) in population of Native American (Acalovschi ,٢٠٠١).

١.٢.٥.٤. Other Risk Factors

I-Obesity:It raises the risk of cholesterol gallstones by increasing biliary secretion of cholesterol (Acalovschi, ٢٠٠١).

II- Diet :The consumption of refined carbohydrate food and lower content of dietary fiber increase risk of chlolesterol gallstone (Thornton *et.al.*,١٩٨٣).

III- Diabetes Mellitus :Gallstones in diabetes may be caused by obesity that often accompanies diabetes rather than diabetes itself (Haber and Heatan ,١٩٧٩).

IV- Rapid weight loss : Bile lithogenicity is further enhanced by increased excretion of cholesterol due to decrease in synthesis of bile acids (Gebhard *et.al.*,١٩٩٦).

V-Hypertriglyceridaemia: All patients with hypertriglyceridaemia have supersaturated gallbladder bile even if they are slim (Alberg , ١٩٧٩).

VI- Drugs lowering serum cholestrol concentration :All fibric acid derivatives increase biliary cholesterol saturation (Acalovschi, ٢٠٠١).

VII-Smoking:Through reduction in the plasma high density lipoprotein (HDL) concentration, a risk for gallstones (Acalovschi, ٢٠٠١).

VIII- Spinal cord injury: Lithogenic risk is due to gallbladder and intestinal hypomotility (Apstein and Dalecki-Chipperfield, ١٩٨٧).

١.٢.٦. Clinical Features

The symptomatology of gallstone disease is varied. Two third of gallstones are asymptomatic, and they seldom develop complications (Beckingham,٢٠٠١). Anumber of symptoms and complications can occur with gallstone disease.

١.٢.٦.١. Biliary Colic

Biliary colic is the most common symptoms of cholelithiasis. It results from transient obstruction of cystic duct by a stone . The pain is severe and steady localized to the epigastrum or upper quadrant and radiate to the back or the right scapula (Agrawal and Jonnalagadda, ٢٠٠٠). The pain persists from ١٥ minutes up to ٢٤ hours ,subsiding

spontaneously or with opoid analgesics. Nausea or vomiting often accompanies the pain ,which is visceral in origin and occurs as a result of distention of the gallbladder due to an obstruction or to the passage of a stone through the cystic duct (Beckingham ,٢٠٠١).

١.٢.٦.٢. Acute Cholecystitis

It is mostly caused by impaction of a stone in the cystic duct resulting in distention and inflammation of the gallbladder (Agrawal and Jonnalagadda,٢٠٠٠). Secondary bacterial infection with enteric organisms(most commonly *E.coli, Klebsiella* and *Streptoccocus faecalis*)occurs in about ٢٠ % of cases (Indar and Beckingham, ٢٠٠٢). Clinical features of acute cholecystitis may include features of local inflammation (e.g.right upper quadrant mass, tenderness) and systemic toxicity (e.g.fever ,leukocytosis). Most patients have had previous attacks of biliary colic. The pain typically lasts longer than ٣ hours and after ٣ hours , pain shifts from the epigastium to the right upper quadrant. This sequence includes visceral pain, pain from ductal impaction by stones, progressing to inflammation of the gallbladder with parietal pain (Ahmed *et.al.*,٢٠٠٠). Murphy's sign is a relatively specific physical finding for acute cholecystitis. Jaundice occurs in patients with gallstones when a stone migrates from gallbladder to the common bile duct (Beckingham ,٢٠٠١).

١.٢.٦.٣. Chronic Cholecystitis

The term "chronic choleystitis" should be restricted to gallbladder containing gallstones with varying degree of inflammation. Patients

complain of recurrent attacks of epigastric or right hypochondrial pain, radiating to the right side of the back and less commonly, to the shoulder (Cuscheini, *et.al.*, ٢٠٠١). The pain of biliary colic usually occurs after a greasy meal. The duration of pain is typically ١ to ٥ hours. Pain lasting beyond ٢٤ hours suggests that acute inflammation of gallbladder is present .Other symptoms ,such as nausea and vomiting ,often accompany each episode (٦٠ – ٧٠ %)of cases. Fever and jaundice occur much less frequently with simple biliary colic (Egbert ,١٩٩١).

١.٢.٦.٤. Choledocholithiasis

About ١٥ % of patients with gallstones have choledocholithiasis. Clinical features suspicious for biliary obstruction due to choledocholithiasis (common bile duct stones) include biliary colic, jaundice, lightening of the stools, and darkening of urine (Bilhartz and Horton, ١٩٩٨).

١.٢.٦.٥. Acute Cholangitis

When an obstructed common bile duct becomes contaminated with bacteria, usually from the duodenum, cholangitis may develop. Charcot's triad of symptoms in severe cholangitis include pain in right upper quadrant, jaundice and high swinging fever and chills (Beckingham ,٢٠٠١).

١.٢.٦.٦. Acute Pancreatitis

Acute pancreatitis occurs because the gallstones obstruct the pancreatic duct during its passage into the intestine (Afdhad and

LaMont, ١٩٩٤) . Gallstones are responsible for ٣٠ % to ٧٥ % of all cases of acute pancreatitis (Bilhartz and Horton, ١٩٩٨).

Other complications which occur less frequently include gallstone ileus and Mirizzi's syndrome.

١.٢.٧. Diagnosis

Awide array of laborotary and radiologic studies is used for evaluation of the gallstones located in the gallbladder and the common bile duct(Somnay *et.al.*,٢٠٠٥) .

١.٢.٧.١. Laborotary Tests

In uncomplicated biliary colic, there are usually no accompanying changes in the hematologic and biochemical tests. In acute cholecystitis, leukocytosis is usually observed. Serum aminotransferase , alkaline phosphatase , bilirubin and amylase levels may also be elevated . The most reliable indicator for gallstones as the cause of acute pancreatitis is an elevation of alanine aminotrasferase levels greater than ٢.٥ times above normal (Soetikno and Carr- Loocke ,١٩٩٨). Several blood tests become abnormal with choledocholithiasis, aspartate aminotransferase,alanine aminotrasferase enzymes and alkaline phosphatase are raised. Although elevated levels of aminotransferases occur in ٧٥ % of patients with cholecystitis, the elevation in cholecystitis are almost always smaller than six fold, so

an elevation greater than six fold suggests choledocholithiasis, the elevation levels of bilirubin in choledocholithiasis are higher than the levels in cholecystitis (Afdhad and LaMont, ١٩٩٤).

١.٢.٧.٢. Imaging Technique for Diagnosis

- Ultrasonography

Ultrasonography should be a routine examination for the confirmation or exclusion of gallstone disease. Ultrasonography provides more than ٩٥٪ sensitivity and specificity for the diagnosis of gallstone (Ahmed *et.al.*,٢٠٠٠).

Other techniques include (Somnay *et.al.*,٢٠٠٥):

-Cholescintigraphy

-Oral cholecystography

-Computed Tomography and Magnetic Resonance Imaging

-Endoscopic Retrograde Cholangiopancreatography (ERCP)

-Endoscopic Ultrasonography (EUS)

١.٢.٨. Treatment

Biliary symptoms can be treated medically in the first instance. Biliary colic will respond to parenteral analgesia, where patients have acute cholecystitis, broad spectrum antibiotics are conventionally given in addition to appropriate analgesia (Bateson,١٩٩٩).

Although the inflammation is initially chemical, most surgeons will choose to use systemic antibiotic because of the risk of progression to

an empyaema and septic complications . Also if surgery is preformed, antibiotic prophylaxis will reduce the wound infection rate although this has been questioned in patients undergoing laproscopic cholecystectomy.

If a patient with gallbladder stones refuses surgery or is unsuitable for a general anaesthetic, alternatives may need to be considered. Bile acid therapy is a safe option but is suitable for only a few patients (Neligan *et.al.*, ١٩٨٣), and is achieved only with radiolucent stones of ٥ mm or less (Hood *et.al.*,١٩٩٣). The best bile acid therapy is ursodeoxycholic acid, chenodeoxycholic acid or combination of both. Bile acid therapy can be used in stone prevention in very high risk groups. When gallbladder stones are proved to be the cause of severe symptoms, cholecystectomy is the best treatment for most patients.

The introduction of laproscopic cholecystectomy over the past ١٠ years has changed surgical practice greatly (Lam *et. al.*, ١٩٩٦) . Advantages of the laproscopic cholecystectomy include decreased postoperative pain, shorter hospital stay, and earlier return to work and full activity. The main complication is injury to common bile duct ,there are few contraindications to the laproscopic cholecystectomy,the primary ones are peritonitis ,cholangitis , gangrene and perforation of the gallbladder, Portal hypertension and serious bleeding disorder (Beckingham , ٢٠٠١).

١.٢.٩. Biliary Tract Infection

Bacterial infection of the bile system appears to be an impotant factor in the formation of gallstones . The infection may be ascending in character, from the duodenum through the common bile duct, by blood through arterial vesseles, venous vesseles and the portal system or descending from the liver, through bacteria carried along with bile by lymphatic vesseles , from inflammatory foci present in the adjacent organs, i.e. the stomach , duodenum or appendix (Jasienski, ١٩٥٨).The presence of bacteria in bile is a very serious clinical problem .

The organisms cultured from gallbladder bile are predominantly aerobes such as *E.coli* ,*Klebsiella* spp. , *Streptoccocus* spp. ,a third generation cephalosporins is the antibiotic of choice . Anaerobes such as *Bacteroids fragilis* are associated with more severe, mixed infections particularly in elderly, these required combination chemotherapy using metronidazole with aminoglycosides and , or penicillin (Cuschieni *et.al.*,٢٠٠١).

Infection of postsurgical wounds, septic complications, and other complications are more frequently discovered in patients with positive bile culture.The bacteria present in bile can modify its composition, or indirectly,by changing the motor activity of the extra-hepatic bile ducts. Both mechanisms lead to change in the composition of the bile, and as a result to the appearance of lithogenic bile (Kosowski *et.al.*,٢٠٠٠).

Constriction of bile ducts caused by infectious lesions and infections , and by stasis of bile, has a decisive impact on the formation of stones (Lindelof and Vanderlinden ,١٩٦٥).

١.٢.١٠. The Microorganisms

١.٢.١٠.١. Enterobacteriaceae

The members of Enterobacterieceae are regularly recovered from the human gastrointestinal tract,where they are members of the normal fecal flora or transient colonizers (Leclerc *et.al.,* ٢٠٠٠). Besides, inhabitants of the human gastrointestinal tract, the Enterobacterieceae are most prominent family of gram negative bacteria that cause biofilm-related infections such as biliary tract infections, bacterial prostatitis and catheter associated urinary tract infections (Bouza *et.al.,*٢٠٠١). The gut is considered a primary source for dissemination and transmission of those potential pathogens to susceptible sites (Zogaj *et.al.,*٢٠٠٣).

١.٢.١٠.١.١. *Escherichia coli*

E.coli is one of the most important members of the Enterobacteriaceae.Strains predominate among the aerobic commensal bacteria in the healthy human intestine (Collee *et.al.,*١٩٩٦). Maki, (١٩٦٦) shown that bacterial β-glucuronidase produced by *E .coli* is an important enzyme which deconjugates bilirubin diglucuronide, resulting in the release of free bilirubin and glucuronic acid , the

former precipitates with calcium ion to form calcium bilirubinate, which is the major component of brown pigment stones . It is assumed that one of the factors playing a role in the pathogenesis of gallstones is *E.coli* (Claesson *et.al.,*١٩٨٦).In the pathogenesis of bacterial infections, great significance is assigned to the adherence properties of bacteria (Wadstorm and Trust, ١٩٨٤), the permanent attachment of the microorganism to the cells of the host which causes lasting damage (Spitz *et.al.,* ١٩٩٥). Integrity is lost due to their adherence ,and with it the barrier function of the epithelial cells changes are brought about in the structure of the skeleton of the eukaryotic cell (MacDaniel *et.al.,*١٩٩٤).

١.٢.١٠.١.٢. *Klebsiella pneumoniae*

Klebsiella is a member of the family Enterobacteriaceae , colonies are large and highly mucoid (Abbott ,١٩٩٩). Most *Klebsiella pneumoniae* clinical isolates are fully encapsulated and adhere in vitro to intestinal cell lines with aggregative pattern . The capsule plays an active role during the initial steps of the pathogenesis by interacting with mucus producing cells (Favre-Bonte *et.al.,*١٩٩٥). *Klebsiella pneumoniae* is opportunistic bacteria among gram negative bacilli responsible for nosocomial infections .Extended spectrum β-lactmase production, an event probably linked to a growing use of cephalo-sporins as therapeutic agents (De Champs *et.al.,*١٩٩١).The colonization of mucus membranes by bacteria is linked to an adhesion process

involving specific adhesions on the bacterial surface (Finlay and Falkow , ١٩٨٩). Several pilli involved in either adhesion to gallbladder epithelial and tracheal ciliated cells or adhesion to the kidney epithelium (Tarkkamen *et.al.,*١٩٩٠).

١.٢.١٠.١.٣. *Enterobactor*

It is one of the members of Enterobacteriaceae. This pathogen is recovered from respiratory tract and is often isolated in urine and the gastrointestinal tract (Jravis and Martone, ١٩٩٢). Most isolates involved in nosocomial infections are resistant to multiple antibiotics (Arpin *et.al.,*١٩٩٦). In addition,in some isolates, alteration of the membrane protein composition has led to resistance through impermeability or efflux associated with enzymatic resistance resulting in multidrug resistance and no availability of an alternative antibiotics (Mallea *et.al.,*١٩٩٨). Most of *Enterobacter* strains are resistant to serum bactericidal activity and are able to produce aerobactin and haemagglutinin, all of them could adhere and invade human epithelial cells (Keller *et.al.,*١٩٩٨).

١.٢.١٠.١.٤. *Proteus mirabilis*

Proteus is a member of the family Enterobacteriaceae . It is a motile gram negative bacteria which can not ferment the lactose and can produce disease only when leave the intestinal tract (Farmer, ٢٠٠٠). *Proteus mirabilis* is an important pathogen of the urinary tract and is the primary infectious agent in patients with indwelling urinary

catheters. This bacteria has numerous virulence factors including fimbriae, flagella, outer membrane proteins, lipopolysaccharide, capsule antigens, urease , immunoglobulin A proteases, hemolysin , and finally the most characteristic attribute of *proteus,* swarming growth enabling them to colonize and survive in higher organisms (Rozalski *et.al.,*١٩٩٧).

١.٢.١٠.١.٥. *Citrobacter*

Citrobacter spp. are gram negative commensal bacteria that infrequently cause serious infections in compromised host. Although *Citrobacter* spp. are infrequent pathogens,local or systemic breaches of host defences can allow them to cause a range of infections.These include urinary tract infections, gallbladder infections, neonatal sepsis, brain abscess, blood stream infections, intrabdominal sepsis . *Citrobacter* spp. are considered the largest group of multi-resistant Enterobacteriaceae which carry high mortality rate due to ineffective empirical antibiotic therapy (Pepperell *et.al.,*٢٠٠٢). It is unclear what role these spp.may play in human infections(Luperchio and Scharer, ٢٠٠١).

١.٢.١٠.٢. *Pseudomonas aeruginosa*

It is non fermentive aerobic gram negative rods. It is a ubiquitous pathogen prevalent in the hospital environments, and can cause severe nosocomial infections (Morrison and Wenzel , ١٩٨٤). The latter involve a broad spectrum of infections including the

respiratory, gastrointestinal and urinary tract as well as wound infections, sepsis and others (Pollack ,١٩٩٥). The pathogenicity of bacteria contributes to the virulence factors of it. The capsule or slim layer is associated with adherence and protects the bacteria from phagocytosis.The production of extracellular protease, cytotoxin and hemolysin have an important role in virulence. In addition , the siderophore production under low iron condition helps the growth of pathogen (Woods and Iglewsk, ١٩٨٣). Zhu *et.al.*, (٢٠٠٤) found the relationship of bacteria identified in cholesrol gallstones and gallstone formation and observed that the *Pseudomonas aeruginosa* and *Enterococcus* specifically had pronucleating ability through shorten cholesterol nucleation time .

١.٢.١٠.٣. *Acinetobacter*

They are strictly aerobic , gram negative coccobacillary rods, oxidase negative, non lactose fermentive. They are generally considered to be non pathogenic to healthy individuals but may cause infection in debilitated individuals (Bouret and Grimont, ١٩٨٧) . They are important causes of hospital – acquired infections , and are most likely to involve the respiratory tract, urinary tract and wounds and may progress to septicaemia (Cisneros *et.al.*,١٩٩٦) . the pathogenicity of bacteria is related to presence of small capsule and production of β-lactamase enzyme similar to that of MRSA bacteria with high antibiotic resistance (Iskandar *et.al.*,٢٠٠٣). Moreover, they have no

adhesive agents and some species produced lipase, histamine, siderophore and other produce hemolysin (Hostacka, ٢٠٠٣).

١.٢.١٠.٤. *Salmonella*

They are gram negative bacilli , non lactose fermenting , *Salmonella typhi* is the etiological agent of typhoid fever, ٣-٥٪ of the population that are infected with *S.typhi* will become chronic carriers organism .During chronic carrier state, bacteria reside in the gallbladder . Additionally, there is a strong correlation between chronic carriers and individuals with gallstones (Dutta *et.al.*, ٢٠٠٠). *Salmonella* spp. form biofilms on the surface of gallstones when the bacteria are protected from bile and antibiotics (Prouty *et.al.*,٢٠٠٢).They have other important virulence factors including capsule, surface antigens and fimbria (Collee *et.al.*,١٩٩٦)

١.٢.١٠.٥. *Enterococcus*

Enterococci are gram positive bacteria that usually reside in the gasterointestinal tract as commensal organisms, but they are capable of causing severe infections (Frankenberg *et.al.*,٢٠٠٢). They cause infection at a wide variety of sites including blood stream, abdomen,biliary tract,and urinary tract as well as burn wounds (Jett *et.al.*,١٩٩٤). They are intrinsically resistant to many antimicrobial agents, they are resistant to low levels of aminoglycosides and acquire resistance to chloramphicol and erythromycin. *Enterococcus* resistance is due to β-lactamase (Murray ,١٩٩٠). Several virulence – related

factors have been described for *Enterococci* including cytolysin, a factor called aggregation substance and gelatinase(Franz *et.al.,*١٩٩٩).

١.٢.١٠.٦. Viridans Streptococci

Several streptococcal species may be either α–hemolytic with the production of green coloration around their colonies on blood agar, or non hemolytic. Viridans group comprise a large proportion of the commensal bacteria that colonize on surface , but under certain conditions are associated with oral infection (Fransen *et.al.,*١٩٩١).

These bacteria enter the blood stream following trauma to oral tissues, they are important causes of deep infections and native valve endocarditis ,however the mechanisms of the pathogenesis are poorly understood (Murray *et.al.,* ٢٠٠٣).

١.٢.٣.١٠.٧. *Staphylococcus aureus*

They are gram positive spherical bacteria usually arranged in grape like irregular clusters. 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).

Infection of the gallbladder with this organism has been rarely described and may be associated with gallstones and obstructive disease as well as a calculous cholecystitis in the setting of bacteremia and endocarditis, so the finding of *S . aureus* cholecystitis with bacteremia should prompt an investigation for possible

endovascular focus of infection (Merchant and Falsey, ٢٠٠٢). Treatment of staphylococcal infection has become increasingly problematic due to emergence of multidrug resistant strains (Change *et.al.*,٢٠٠٣).

Materials and Methods

٢.١ Materials

The materials are listed in Tables (٢-١) and (٢-٢) .

٢.١.١ Laboratory Instruments

Table (٢-١): List of Instrument Used .

Instrument	Company
Sensitive electric balance	A & D-Japan.
Autoclave	Stermite- Japan.
Incubator	Memmert- Germany.
Distillator	GFL- Germany.
Centrifuge	Hermle- Japan.
Oven	Memmert-Germany.
Refrigerator	Concord- Italy.
Benson burner	Germany.
Millipore filter paper	Satorius membrane filters GmbH- W. Germany.
Light microscope	Olympus-Japan.
Micropipette	Oxford, USA.
pH meter	Hoeleze&Cheluis,KG-Germany.
Inoculating loop	Oxford-Japan.
Inoculating needle	Oxford-Japan.
Sterile syringe	Discardit-Spain.
Biconcave slide	GFL-Germany.
Hot plate	Classico-India
Vacuum	Classico-India

٢.١.٢. Chemical and Biological Materials

Table (٢-٢): Chemical and Biological Materials Used.

Name of Material	Company
A-Chemical Materials -$CaCl_2$, KOH, KH_2PO_4, $MgSO_4$, Na_2HPO_4, , NaCl, NH_4Cl,	Merk-Darmstadt.
- α-naphthol, Chloroform, Deoxycholate bile salt, esculin, HCl, Isopropyl Alcohol, Methyl red, Petroleum ether BP ٣٨-٥٣ c°, Tetramethyl-P-paraphenylene diamine dihydrochloride.	B.D.H.- UK.
-٩٩٪ Ethanol, Glucose, H_2O_2, Kovac's reagent, ٩٩٪ Methanol, Urea solution.	Fluka chemika-Switzerland.
- Cholesterol powder	Sigma-USA.
-Cholesterol kit -Bilirubin kit	BioMerieux-USA Randox-UK.
B-Biological Materials - Culture media	
- Agar-agar , Blood agar base, Brain heart infusion agar, MacConcky agar, Nutrient agar, Mὺller-Hinton agar, Nutrient broth, peptone broth,	Mast Lab.- UK .
- Kliglar iron agar, MR-VP broth, Simon citrate agar ,Urea agar base .	Diffco-Michigan

٢.٢. Patients and Methods

٢.٢.١. Patients and Specimen collection

During the period from October /٢٠٠٤ to June /٢٠٠٥, ٨٠ patients with symptomatic gallstone underwent elective cholecystectomy done in surgical unit at Hilla Teaching Hospital.

During surgery , gallstones and gallbladder biles were collected . Those stone formers were of different ages (١٨ – ٨٧ years) and different sexes (٦٤ females and ١٦ males). Samples of gallbladder biles were aspirated aseptically by sterile syringe with rupper stopper needle, during the operation , and plated within ٣٠ min of collection.

The stone was washed with normal sterile saline to remove surface contaminants and each core of gallstone was scopped for bacterial culture,a portion of stone was taken for determination of cholesterol and bilirubin percentage in each stone .

٢.٢.٢. Methods

٢.٢.٢.١. The Preparation of Reagents

I-Methyl red reagent: ٠.١ gm 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 organisms produced acid end products when fermenting dextrose (MacFaddin, ٢٠٠٠) .

II-**Voges –Proskauer reagent:**

Reagent A- ٥ gm of alpha - naphthol was dissolved in ١٠٠ ml of ٩٩٪ ethanol .

Reagent B- ٤٠ gm 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 gm of tetra-P-paraphenylene diamine dihydrochloride in ١٠ ml of distilled water and then was stored in a dark container. It was used for the detection of bacteria of certain oxidase and the exclusion of Enterobacteriacae (Baron *et.al.,*١٩٩٦) .

IV-**Catalase reagent :**It was prepared by dissolving ٣ gm of H_2O_2 in ١٠٠ ml of distilled water and then it was stored in a dark container, it was used for detection of bacteria with certain catalase enzyme (Baron *et. al.,*١٩٩٦) .

٢.٢.٢.٢. The Preparation of Media

I-**M٩ media :** ٦ gm of Na_2HPO_4 , ٣ gm of KH_2PO_4, ٠.٥ gm of NaCl, ١ gm of NH_4Cl, all of them were dissolved in ٩٥٠ ml of distilled water with ٢٪ agar and then sterilized by autoclave and after cooling the mixture to ٥٠ C° ,٢ ml of $MgSO_4$(١M), ١٠ ml of ٢٠ % glucose and ٠.١ ml of $CaCl_2$ (١M) (sterilized separately by

filtrations) were added to it , then the volume was completed to ١٠٠٠ ml of distilled water(Miniatis *et.al.*,١٩٨٢).

II-**Esculin media :** The esculin is ٦,٧-dihydroxycoumarin ٦-glucoside which has inhibitory effect on xanthine oxidase enzyme (Capell *et.al.*, ١٩٩٥). Esculin media was made from nutrient agar with ٠.٤ gm ferric ammonium citrate and ٥ gm esculin then the volume was completed to ١٠٠٠ ml of distilled water. Afterwards the media was poured in tubes and sterilized by autoclave , it was prepared as slants (MacFaddin , ٢٠٠٠).It was used for differentiation group D streptococci from other streptococci.

٢.٢.٢.٣. Identification of Bacteria

A single colony was taken from each primary positive culture on blood agar and on macConcky agar and it was identified depending on its morphology (shape, size , borders, and texture) and then it was examined under microscope after staining it with Gram stain . After staining , the biochemical tests were done on each isolate to complete the final identification(Collee *et.al.*, ١٩٩٦).

٢.٢.٢.٤. Biochemical Tests

٢.٢.٢.٤.١. 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 (Collee *et .al.*,١٩٩٦).

٢.٢.٢.٤.٢. 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 (Collee *et.al.,*١٩٩٦) .

٢.٢.٢.٤.٣. Coagulase Test : Several colonies of bacteria were transferred with a loop to a tube containing ٠.٥ ml of plasma .The tube was covered to prevent evaporation and incubated at ٣٧C° overnight.The test was read by tilting the tube and observing for clot formation in the plasma . A negative test resulted in the plasma remaining free-flowing with no evidence of a clot (Collee *et.al.,* ١٩٩٦).

٢.٢.٢.٤.٤. Indole Test: A ١٪ solution tryptone broth was prepared in the tubes . After that inoculated the broth with bacterial colonies and it was incubated for ٤٨ - ٧٢ hours at ٣٧ C°. Testing for indole production was done by adding ٦-٨ drops of Kovacs Reagent (p-dimethylamin-benzaldehyde in amyl alcohol).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) .

٢.٢.٢.٤.٥. Methyl Red Test : The test was perfomed on ٥ ml of MR-VP broth cultured by the organism and then it was incubated for ٢٤ hours at ٣٧ C°. After that the ٦-٨ drops of methyle red reagent was added to culture . The change of color to orange was a positive reaction (Collee *et.al.,*١٩٩٦).

٢.٢.٢.٤.٦. Voges-Proskaur Test: The test was perfomed on ٥ ml of MR-VP broth cultured by the organism and then it was incubated for ٢٤ hours at ٣٧ C°. After that ١٥ drops of ٥٪ alpha naphthol (reagent A) was added and followed by ١٠ drops of ٤٠٪ KOH (reagent B)and the mixture was shacked well and allowed standing for up to ٣٠ min before calling the reaction negative .The positive culture was turning to red at the surface of the liquid , and the color spread gradually throughout the tube (Baron *et.al.*,١٩٩٦) .

٢.٢.٢.٤.٧. Simon Citrate Test: After the sterilization of simon citrate slants by autoclave , the bacterial culture was inoculated and inocubated for ٢٤-٤٨ hours at ٣٧ C°.The positive result was a change of the color of media from green to blue . The unchanging of the color was a negative reaction (Benson ,١٩٩٨).

٢.٢.٢.٤.٨. Kliglar Iron Agar (KIA) Test: The aim is to differentiate the Enterobacteriaceae according to carbohydrate fermentation and hydrogen sulfide production . The organism was grown on KIA slant by stab and streak and then it was inoculated at ٣٧ C°for ٢٤-٤٨ hours. The changing of the color of the media from orange-red to yellow was due to carbohydrate fermentation with or without gas formation at butt of slant . In addition , the formation of hydrogen sulfide was given a black color precipitation at butt (MacFaddin ,٢٠٠٠) .

٢.٢.٢.٤.٩. Urease Test: The urea base agar was sterilized by autoclave. After cooling it to ٥٠ C°, the urea substrate was added

and was poured in sterile tubes then it was inoculated by bacterial cultures and all were incubated at ٣٧ C° for ٢٤-٤٨ hours . The positive reaction was a deep pink color. Failure of deep color to develop was a negative reaction (Benson,١٩٩٨).

٢.٢.٢.٤.١٠. 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 (Capell *et.al.*١٩٩٥).This reaction presumptively identifies group D streptococci (Facklam and Moody ١٩٧٠).

٢.٢.٢.٤.١١. Mannitol Salt Agar: The medium turns from pink to bright yellow if the bacteria are mannitol fermented and the test is positive (MacFaddin ,٢٠٠٠).

٢.٢.٢.٤.١٢. Eosin Methylene Blue (EMB) Agar: Lactose fermenting colonies were either dark or possessed dark centers with transparent colorless peripheries, while organisms that did not ferment lactose remained uncolored . This purple color was due to the absorption of the eosin methylene blue complex , which was formed in the presence of acid. Certain numbers of the coliform group , especially *E-coli,* exhibited a greenish metallic sheen by reflected light (Collee *et.al.,*١٩٩٦) .

٢.٢.٢.٤.١٣. Motility Test by Using Semisolid Media:١٠ ml of semi-solid media was dispensed in test tubes and left to set the vertical position, inoculated with a straight wire, making a single stab down the

center of the tube to about half the depth of the medium . The culture was incubated at ٣٧ C° and examined at ٦ hours, ١ and ٢ days.Non motile bacteria had generally been confined to the stab-line and given sharply defined margines leaving the surrounding medium clearly transparent. Motile bacteria typically gave diffuse hazy growth that spread throughout the medium rendering it slightly opaque (MacFaddin, ٢٠٠٠).

٢.٢.٢.٤.١٤. Growth in ٦.٥٪ NaCl: Two or three colonies were inoculated into a tube of nutrient broth with ٦.٥٪ NaCl and incubated at ٣٥ C° for ٣ days.The growth was judged by the turbidity seen after dispersing any sediment that indicated positive growth, otherwise the growth was negative (Collee *et.al.,*١٩٩٦) .

٢.٢.٢.٥. Antibiotics Diffusion Tests by Kirby-Bauer Susceptibility Test: -

It was perfomed by using a pure culture of previously identified bacterial organism. The inoculum to be used in this test was prepared by adding growth from ٥ isolated colonies grown on a blood agar plate to ٥ ml of broth. This culture was then incubated for ٢hours to produce a bacterial suspension of moderate turbidity.

Asterile swab was used to obtain an inoculum from the standardized culture.This inoculum was then streaked on a Muller-Hinton plate.The antibiotic discs were placed on the surface of the medium at evenly spaced intervals with flamed forceps or a disc applicator.

Incubation was usually over night with an optimal time being ١٤ hours at ٣٧ C°. Antibiotics inhibition zones were measured using a caliber. Zone size was compared to standard zones to determine the susceptibility or resistance of the organism to each antibiotic (MacFaddin, ٢٠٠٠).

Table (٢-٣): Antibiotic Disc Potency by µg/ml (OXIOD-England and TROGE-Germany).

Antibiotics	**AM**	**GM**	**CF**	**CE**	**TMR**	**ATM**	**DO**	**KF**
Potency	١٠	٣٠	٣٠	٣٠	٥	٣٠	٣٠	٣٠

AM=Ampicillin,GM=Gentamicin,CF=Ciprofloxacillin,CE=Cefotaxim TMR=Trimethoprim,ATM=Aztreonam,DO=Doxycycline, KF=Keflin (Cephalothin).

٢.٢.٢.٦. The Effect of Deoxycholate on Bacterial Growth:

١) Nutrient broth was prepared and distributed in tubes and deoxycholate bile salt was added to each tube at various percenttage (٠.٧٪ , ١.٥٪, ٣٪,).

٢) Positive control was prepared by using nutrient broth free from deoxycholate .

٣) The tubes were inoculated with ٠.٥ ml of bacterial suspension and then incubated at ٣٧C° for ٢٤ hours .

٤) After incubation, the absorbance was read at wave length ٥٢٠ nm by using spectrophotometer to show the effect of deoxycholate on the growth of bacterial strain.

٢.٢.٢.٧. The Effect of Cholestrol on Bacterial Growth:

١) The gram positive and gram negative bacterial isolates were cultivated on nutrient broth containing cholesterol with concentration (٢٥٠ mg /dl)and incubated over night at ٣٧ C° .

٢) The bacterial cultures of gram positive and gram negative were diluted by using of physiological normal saline in a manner of serial dilution as (١/١٠,١/٢٠,١/٤٠,١/٨٠,١/١٦٠,١/٣٦٠).

٣) Each dilution was cultivated on M٩ media at ٣٧C° for ٢٤ hours.

٤) These cultures were used to measure the effect of cholesterol on the bacterial growth by using CFU technique.

٥) The bacterial cultures of gram positive and gram negative were diluted as above and each dilution was cultivated on M٩ media at ٣٧C° for ٢٤ hours and these cultures represented the control.

٢.٣. Gallbladder Stones Preparation and Extraction:

Stone samples were prepared for the analysis according to the method of (Nakayama ,١٩٦٨) ; (Trotman *et.al.*,١٩٧٧).

٢.٣.١. Stone Extraction:

٢.٣.١.١. Reagent

١) Acidified methanol: chloroform ١:٢ v/v. (Acidified methanol is ٠.٩ ml volume of ١N HCL with ٩٩.١ ml volume of ١٠٠ % methanol).

٢) Petrolum ether BP ٣٨-٥٣ C°.

٣) Ethanol ٧٠٪.

٢.٣.١.٢. Procedure

The following preparation of solutions was made:

A) ٢٠ mg of dried homogeneous powdered stone was weighed and transferred to ١٥ ml graduated centrifuge tube .

B) ١٥ ml of acidified methanol : chloroform ١:٢ (v/v) mixture was added and mixed vigorously.

C) After being kept for ٣٠ min at room temperature in the dark , the tube was centrifuged for ١٠ min at ١,٠٠٠ g, the supernatent was aspirated and collected aside.

D) The extraction step was repeated twice , and the supernatant was added to previous one, leaving a small amount of sediment designated as residue left in the centrifuge tube .

E) After completion of extraction , the total collected volume was measured and recorded ,immediately an a liquot part was taken for bilirubin estimation .

F) The remaining extract was evaporated to minimal volume at ٥٠ C° under vacume and partitioned between ٥٠ ml of petrolume ether and successive volumes (٢٠,١٥,and ١٥ ml) of ٧٠٪ ethanol. By using a separatory funnel , the two phases were allowed to separate and cholesterol was determined on petroleum ether phase. This phase was reduced to minium volume by evaporation at ٥٠ C° under vacume. (Nakayama ,١٩٦٨) ; (Trotman *et.al.*,١٩٧٧).

٢.٣.٢. Gallstone Analysis

٢.٣.٢.١. Estimation of Bilirubin

The estimation of bilirubin was carried out according to the instructions of manufacture company (Randox).

Weight/weight % = concentration of total bilirubin(mg/dl) × volume of the acidified stone extract × ١٠٠ / weight of the powdered stone analysed(dry weight). (Nakayama ,١٩٦٨) ; (Trotman *et.al.*,١٩٧٧).

٢.٣.٢.٢. Estimation of Cholesterol

The estimation of cholesterol was performed according to the manufacture company (bioMerieux).

Weight/weight % = concentration of cholesterol (mg/dl) × volume of the acidified stone extract × ١٠٠ / weight of the powdered stone analysed(dry weight). (Nakayama ,١٩٦٨) ; (Trotman *et.al.*,١٩٧٧).

٣.١. Clinical Study

٣.١.١. Sex Related Disease

This study included (٨٠) patients suffering from symptomatic gallstones who were admitted to Hilla Teaching hospital–Department of Surgery .The incidence of this disease was found to be higher in females than in males as shown in Table(٣-١) as ٨٠ % were females and ٢٠ % were males with ratio of female :male is ٤:١.

Table (٣-١) Number and Percentage of Sex Distributed Disease.

Sex	Number	Percentage
Female	٦٤	٨٠ %
Male	١٦	٢٠ %
Total	٨٠	١٠٠ %

The results agreed with the study results obtained by Baig *et.al.*, (٢٠٠٢) who showed that ٧٢.٥ % of patients with gallstones were females and ٢٧.٥ % were males. There is consistent evidence that gallbladder disease is more common in females at all ages, although the female: male proportion decreases slightly with age (GREPCO, ١٩٨٨).

It is tempting to explain the increased frequency of gallstones in women as an effect of female sex hormones on hepatic function , bile secretion and gallbladder function .

In contrast to earlier reports, recent studies of biliary lipids during the menstrual cycle show no effect on cholesterol saturation (Whiting *et.al.,*١٩٨١). Bile tends to be more saturated in women than in men and this cannot be explained by differences in body weight or age (Hofmann *et.al.,*١٩٨٢).

An increase in the number of pregnancies is associated with an increased risk of gallstones (Moro *et.al.,*٢٠٠٠). Tierney *et.al.,* (١٩٩٩) believe that the progesterone component rather than the oesterogen is responsible for the changes in biliary lipids. Bile is more saturated during the second and third trimester of pregnancy (kern *et.al.,*١٩٨١).
The use of oral contraceptive induces an increased risk of gallbladder disease. Oral contraceptive therapy may be associated with an increase in cholesterol saturation (Dourakis and Tolis, ١٩٩٨). Oesterogen increases the biliary secretion of cholesterol and the lithogenicity of bile, there may be stimulation of hepatic lipoprotein receptors and increased hepatic cholesterol uptake. Synthesis of chenodeoxycholic acid is inhibited and the pool of chenodeoxycholic acid is reduced . Thus , the overall effect of oesterogen is to increase biliary cholesterol and promote the secretion of lithogenic bile (Bouchier,١٩٩١).

Apart from an effect on bile chemistry , hormones also influence gallbladder function which , in turn, might affect biliary lipids

secretion or predispose the organ to cholecystitis . In pregnancy the fasting and residual gallbladder volumes are larger and the emptying rate is slower than in non pregnant women (Braveman *et.al.,*١٩٨٠). Thus the effect of female sex hormones on the biliary system is complex and diverse but,by adversely influencing cholesterol and bile salt secretion into bile as well as gallbladder function, they predispose to cholesterol gallstone disease .The strong relationship of femininity and parity to cholesterol gallstones does not exist for pigment lithiasis both men and women are affected equally (Soloway *et.al.,*١٩٧٧). The results of our study are very much in agreement with the above view, whereby the incidence of pigment stones in both sexes is almost equal.

٣.١.٢. Age Related Disease

This study showed that the more affected age group in females was between ٤٠-٤٩ years old (٣٥.٩ %) while in male , the more affected age group was between ٥٠-٥٩ years old (٤٣.٧٥ %)as shown in Figure (٣-١). This study revealed that in both sexes , hospitalization of patients for gallstones were rare under ١٠ years age, and increased significantly with age , more so in females and thereafter declined gradually, the affected patients over ٦٠ were rare . These results agreed with the results obtained by Channa *et.al.,*(٢٠٠٤).

The low incidence of gallstone disease over the age of ٦٠ might be

due to partly the fact that old people are poor candidate for surgery and anaesthesia in addition to lesser easy access of medical assistance to this age group. The propotion of patients below ٦٠ years was high.That is, operation was performed at an earlier stage of the disease thus favouring the admission of young patients with fewer anticipated complications.

In general, gallstones of any type are rare before the age of ١٠ years . Many factors contribute to gallstones in childrens. About ٢٥٪ of children with gallstones have hemolytic diseases , other possible predisposing factors are cystic fibrosis , liver disease , bowel resection and heart disease (Henschke and Teele , ١٩٨٣).

The prevalence of gallstones remains rare until the onset of adolescence and then begins to increase in frequency , particularly in females. This is in agreement with the finding of Bennion *et.al.*,(١٩٧٩) who demonstrate that cholesterol saturation increases significantly in both sexes at the time of puberty,being more marked in females than males among Pima Indians. Studies on the change in the biliary lipids with aging suggest that the cholesterol content increases (Fujiyama *et.al.*,١٩٧٩). This effect is more marked in women and is unrelated to any influence of obesity. The increased lithogenicity of bile is due to an increased output of cholesterol, but in addition the bile acid pool size and secretion rate diminish with age. Normal infants and children have secretion ratio of cholesterol : bile salts that are lower than adults (Heubi *et.al.*,١٩٨٢).

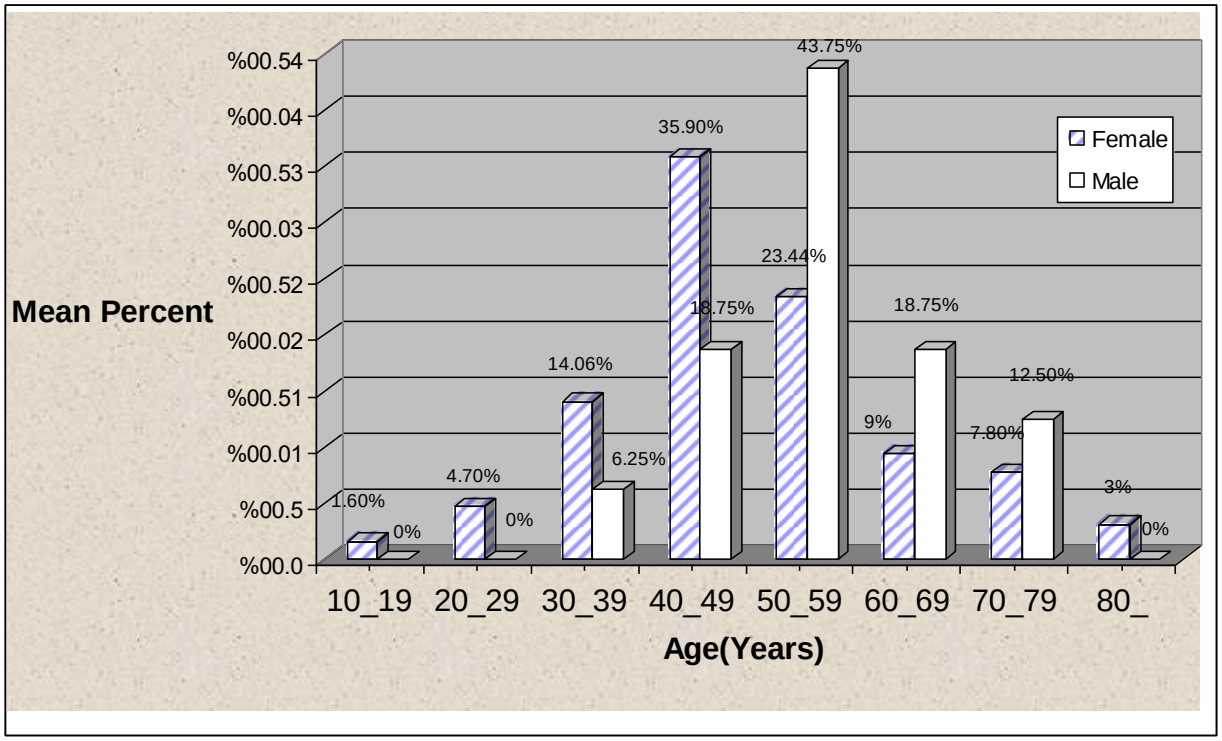


Figure (٣-١) Percentage of Age Related to Sex of Patients with Gallstones.

٣.٢. Physical Criteria of Gallstones

Prior to chemical analysis, the ٨٠ gallstones were classified by gross visual inspection as pigment stones which were black to dark brown in colour, with different shapes of almost all stones when they occured in multiple , they had irregular shape and were often speculated with shiny or dull black colour. The ovoid pigment stones were solitary. On cross section pigment stones had amorphous appearance, or cholesterol stones which were yellow to light brown, cholesterol stones were either solitary or multiple . The shape of the solitary stones was usually round or mulberry,when multiple they were

faceted and smooth.On cross section they were crystalline or laminated with a distinct dark nucleus. The results of this study agree with the results obtained by Whiting *et.al.*,(١٩٨٣).

The numbers of gallstones found in each patient ranged from a single solitary stone , which was either a cholesterol stone or a pigment stone , to multiples. In general, multiple stones occurred more frequently (٥٧.٥٪) while solitary stones had (٤٢.٥٪) incidences when analysed chemically,out of ٣٤ solitary stones found in this study; (٥٠ %) were pigment stones, (٣٥.٣ %) cholesterol stones and (١٤.٧ %) were mixed stones. The remaining ٤٦ stones were multiple and were mostly cholesterol stones(٤٣.٤ %),pigment stones (٢٣.٩٪) and mixed stones(٣٢.٧٪) as shown in Table (٣-٢).

Table(٣-٢):Number of Gallstones Per Patients in Relation to Their Types .

Types	Cholestrol stone		Pigment stone		Mixed stone		Total	
	No.	%	No.	%	No.	%	No.	%
Solitary	١٢	(٣٥.٤٪)	١٧	(٥٠٪)	٥	(١٤.٧٪)	٣٤	(٤٢.٥٪)
Multiple	٢٠	(٤٣.٤٪)	١١	(٢٣.٩٪)	١٥	(٣٢.٧٪)	٤٦	(٥٧.٥٪)
Total	٣٢	(٤٠٪)	٢٨	(٣٥٪)	٢٠	(٢٥٪)	٨٠	(١٠٠٪)

The number of stone did not correlate with the cholesterol saturation index or the total lipid concentration, while the nucleation was determined to be negatively correlated with the number of gallstones. Patients with solitary stone had a significantly longer

nucleation time than those with multiple gallstones , so that there existed a nucleation promoting activity, which seemed to be more pronounced in patients with multiple stones than in those with solitary stones, indicating a major risk factor for the recurrence rate after dissolving or removal of gallstones in those patients (Tudyka *et. al.,*١٩٩٤). Also there was no relation between the number of stones and the percentage of positive bacterial culture (Csendes *et.al.,*١٩٩٦a).

٣.٣. Classification of Gallstones in Relation to Chemical Analysis

Visual distinction of pigment stone from cholesterol stone was uniformly confirmed by biochemical analysis.

The cholesterol and bilirubin content of gallstone was quantitatively measured, and the results were expressed as the percentage of dry weight(w/w%).The percentage composition of pigment stones differed strikingly from that of cholesterol stones as shown in Table (٣-٣).

Table (٣-٣) The Percentage of Cholesterol and Bilirubin in Three Types of Gallstones.

Component	Stone Type		
	Cholesterol stone No.= ٣٢	**Pigment stone No.=٢٨**	**Mixed stone No.=٢٠**
Cholesterol	Range=٦٥-١٠٠٪	Range=٠.٠١-١٩.٣٪	Range=٢٦.٩-٥٨.٥٪
Bilirubin	Range=٠.٠١-١٦.٤٪	Range=٠.٦٣-٣٠.٨٪	Range=٠.٣٢-١٧.٣٪

According to Morrissey,(١٩٨٢), gallstones having more than ٦٠٪ of its dry weight as cholesterol were considered as cholesterol stones. In the present study, ٣٢ patients had cholesterol stones(٤٠٪) while ٢٨ patients had pigment stones (٣٥ %)defined on the basis of cholesterol content lower than ٢٥ %. Those cases with cholesterol content between ٢٥-٦٠ % were classified as mixed –cholesterol stones ٢٠ patients (٢٥ %).

These results are in agreement with the results of related study carried out in south of Iraq by Al-Kass , (١٩٨٩) who showed that the most common type of gallstones was of cholesterol type (٤٢.٨٦%) followed by pigment stones (٣٨.٦٪) and then by mixed stone(١٨.٦٪). While the results of similar studies done in other countries like the study done in southern India by Jayanthi *et.al.*,(١٩٩٨) who showed that the most common type of gallstones was pigment stone (٦٣.٨٪) followed by mixed type (٣٤.٨٪) and then by cholesterol stone (١.٩٪) while in northern India gallstones were predominantly of cholesterol type. The comparsion of our data with those in similar studies in different countries may not be valid because of geographic,dietary and ethnic differences . When all the stones were taken together there was significantly more cholesterol contained among stones from female patients than in males as in Table (٣-٤). This is similar to the results obtained by Al-Kass, (١٩٨٩).

Table (٣-٤) Distribution of Gallstones Types According to the Sex.

Sex	Stone type							
	Cholesterol stone		Pigment stone		Mixed stone		Total	
	No.	%	No.	%	No.	%	No.	%
Female	٣٠	٩٣.٧٥	١٦	٥٧.١٤	١٨	٩٠	٦٤	٨٠
Male	٢	٦.٢٥	١٢	٤٢.٨٦	٢	١٠	١٦	٢٠
Total	٣٢	٤٠	٢٨	٣٥	٢٠	٢٥	٨٠	١٠٠

٣.٤. Clinical Features Related to Disease

According to the clinical features of gallstones disease shown in Table (٣-٥), the most common presentation of symptomatic gallstones was chronic cholecystitis (٥٢.٥ %) followed by acute exacerbation of chronic cholecystitis and then acute cholecystitis. These results are in agreement with the results obtained by Balasundaram *et.al.*, (٢٠٠٠). The clinical presentation depends on the severity of the disease . Abdominal pain, varying in intensity from mild to severe was the major complaint in ٩٨.٧٥٪ of patients. The pain most frequent in the right hypochondrial area was sometimes noted in the epigastrium. This result is similar to the result of similar study obtained by Ballal *et.al.*,(٢٠٠١). Right hypochondrial pain and intolerance to fatty meal with absence of heart burn were significantly related to gallstones this is in agreement with the result obtained by Festi *et.al.*,(١٩٩٩).

Symptoms of acute cholecystitis are usually sudden and associated with signs of acute inflammation, while in chronic cholecystitis the symptoms are frequently recurrent and persistent without signs of acute inflammation. The symptoms of gallstones diseases are not always specific and the differential diagnosis should include peptic ulcer – gastroesophageal reflux –hepatitis –appendicitis –renal calculous and irritable bowel (Hermann and Walsh,١٩٩٤). Only ١٠-٣٠٪ of gallstones diseases have symptoms and the majority of gallstones remain silent which need no treatment (Indar and Bechingham,٢٠٠٢). The probability of patients with silent gallstones developing biliary related pain ١-٢ % per year and the risk of developing complications is less (approximately ٠.١ % per year) (Gracie and Ransohaft,١٩٨٢).

Table (٣-٥) Clinical Features of Gallstone Disease.

Clinical features	Chronic cholecystitis		Acute on chronic cholecystitis		Acute cholecystitis	
	No.=٤٢	%	No.=٢٥	%	No.=١٣	%
Right hypocho-ndrial pain	٣٧	٨٨.١	١٦	٦٤	١٢	٩٢.٣
Epigastric pain	٤	٩.٥	٩	٣٦	١	٧.٦
Abdominal distention	١	٢.٤	٠	٠	٠	٠
Fever	١٠	٢٣.٨	١٩	٧٦	٧	٥٣.٨
Jaundice	١٠	٢٣.٨	٦	٢٤	٣	٢٣
Nausea	٢٨	٦٦.٦	١٩	٧٦	٥	٣٨.٥
Vomiting	٢٢	٥٢.٣	٨٠	٢٠	٧	٥٣.٨

٣.٥. Isolation of Bacteria Associated with Disease

The results of this study showed that ٣٤ patients expressed out of ٨٠ (٤٢.٥٪) had either bile or gallstone ,or both positive bacterial cultures which were in agreement with the result of other related study obtained by Balasundaram *et.al.*,(٢٠٠٠).The result of bile culture in our study as in Table (٣-٦) showed that ٢٥٪ of patients had positive bile culture and ٧٥٪ of them had negative bile culture, this result agreed with other results obtained by Al-Harbi *et.al.*,(٢٠٠١) who showed that only ٢٥٪ of patients undergoing cholecystectomy for gallstones with positive bile culture.

The isolation rates of bacteria in bile of patients with gallstone ranging from ٣-٥٨% have been reported by Csendes *et.al.*,(١٩٩٦a), who simultaneously assessed bile from the gallbladder and the common bile duct in control subjects and patients with gallstones and common bile duct stones. Of ٢٢١ patients with symptomatic gallstones, ٤٩(٢٢.٢٪) patients had positive cultures in the gallbladder bile. Although there was no relationship between the numbers of stones in the gallbladder or common bile duct and the percentage of bacteria present in the bile, the presence of bacteria in the gallbladder bile varied according to the severity of the biliary tract disease and increased significantly with age. They concluded that one stone was enough to facilitate colonization of bile and the risk of a positive culture did not increase when more stones were present.

Table (٣-٦) Number and Percentage of Bile Culture Results.

Culture	Number	Percentage
Culture positive	٢٠	٢٥ %
Culture negative	٦٠	٧٥ %
Total	٨٠	١٠٠ %

Table (٣-٧) shows that ١٧.٥٪ of patients had positive gallstone culture and ٨٢.٥ % of them had negative stone culture. This result differs from other results done in other countries because pigment stones and mixed stones are the most common types while in our country the cholesterol stone is the commonest type and the bacterial infection is only important in the formation of pigment and mixed stones.

Table (٣-٧) Number and Percentage of Gallstone Culture Results According to Their Types.

Culture	Stone Type							
	Cholesterol stone		Pigment stone		Mixed stone		Total	
	No.	%	No.	%	No.	%	No.	%
Culture positive	٠	٠	٦	٢١.٤	٨	٤٠	١٤	١٧.٥
Culture negative	٣٢	١٠٠	٢٢	٧٨.٥٧	١٢	٦٠	٦٦	٨٢.٥
Total	٣٢	١٠٠	٢٨	١٠٠	٢٠	١٠٠	٨٠	١٠٠

There are conflicting reports regarding the significance of bacterial infection in both normal subjects and patients with cholelithiasis . It has been suggested that the discordant reports are because of the differences in grouping of patients in sampling and cultural technique (Csendes *et.al.,*١٩٩٦a), but undisputed is the fact that clinical risk factors exist and this favours the isolation bacteria in bile, especially in calculous cholecystectomy, with a corresponding increased risk of postoperative sepsis. These risk factors include patients older than ٧٠ years, previous biliary tract operation, jaundice, chills and fever within one week of operation, and operation performed within one month of an acute attack of cholecystectomy (Nord,١٩٩٠). Antimicrobial prophylaxis is recommended when one or more of these risk factors are identified pre-operatively, although their relative importance in laproscopic cholecystectomy is undetermined (Gold-Deutch *et.al.,* ١٩٩٦); (Den Hoed *et.al.,*١٩٩٨).

In our study, the age over ٥٠ years was the only significance pre-operative factor associated with positive bile cultures. Asimilar finding was reported by Csendes *et.al.*(١٩٩٦a).

٣.٥.١. Types of Bacterial Isolates

Table (٣-٨) showed that the most common organism cultured from bile was *E.coli* (٣٠٪) followed by *Klebsiella pneumoniae*(٢٠%),

Enterococcus faecalis (١٥٪), *Enterobacter aerogenes*(١٥٪), *Acinetobacter* (١٠٪) and lastly one isolate of *Staphylococcus aureus*(٥٪) and one isolate of viridans streptococci(٥٪) were obtained. These results can be compared with those of another study obtained by Al-Harbi *et.al.*,(٢٠٠١)who showed that *E.coli* was also the most common organism (٢٨.١٪) ,followed by *Enterococcus fecalis* (١٥.٦٪), and *Pseudomonus aeruginosa* (٩.٤٪) and had four isolates contained more than one organisms , one sample grew amixture of *Enterococci* and mixed coliform, the second had *Acinetobacter* and *E.coli* , the third *Pseudomonus aeruginosa* and *Morganella morgani* and the fourth *Aeromanas hydrophilia* and *Enterobacter Cloacae.* In addition Ohdan *et.al.*,(١٩٩٣) in Japan, Samy and MacBain , (١٩٩٥) in Glasgow, and Darko and Archampong ,(١٩٩٤) in Ghana investigated the microflora in bile from the gallbladder of patients who underwent surgery for cholelithiasis and the predominant organisms were *E.coli* and Enterobacteriaciae as well as *Enterococci.* Our bile flora was different from that of Csendes *et.al.*,(١٩٩٦a) and Ohdan *et.al.*,(١٩٩٣) in that *Pseudomonus aeruginosa* was the second most common organism isolated, an organism which was unlikely to be sensitive to the antibiotics commonly used for prophylaxis in biliary surgery.

Table (٣-٨) Number and Percentage of Bile Bacterial Isolates.

Bacteria	Number	Percentage
E.coli	٦	٣٠ %
Klebsiella pneumoniae	٤	٢٠ %
Streptococcus fecalis	٣	١٥ %
Enterobacter aerogenes	٣	١٥ %
Acinetobacter	٢	١٠ %
Staphylococcus aureus	١	٥ %
Viridans streptococci	١	٥ %
Total	٢٠	١٠٠ %

Table (٣-٩) shows that the most common organisms isolated from gallstones were *Enterobacter aerogenes* (٣٥.٨%), *Klebsiella pneumoniae* (٢٨.٦%),*E.coli* (٢١.٤٪) and *Enterococcus faecalis* (١٤.٢٪). This result can be compared with the results of another study obtained by Balasundaram *et.al.*,(٢٠٠٠) who showed that the most common organism in gallstone culture was *E.coli* (٦٠٪) followed by *Citrobactor* (٢٤٪), *Klebsiella* (١٦٪), *Bacillus subtilis* (٨٪) and *Staph.aureus* (٤.٤٨٪). Also, they showed that most of pigment and mixed types of gallstones were positive cultures, none of cholesterol stone were culture positive in their study which is in agreement with the result of our study.

Table (٣-٩) Number and Percentage of Gallstones Bacterial Isolates According to their Types.

Bacterial isolate	Stone type			Total	
	Pigment stone	Mixed stone	Cholesterol stone	No.	%
Enterobacter aerogenes	١	٤	٠	٥	٣٥.٨
Klebsiella pneumoniae	٣	١	٠	٤	٢٨.٦
E.coli	٢	١	٠	٣	٢١.٤
Strep. faecalis	٠	٢	٠	٢	١٤.٢
Total	٦	٨	٠	١٤	١٠٠

The predominance of pigment and mixed stones positive cultures as compared to a negative culture for cholesterol stones does imply a specific role for infection in the formation of brown pigment stones and mixed stones. Failure to isolate organisms from gallstones does not indicate that the aetiology is unrelated to infection but the organisms which have initiated the stone formation are not in viable form in the stone or in bile till surgery (Lygidakis ,١٩٨٢). The culture of the organisms from the bile from patients with symptomatic gallstones

at time of operation does not necessarily indicate a cause, may be secondary to calculous formation(Ballal *et.al.*,٢٠٠١).

The role of bacterial infection in the pathogenesis of gallstones remains controversial (Leung *et.al.*,١٩٨٩). Maki , (١٩٦٦) had shown that the bacterial β-glucouronidase produced by *E.coli* and other bacteria was an important enzyme which deconjugated bilirubin diglucuronide, resulting in the release of free bilirubin and glucuronic acid. The former precipitates with calcium ion to form calcium bilirubinate, which is the major component of brown pigment stones. Wong *et.al.*,(١٩٨١) had also found a strong association between the presence of bacteria and the occurance of ductal stones in patients with recurrent pyogenic cholangitis. Stewart *et.al.*, (٢٠٠٥) found that less than half of bacteria obtained from gallstones containing calcium bilirubinate produced β-glucuronidase, so the widely accepted theory of the pathogenesis of these stones must be reevaluated.

٣.٥.٢. Identification of Bacteria

٣.٥.٢.١.Enterobacteriaceae and *Acinetobacter*

Members of Enterobcteriaceae are gram negative, non spore forming bacilli that grow both aerobically and anaerobically on ordinary laboratory media including MacConkey's lactose –bile salt agar. They are oxidase negative and catalase positive , they ferment glucose and other carbohydrates in peptone water with the production of acid or acid and gas, they reduce nitrate to nitrite,and are either

motile with peritrichous flagella or non motile (Collee *et.al.*,١٩٩٦). The isolation of these bacteria depended on the differences in specific biochemical tests like indole ,MR, VP,urease ,citrate, motility, growth on TSI agar and production of H٢S gas (MacFaddin ,٢٠٠٠).

Acinetobacter produced non –lactose fermented colonies, it was pale on EMB agar. Its biochemical tests showed that catalase positive, oxidase negative,MR positive, VP negative, indole negative, on KIA agar: H٢S negative, motility negative, urease negative, citrate negative.

Table (٣-١٠) The Diagnostic and Biochemical Tests of Enterobacteriaceae and *Acinetobacter*.

Test	***E.coli***	***K.pneumoniae***	***Enterobacter***	***Acinetobacter***
EMB	Metallic	Centrally dark	Centrally dark	Pale
Indole	+	–	–	–
L F	+	+	+	–
H٢S	–	–	–	–
Catalase	+	+	+	+
Oxidase	–	–	–	–
Urease	+	+	–	–
Citrate	–	+	+	–
MR	+	–	–	+
VP	–	+	+	–
Motility	+	–	+	–

E.coli produced lactose fermented colonies, it was metalic sheen on EMB agar . Its biochemical tests showed that catalase positive, oxidase negative, MR positive, VP negative, on KIA agar :H_2S negative, motility positive , urease was positive, citrate was negative. Almost all strains possessed the enzyme β-glucuronidase(Murray *et.al.*,٢٠٠٣).

K .pneumoniae produced a mucoid lactose fermented colonies , it was centrally dark on EMB agar. Its biochemical tests showed that indole was negative, on KIA agar:H_2S was negative, motility was negative, urease was positive and citrate was positive.

Enterobacter aerogenes produce a mucoid weak lactose fermented colonies, it was centrally dark on EMB agar. Its biochemical tests showed that catalase was positive , oxidase was negative, MR was negative,VP was positive, indole was negative , on KIA agar: H_2S was negative , motility was positive, urease was negative , and citrate was positive for all .

٣.٥.٢.٢. *Staphylococcus aureus*

It was gram positive cocci arranged in clusters which produced a large hemolytic golden yellow colonies in appearance.Its biochemical tests were catalase positive, oxidase negative, coagulase positive, mannitol fermentation positive, motility negative.

٣.٥.٢.٣. *Enterococcus faecalis*

It was gram positive cocci arranged as small chains, mostly non hemolytic ,grew on blood and MacConkey agars as faint colonies. It was able to grow in broth containing ٦.٥ % NaCl.

The biochemical tests of them were catalase negative, oxidase negative, esculin test positive and motility negative .

٣.٥.٢.٤. Viridans Streptococci

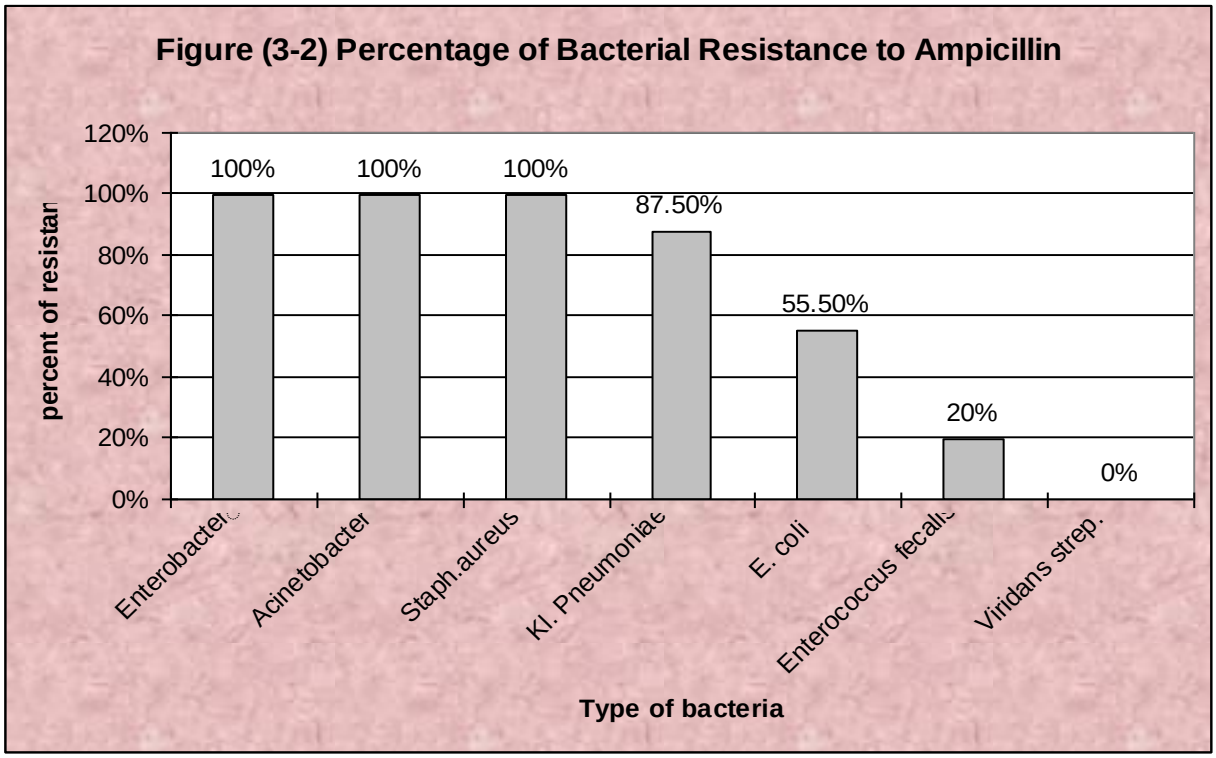
They were gram positive cocci arranged as short chains, mostly α-hemolytic. Most grew well on blood agar but failed to grow on MacConkey bile-salt agar. It was not able to grow in broth containing ٦.٥ % NaCl . The biochemical tests of it were catalase negative, oxidase negative, esculin test negative .

Table (٣-١١) The Diagnostic and Biochemical Test of *Staph.aureus*, *Enterococcus faecalis* and Viridans Streptococci.

Test	***Staph.aureus***	***Enterococcus faecalis***	**Viridans streptococci**
Morphology	Clusters	Pairs,short chains	Short chains
Haemolysis	β-haemolysis	Non haemolytic	α-haemolytic
Catalase	+	–	–
Oxidase	–	+	+
Coagulase	+	–	–
Esculin	–	+	–
Mannitol fermentation	+	–	–
Growth on MacConkey	–	+	–
Growth in ٦.٥٪ Nacl	–	+	–

٣.٦. Effect of Antibiotics on Bacterial Isolates

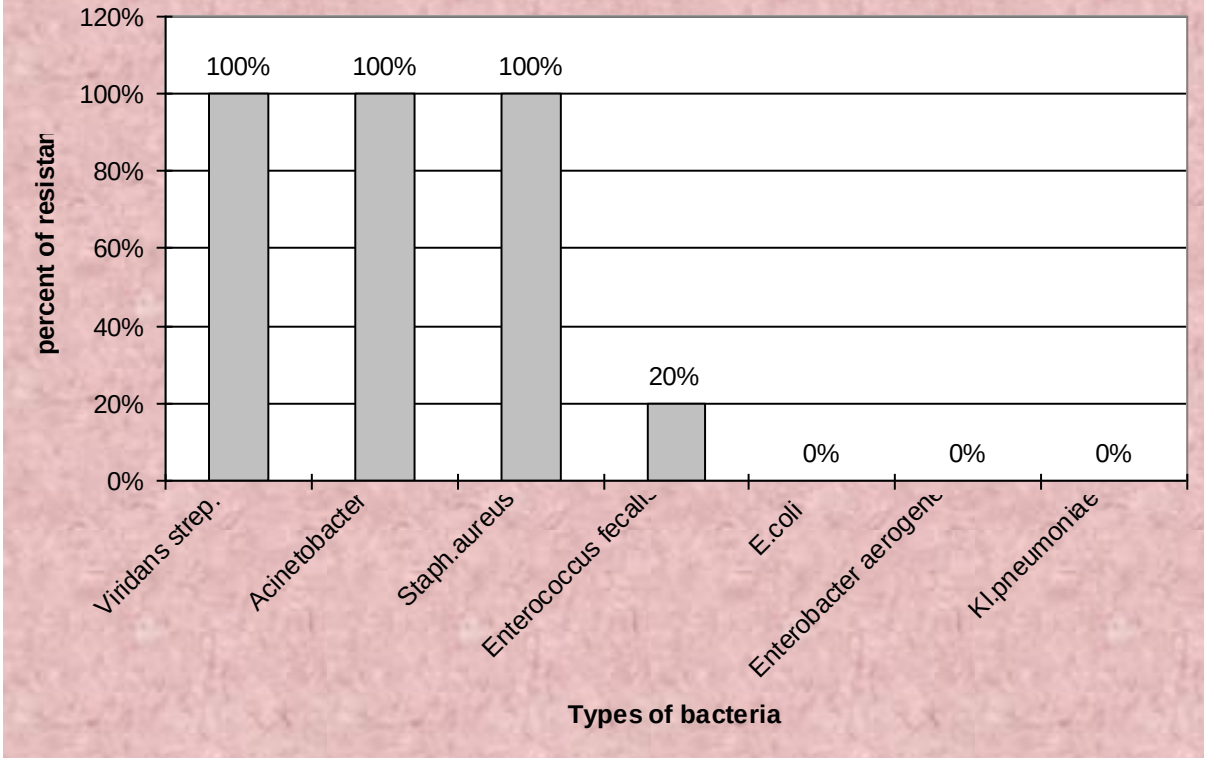
Regarding ampicillin , ١٠٠ % of *Acinetobacter , Staph.aureus ,* and *Enterobacter* were resistant and these results were correlated with results obtained by Savov *et.al.,* (٢٠٠٣), Rashmi *et.al.,* (٢٠٠٥) and Ohara *et.al.,*(١٩٩٨)who observed that these bacteria had high resistance to ampicillin. In addition ٥٥.٥ % of *E.coli* , ٨٧.٥ % of *Klebsiella pneumoniae* were resistant to ampicillin and these results agreed with the results obtained by Oteo *et.al.,*(٢٠٠٥) who found that ٥٩.٩ % of *E.coli* were resistant to ampicillin and Bergeron *et.al.,*(١٩٨٨) who found that ١٠٠ % of *K.pneumoniae* were resistant to ampicillin that caused severe biliary tract infections , ٢٠ % of *Streptococcus fecalis* were resistant to ampicillin. This result agreed with the results obtained by Bergeron *et.al.,* (١٩٨٨) who found that ١١.١ % of *Strep.fecalis* resist to ampicillin. One isolate of Viridans streptococci was sensitive to ampicillin , this result agreed with the results obtained by Prabhu *et.al.,*(٢٠٠٤) who found that high rate susceptibility of Viridans group streptococci were sensitive to ampicillin . Generally ٧٠.٦ % of bacteria isolated from patients in this study were resistant to ampicillin and these results are in agreement with the results obtained by Al-Harbi *et.al.,*(٢٠٠١) who found that ٧٧.٣ % of bacteria with biliary tract infections were resistant to ampicillin. The resistances of these bacteria is mostly due to either production of β-lactamase enzyme or lack of penicilln receptors on cell wall or even alteration in their permeability to β-lactam antibiotics (Yu *et.al.,*١٩٩٩).



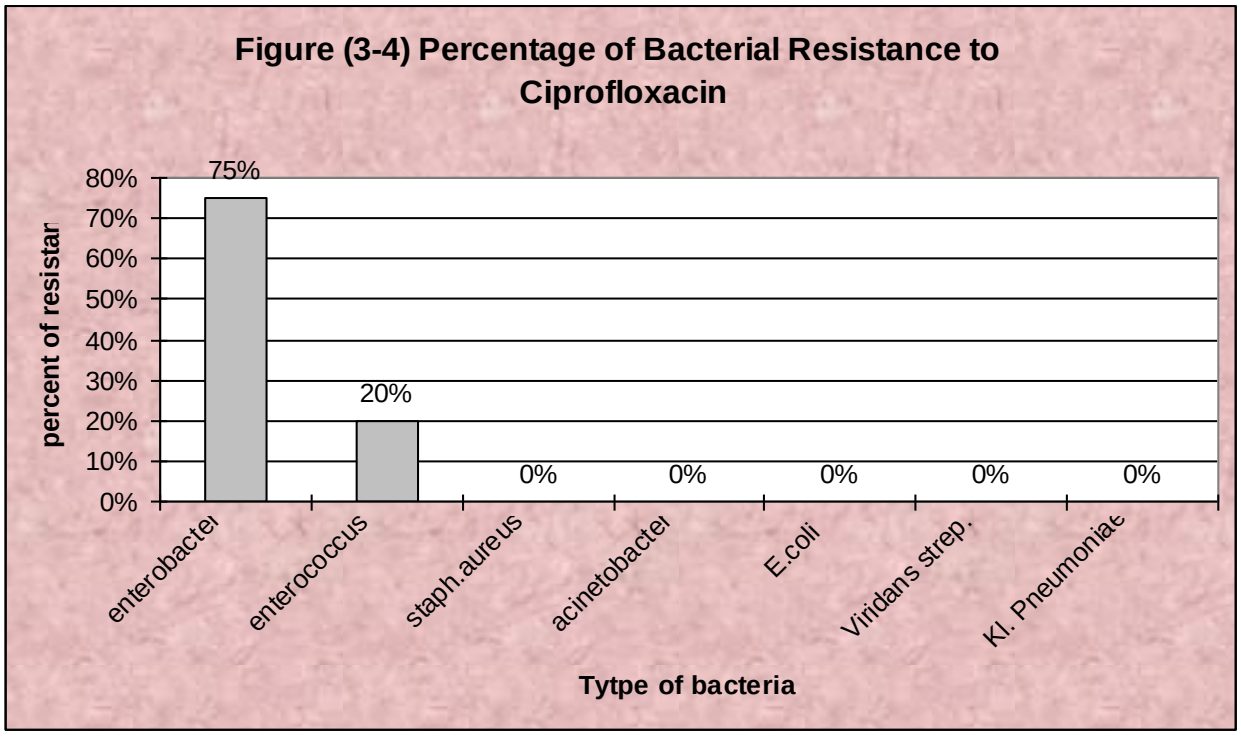
Regarding gentamicin, ١٠٠ % of *Acinetobacter, Staph. aureus,* and Viridans streptococci were resistant to gentamicin, these results agreed with the results obtained by Fernandez-Cuenca *et.al.,*(٢٠٠٤) and Rashmi *et.al.,*(٢٠٠٥) and Smith *et.al.,*(٢٠٠٤) who showed that these bacteria had high resistance to gentamicin. In addition, all isolates of *E.coli, Klebsiella pneumoniae, Enterobacter* were sensitive to gentamicin. These results agreed with the results obtained by Tonkic *et.al.,*(٢٠٠٥), Karlowsky *et.al.,*(٢٠٠٤) and Petrov *et.al.,*(٢٠٠٥) who found that ٥ % of *E.coli* were resistant to gentamicin, more than ٩٠ % of *Klebsiella pneumoniae* were sensitive to gentamicin while ٢٨.٥ % of *Enterobacter* were resistant to gentamicin.

٢٠ % of *Enterococcus fecalis* were resistant and this agreed with the results obtained by Sanchez-Mohina *et.al.*,(٢٠٠٤) who found that ٣٢ % of *Enterococcus fecalis* were resistant to gentamicin. Generally ٢٠.٨ % of bacteria isolated from patients in this study were resistant to gentamicin but Al-Harbi *et.al.*,(٢٠٠١) showed that only ٤.٥ % of bacteria isolated from patients with cholelithiasis were resistant to gentamicin. The production of aminoglycoside modifying enzyme is the most important mechanism of its resistance commonly due to plasmid transfer but the alteration in cell wall permeability is another cause of resistance (Mims *et.al.*,٢٠٠٤).

Figure (3-3) Percentage of Bacterial Resistance to Gentamicin

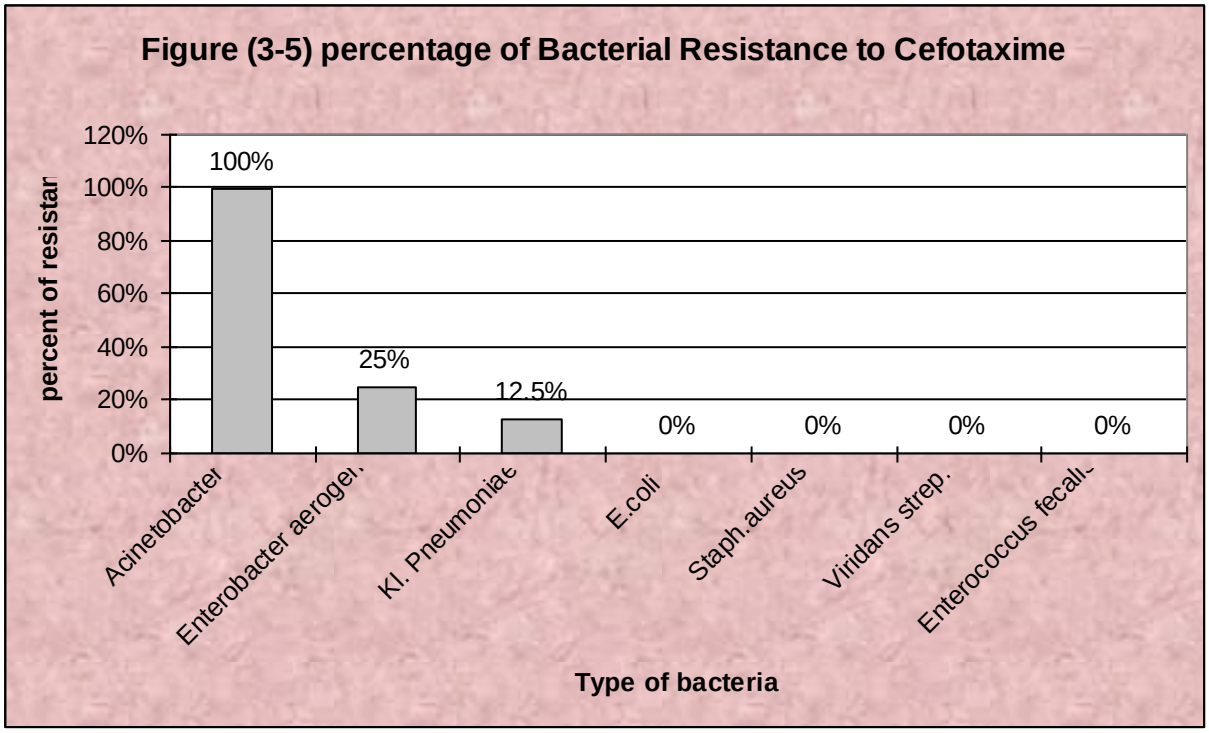


Regading ciprofloxacin, all isolates of *E.coli, Acinetobacter, Klebsiella pneumoniae, Staphylococcus aureus* were sensitive to ciprofloxacin but Tonkic *et.al.*,(٢٠٠٥) found that ٤.٤ % of *E.coli* and ٤ % of *Klebsiella pneumoniae* were resistant to ciprofloxacin and Oteo *et.al.*,(٢٠٠٥) showed that ١٩.٣ % of *E.coli* were resistant, Fernandez-Cuenca *et.al.*,(٢٠٠٤) observed that less than ٢٠ % of *Acinetobacter* were resistant to ciprofloxacin , and Rashmi *et.al.*(٢٠٠٥) found that ciprofloxacin is the drug of choise against *Staph.aureus* . ٧٥ % of *Enterobacter* were resistant to ciprofloxacin ,this is in accordance with the results of Ohara *et.al.*,(١٩٩٨) who found that this bacteria had high rate of resistance to ciprofloxacin . ٢٠ % *Streptococcus fecalis* were resistant to ciprofloxacin while Lynette and Jaykus, (٢٠٠٤) found that only ٥ % of *Strep.fecalis* were resistant but Karlowsky *et.al.*,(٢٠٠٤) observed that ٥٢.١ % of *Strep.fecalis* were resistant. The alone isolate of Viridans Strep. was susceptible to ciprofloxacin. This results is in agreement with the results of Seppala *et.al.*,(٢٠٠٣). Generally ٢٩.٢ % of bacteria isolated from patients in this study were resistant to ciprofloxacin .The resistance rate to ciprofloxacin was increased this may be due to abuse of antibiotics leading to transferring the resistant through genetic factors such as plasmids and transposons or due to changing in cell wall permeability (Mims *et.al.*,٢٠٠٤).



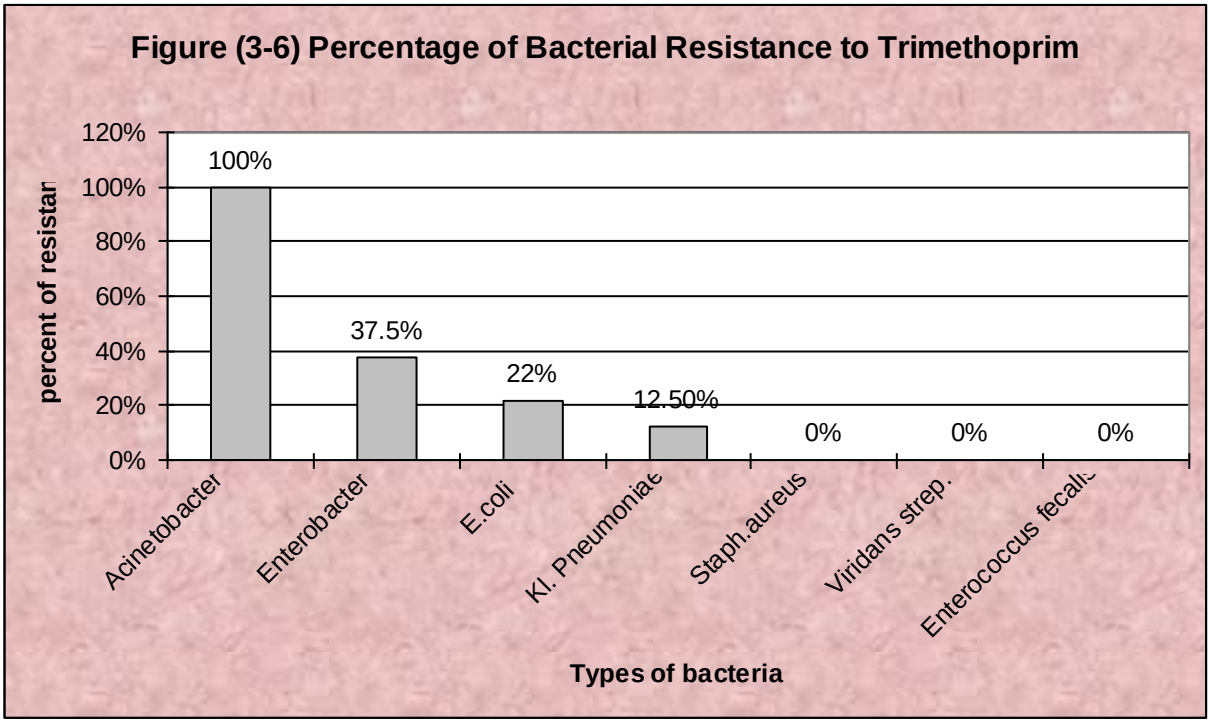
Regarding cefotaxime ,all isolates of *Staph.aureus,* Viridans strep., *Enterococcus* and *E.coli* were susceptible to cefotaxime and this is in agreement with the results obtained by Karlowsky *et.al.*,(٢٠٠٤) who showed that these bacteria are highly susceptible to cefotaxime. ١٠٠ % of *Acinetobacter* were resistant to cefotaxime , and this is in agreement with the results obtained by Blahova *et.al.*,(٢٠٠١) who found that all isolates of *Acinetobacter* were resistant to cefotaxime . ٢٥ % of *Enterobacter,* ١٢.٥ % of *Klebsiella pneumoniae* were resistant to cefotaxime, and this is in agreement with the results of Mathai

et.al.,(٢٠٠١) and Lee *et.al.*,(٢٠٠١) who found that ٢٠.٤ % of *Enterobacter* were resistant to cefotaxime and ٢٠ % of *Klebsiella pneumoniae* were resistant to cefotaxime. Generally ١٧.٦ % of bacteria isolated from patients in this study were resistant to cefotaxime.This due to production of extracellular materials with low affinity to β-lactam antibiotics (Katzung, ٢٠٠٢).



Regarding trimethoprim , ١٠٠ % of *Acinetobacter* were resistant to trimethoprim , this result differs from the results of Frenandez – Cuenca *et.al.*,(٢٠٠٤) who found that less than ٢٠ % of *Acinetobacter* were resistant to trimethoprim. *Enterobacter* ٣٧.٥ % were resistant to trimethoprim while Ohara *et.al.*,(١٩٩٨) who found that the organism was susceptible to trimethoprim-sulfamethoxazole . The alone isolate of *staph.aureus* was susceptible to trimethoprim ,this result agrees with

the results obtained by Stratchounski *et.al.*,(٢٠٠٥) who found that ٠.٨% of *Staph.aureus* were resistant to trimethoprim . ١٢.٥ % of *Klebsiella pneumoniae* were resistant to trimethoprim and ٢٢.٢ % of *E.coli* were resistant to trimethoprim , and this is in agreement with the results of Tonkic *et.al.*,(٢٠٠٥) who observed that ١٣.٣ % of *Klebsiella pneumoniae* were resistant but Oteo *et.al.*,(٢٠٠٥) found that ٣٢.٦ % of *E.coli* were resistant. The alone isolate of Viridans streptococci was susceptible to trimethoprim, this result agreed with the results of Karlowsky *et.al.*,(٢٠٠٤) who found that the Viridans group streptococci were susceptible to trimethprim-sulfamethoxazole . All isolates of *Enterococcus* were sensitive to trimethprim and this result agreed with these obtained by El-kholy *et.al.*, (٢٠٠٣). Generally ٢٩.٢٪ of bacteria isolated from patients in this study were resistant to trimethoprim, this resistance rate emerged due to previous longer use of it leading to pumping it from the resistance cell (Murray *et.al.*,١٩٩٩).

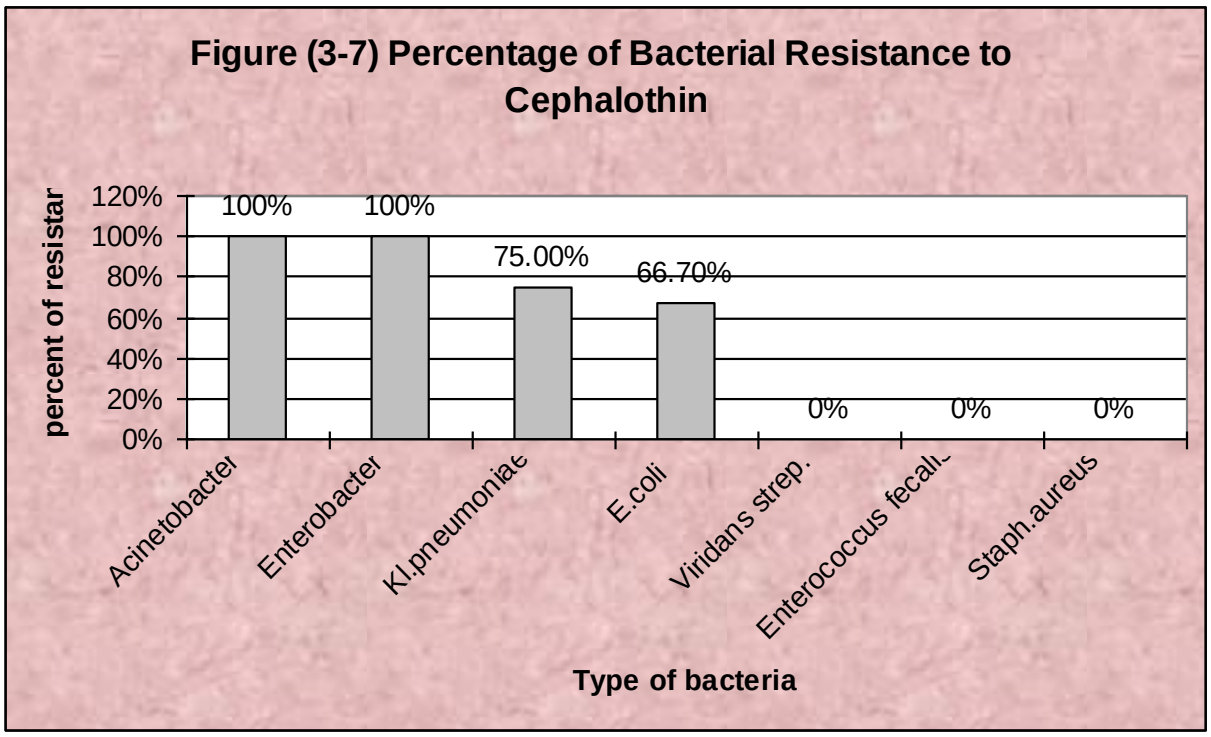


Regarding cephalothin,١٠٠٪ of *Acinetobacter* and *Enterobacter* were resistant to cephalothin , and this result agreed with the results of Fernandez-Cuenca *et.al.*, (٢٠٠٤) and ManFredi *et.al.*, (٢٠٠١) who showed that these bacteria were highly resistant to cephalothin .

٧٥ % of *Klebsiella pneumoniae* and ٦٦.٧ % of *E.coli* were resistant to cephalothin. These results can be compared with the results of Yeh and Chi, (٢٠٠١) who observed that ٧٢ % of *E.coli* were resistant,the resistant rate of *Klebsiella pneumoniae* range from ٢٤-٤١ % .

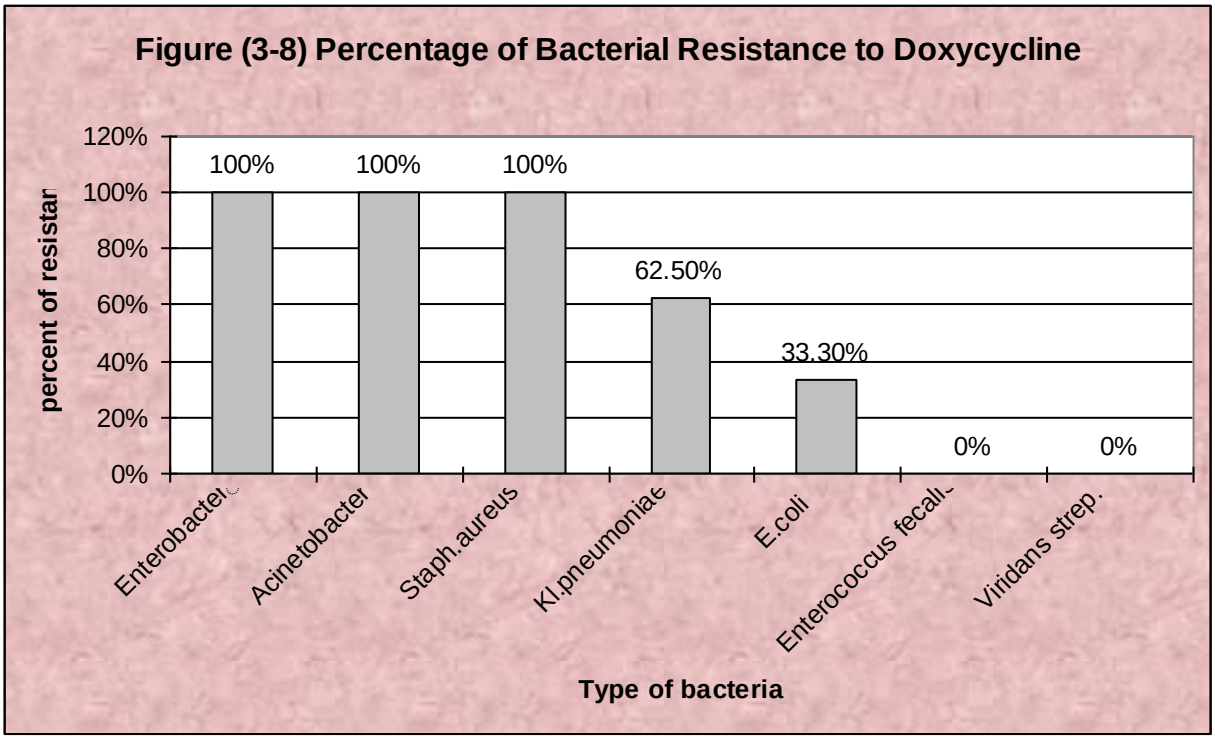
All isolates of *Enterococcus, Staph.aureus* and Viridans streptococci were sensitive to cephalothin , and this is in agreement with the results of Astra and Amanuel, (٢٠٠١) who found that the gram

positive cocci were highly resistant to cephalothin.Generally ٤٤.١٪ of bacteria isolated from patients were resistant to cephalothin. Antibiotic resistance is linked to prior exposure to antibacterial drugs because the first and second generation cephalosporins are commonly prescribed for hospitalized patients (Liu *et.al.*,١٩٩٩).



Regarding doxycycline, ١٠٠ % of *Enterobacter, Staph.aureus* and *Acinetobacter* were resistant to it, and this was in agreement with the results obtained by Zhou *et.al.*,(٢٠٠٣), El-kholy *et.al.*, (٢٠٠٣) who found that these bacteria were resistant to doxycycline while Fernandez-Cuenac *et.al.*,(٢٠٠٢) showed that ٣٢ % of *Acinetobacter* were resistant to doxycycline . In addition , ٣٣.٣ % of *E.coli* and ٦٢.٥ % of *Klebsiella pneumoniae* were resistant . These results can be`compared with the results of Leegard *et.al.*, (١٩٩٩) and Aggarwal

et.al.,(٢٠٠٣) who found that half of these bacteria were resistant to doxycycline. All isolates of *Enterococcus faecalis* and Viridans streptococci were sensitive to doxycycline,and these results were in agreement with Low *et.al.*,(٢٠٠١)and Feres *et.al.*,(١٩٩٩) who observed that these bacteria were highly sensitive to doxycycline . Generally ٦٤.٧ % of these bacteria isolated from patients were resistant to doxycycline. This resistance was transported by a plasmid, although it is a new drug with complete absorption and not affected by gut flora (Murray *et.al.*١٩٩٩).



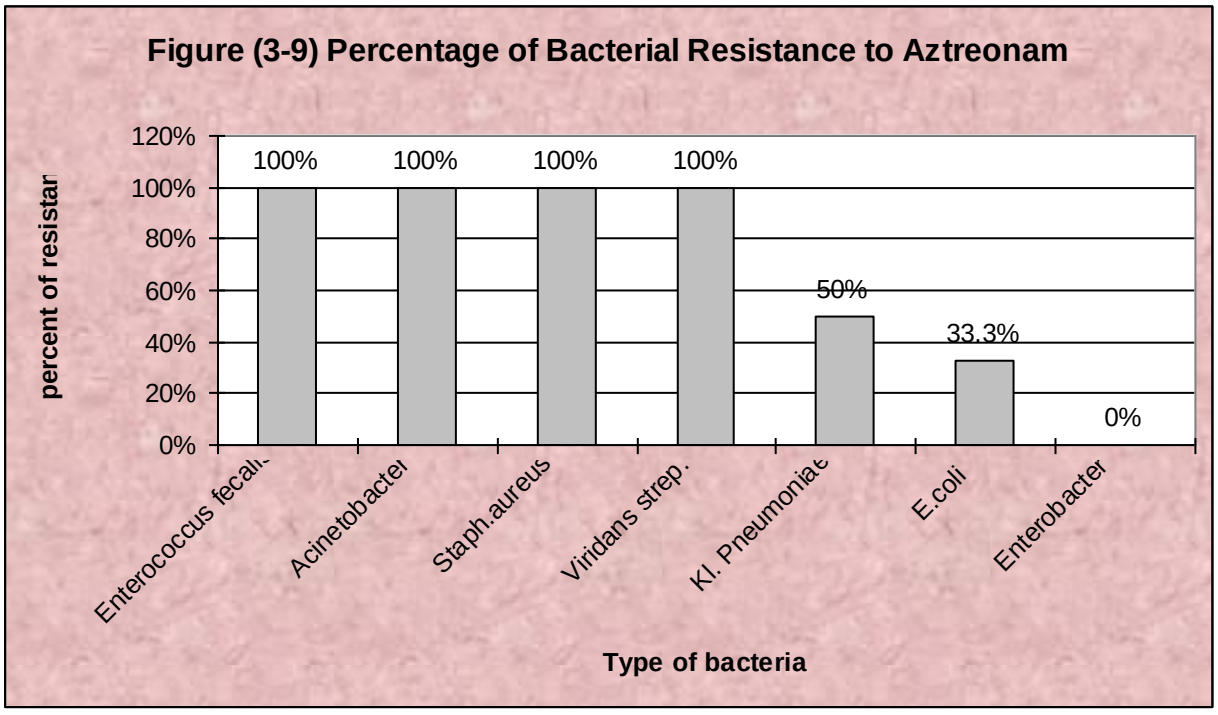
Regarding aztreonam,١٠٠٪of *Acinetobacter,Staph.aureus,*Viridans strepcocci and *Enterococcus fecalis* were resistant to aztreonam .This result agreed with the result of El-kholy *et.al.*, (٢٠٠٣) and Fuchs *et.al.*,(١٩٩٨) who showed that this drug had poor activity against these

bacteria . ٣٣.٣ % of *E.coli*, ٥٠ % *Klebsiella pneumoniae* were resistant to aztreonam while El-kholy *et.al.*, (٢٠٠٣) showed that

٤٤ % of *E.coli* and ٦٤ % of *Klebsiella pneumoniae* were resistant to aztreonam . All isolates of *Enterobacter* were sensitive to aztreonam, and this result agreed with the results of Capdevila *et.al.*,(١٩٩٨) who observed that *Enterobacter* was highly susceptible to aztreonam.

Generally ٤٤ % of bacteria isolated from patients were resistant to aztreonam. Aztreonam is a bactericidal antibiotic, which interfere with the synthesis of the bacterial cell wall. It has an excellent activity against major gram negative pathogen, it is not absorbed orally, it is of limited use because it is expensive and not available (Kapour and Gathwala ,(٢٠٠٤).

Figure (3-9) Percentage of Bacterial Resistance to Aztreonam



٣.٧. The Effect of Bile Salts (Deoxycholate) on the Growth of Bacterial Isolates

Deoxycholic acid decreases biliary secretion of cholesterol and is therefore used for the dissolution of cholesterol gallstones (Sauter *et.al.*, ٢٠٠٤). In the present work we studied the activity of deoxycholate against gram negative and gram positive bacteria,which showed that the *Acinetobacter* was more resistant to this reagent at all concentration , whereas other types showed variable degree of resistance (*E.coli, Klebsiella pneumoniae, Enterobacter, Enterococcus faecalis,* Viridans streptococci, *Staph.aureus*).

Figure (٣-١٠) shows the effect of bile salts (deoxycholate) at different concentrations on the growth of bacterial isolates.All isolates showed decrease in the optical density propotionally to the increase in the concentration of deoxycholate. So we concluded that the advantage of this therapy in addition to dissolution of gallstones also had an effect on bacteria if it was associated with the formation of stone or with biliary tract infection. Also the advantage of ursodeoxycholic acid over the other type of bile acids are lower dosage, shorter treatment period required and it is virtually free of any side effect (Erlinger *et.al.*,١٩٨٤).

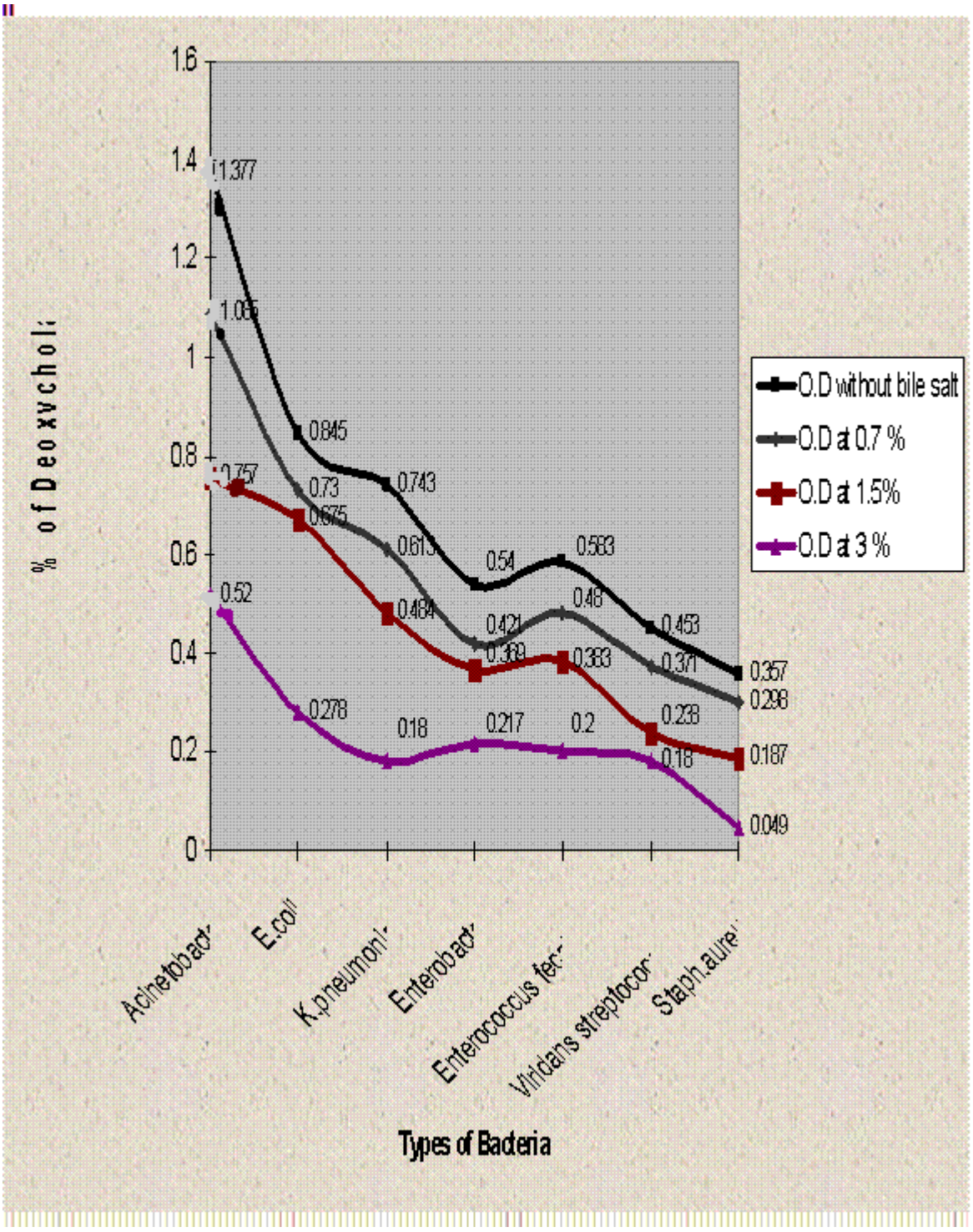


Figure (٣-١٠) The Effect of Bile Salt (Deoxycholate) on the Growth of Bacterial Isolate.

٣.٨. The Effect of Cholesterol on the Growth of Bacteria

Table (٣-١٢) shows the effect of cholesterol (٢٥٠ mg/dl)(the lower limit of cholesterol concentration in the pure cholesterol stone) on the growth of bacterial isolates (gram negative and gram positive bacteria). No growth was detected on the plate cultivated with gram negative when treated with cholesterol, whereas no effect was noted on gram positive when compared to the control.

This effect may be due to the structure of cell wall composition in gram positive which consists mainly of peptidoglycan which show a degree of resistance to cholesterol. Although several factors were known to trigger nucleation and /or growth of cholesterol crystals, the role of bacterial etiology was usually neglected. Recently, it has been reported that bacterial DNA has been detected in pure cholesterol stones with negative bacterial cultures (which were not previously believed to be related to bacteria using polymerase chain reaction (PCR) (Xiao-Ting *et.al.,*١٩٩٨);(Kawai *et.al.,* ٢٠٠٢) who concluded that the gram positive cocci were associated with the formation of pure cholesterol stones, while gram negative rodes were associated with the formation of brown pigment stones, and gram positive cocci with gram negative rods were associated with the formation of mixed cholesterol stones.

Table (٣-١٢) Effect of Cholesterol on the Growth of Bacteria.

Bacteria	Control	Growth with cholesterol(٢٥٠ mg/dl)
Gram positive	Positive(heavy growth)	Positive growth
Gram negative	Positive(heavy growth)	Negative growth

٤.١. Conclusions

١- Gallstone disease is more in females than males and secondly the higher incidence of gallstone is in the age group ٤٠-٦٠ years.

٢- Morphological and chemical analysis of gallstone demonstrates that the predominant type of gallstone is cholesterol stone rather than pigment stone.

٣- The patients with symptomatic gallstones are commonly presented with chronic cholecystitis, and least with complications like pancreatitis, cholangitis…..etc.

٤- Age over ٥٠ years is the only significant preoperative factor associated with positive bile cultures.

٥- The bacteria isolated from brown pigment stones are gram negative rods, while in mixed cholesterol stone the bacteria consist of gram negative rods and gram positive cocci. No bacteria are isolated from pure cholesterol stone.

٦- Gentamycin and cefotaxime are the most effective antibiotics against isolated bacteria.

٧- In vitro, pure cholesterol has no effect on gram positive cocci while gram negative rods are sensitive to it, so gram positive cocci may be associated with the formation of completely pure cholesterol stones.

٨- Deoxycholic acids have an effect on growth of isolated bacteria in addition to its ability to dissolve gallstone.

٤.٢. Recommendations

١- In view of microflora of the gallbladder and the susceptibility pattern of our isolates we would suggest that antibiotics prophylaxis recommended for cholecystectomy.

٢- The role of bacterbilia in the surgical management of cholelithiasis requires further study.

٣- More work is needed to assess the detailed mechanisms of stone formation which might reveal some of the yet undiscovered facts in gallstone pathogenesis.

٤- Introduction of new molecular techniques for the detection of fastidious microorganisms other than bacteria isolated by conventional cultural technique associated with gallstones is recommended.

٥-In the light of conclusions there is a need to recommend to increase utilization of bile acid therapy in the treatment of gallstone disease due to dual activity on the bacteria and dissolution of gallstones.

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