جامعة بابـل

كلية الطب

دراسة بكتيرولوجية على إلتهاب الزائدة الدودية

رسالة

مقدمة إلى فرع الأحياء المجهرية- كلية الطب -جامعة بابل كجزء من متطلبات نيل

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بكالوريوس - علوم - ١٩٩٥م

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بسمِ اللهِ الرَّحمِن الرَّحِيمِ

﴿ وَءَاتَكُم مِّن كُلِّ مَا سَأَلتُمُوهُ وَإِن تَعُدُّوا نِعمَتَ آللهِ لا تُحصُوهَا إِنَّ الإِنسان لَظَلُوم' كَفَارٌ﴾.

صدق الله العلي العظيم

سورة إبراهيم

آية (٣٤)

University of Babylon

College of Medicine

BACTERIOLOGICAL STUDY REGARDING ACUTE APPENDICITIS

A THESIS SUBMITTED TO THE COUNCIL OF THE COLLEGE OF MEDICINE, UNIVERSITY OF ,BABYLON

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE MASTER DEGREE OF SCIENCE IN MEDICAL MICROBIOLOGY

SUBMITTED BY

Faiz Kamil Ktab – AL Salami

A.H ١٤٢٧A.D ٢٠٠٦JUN

الخلاصة

تضمنت هذه الدراسة ١١٠ مريضا كانوا مصابين بالتهاب الزائدة الدودية الحاد وزاروا مستشفى- الفرات الاوسط - وحدة الجراحة البولية وكانت اعمارهم تتراوح من ١-٦٠ سنة خلال فترة ١٠ اشهر(من شهر تشرين الاول ٢٠٠٤- الى تموز ٢٠٠٥) وكان ٦٩(٦٢.٧%) منهم ذكور و ٤١ (٣٧.٣%) منهم أناث وأظهرت النتائج إن الفئة العمرية الأكثرعرضة للاصابة بين(١١-٢٠) سنة من كلا الجنسين منهم ٣٥(٣١و٨%) ذكوراً و٢١(١٩%) اناثاً. فمن الناحية السريرية كانوا يعانون الآم الجهة اليمنى مع الم بصورة عامة وحمى وفقدان الشهية وغثيان وإمساك وأحيانا حالات إسهال وصداع وصعوبة التبول. أما التشخيص المختبري فقد تضمن حساب عدد كريات الدم البيضاء و C. Reactive protein وفحص الادارا العام بالاضافة الى فحوصات اخرى مثل الفحص بجهاز السونار. وأظهرت النتائج بأن هناك ١١١ عزلة تم تشخيصها من ١١٠ مريضا، وكانت البكتريا الممرضة ٨٧ عزلة(٧٨.٣%) هوائية و ٢٤(٢١.٧%) عزلة لا هوائية، وقد تم الحصول عليها من ١١٠ مسحة، اما بالنسبة لنتائج زرع المسحات كانت موجبة ٩٠(٨١.٨%) مريض فقط وفي ٢٠(١٨.٢%) مريضا كانت سالبة، وكانت انواع البكتريا الظاهرة في الزرع ونسبتها كما يلي ٣٦(٣٢.٦%) *E. coli* و٢١(١٨.٩%) *Bacteroides* spp و ١٨(١١.٢%) *Klebsiella pneumoniae* ١١(٩.٩%) *Pseudomones aeruginose* و٧ (٦.٣%) *Citrobacter freundii* و٥ (٤.٥%) كل من *Salmonella typhi* و Proteus *mirablis* و ٤(٣.٦%) *Enterobacter aerogenes*و ٢(١.٨%) *Peptostreptococus* spp و ١(٠.٩%) كل من *Staphylococcus aureus* و *Clostridium perfringes* . وقد اضهرت الدراسة وجود حالات مزدوجة(ثنائي الاصابة) ٢١ عزلة حيث كانت فيه بكتريا*E.coli* مع بكتريا لاهوائية هي الاكثر سيادة. وقد اضهرت النتائج زيادة في حالات الاصابة بألتهاب الزائدة الدودية خلال اشهر الصيف وقلت خلال أشهر الشتاء. حيث سجلت اعلى إصابة

٢٦(٢٣.٦%) خلال الشهر السابع و١٨(١٦.٢%) أصابه خلال الشهر السادس. واقل نسبة ٤(٣.٦%) خلال شهر كانون الثاني. اما بالنسبة لاختبارات الحساسية تم استخدام طريقتين وهما طريقة الانتشار بالاقراص وطريقة التخافيف الدقيقة لقياس التراكيز المثبط الادنى. اما بالنسبة لتأثير بعض المضادات الحيوية على تلك العزلات البكترية. فقد أضهرت النتائج ان مقاومة العزلات للاموكساسلين(٨٤.٦%) وللكرومايسن(٦٢%) وللاميكاسين(٥٧.٦%) وللسيروفلوكساسلين(٣٨.٧%) وللسينوتكسام(٤١.٤%) وللسيفوكسيم(٤٧.٧%) وللمثبريم(٨٢.٨%) وللفانكومايسين(٩٦.٣١%) وللنورفلوكساسلين(٧٦.٥%) وللتترسايكلين(٧٦.٥%).

قرار لجنة المناقشة

نحن أعضاء لجنة المناقشة نشهد بأننا اطلعنا على الأطروحة الموسومة من الطالب فائز كامل كتاب السلامي وقد ناقشنا الطالب في محتوياتها، وفيما له علاقة بها، ووجدنا بأنها مستوفيه بالقبول بدرجة (أمتياز) لنيل درجة الماجستير في علوم الحياة / الأحياء المجهرية.

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Summary

The subjects of this study were ١١٠ patients having acute appendicitis who were referred to kufa Teaching Hospital, Department of urology whose ages ranged from ١ to ٦٠ years old. Those subjects were investigated through a period of ١٠ months from October, ٢٠٠٤ to July, ٢٠٠٥. Patients were ٦٩(٦٢.٧٪) males and ٤١(٣٧.٣٪) females. The age group of both sexes being more susceptible for appendicitis ranged from (١١-٢٠) yeas old. The clinical features of patients being observed by physicians were studied. Those were right iliac fosia pain, generalized abdominal pain, fever, loss of appetite, nausea, constipation, diarrhea, headache and dysuria. White blood cells count, C. reactive protein and general urine analysis were also studied, in addition to abdominal ultrasongraphy and computer tomography (CT). A total of ١١١ bacterial isolates were identified from inflammed appendix of ١١٠ patients with acute appendicitis. Positive bacterial cultures were detected in ٩٠(٨١.٨٪) patients while ٢٠(١٨.٢٪) patient showed no growth. The aerobic bacteria accounted for ٨٧(٧٨٣٪) isolates whereas anaerobic were ٢٤(٢١.٦%) isolates. Gram negative bacteria were presented in ١٠٧(٩٦.١٪) while Gram positive bacteria were accounted ٤(٣.٦%). *Escherichia coli* were the predominant pathogens, since it accounted for ٣٦(٣٢.٤%) of all isolates followed

by *Bacteroides* spp ٢١(١٨.٩%), *Klebsiella pneumoniae* ١٨(١٦.٢%), *Pseudomonas aeruginosa* ١١(٩.٩%), *Citrobacter freundii* ٧(٦.٣٪), *Salmonella typhi* ٥(٤.٥٪), *proteus mirabilis* ٥(٤.٥٪), *Enterobacter aerogenesa* ٤(٣.٦٪), *peptostreptococcus* ٢(١.٨٪), *Staphylococcus aureus* ١(٠.٩٪) and *Clostridium perfringns* ١(٠.٩٪). Mixed cultures were detected in ٢١ cases, in which more than one organism were detected. Most of mixed bacterial isolates were anaerobic with aerobes (١٣, ٦١.٩٪), in which *Escherichia coli* was the common (١٥, ٧١.٤٪).

Aremarkable an increased occurance of acute appendicitis was observed at warm season, while the incidences were decreased during the winter season. The peak of appendicitis occurance was observed during July ٢٦(٢٣.٦٪) and June ١٨(١٦.٤%), whereas the lowest frequency of appendicitis was observed in January ٤(٣.٦٪).

In this study two methods disk diffusion method and minimum inhibitory concentration were used to determine the susceptibility of bacterial isolates to ١٣ antibiotics. The results showed that (٨٤.٦٪) of total isolates were resistant to (٩٦.٣٪), to vancomycin, (٩٥.٤%) to, (٨٤٪) to, tetracycline (٨٣.٨٪) to, cefixime, (٧٦.٥٪), to norfloxacin, (٦٢٪) to, amoxycillin, (٥٨.٥%) to Garamycin, (٤٦.٨%), to trimethoprime (٤٢.٣%), to cefotaxime, to amikacin, (٣٨.٧٪), to ciprofloxacin.

CERTIFICATION

I certify that this thesis was prepared under my supervision at the College of Medicine, University of Babylon, as a partial fulfillment of the requirements for the degree Master of Science in **Medical Microbiology**.

Advisor

Signature

Prof. Dr. Habeeb Sahib. Naher

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Date: / / ٢٠٠٦

In view of the available recommendation, I forward this thesis for debate by the examining committee

Signature

Ass. Prof. Dr. Mohammed Sabri AL- Saeed

M. Sc., ph. D. Microbial

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Date: / /٢٠٠٦

Examination committee certificate

We, the examining committee, certify that we have read this thesis "Bacteriological study on acute appendicitis" and have examined the student Faiz Kamil Al-Salami in its contents, and that in our opinion it is adequate as a thesis for the degree of master in biology (microbiology).

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HOLY IMAM

AL- HUJJA

TO MY DEAR WIFE

TO MY DEAREST DAUGHTERS

SARA, ZAHRA AND FATMA

List of Contents

Chapter one/Introduction and Literature Reviews

Chapter Two / Materials and Methods

Chapter Three/ Results and Discussion

Conclusions and Recommendations

List of Tables

List of Figures

Abbreviations

ART	Antimicrobial resistance testing
BHI	Brain heart infusion
C	Centigrade
CFA	Colonization factor antigen
CFU	Colony forming unit
CM	Cubic millimeter
CT	Computerized tomographic study
EMB	Eosin methylene blue
G	Gram
Hrs	Hours
KIA	Kliglar iron agar
LF	Lactose fermentation
µg	Micro gram
µl	Micro liter
MIC	Minimum Inhibition concentration

ml	Mili liter
mm	Mili meter
NCCLS	National Commetti of Clinical Laboratory Standards
No	Number
Spp	Species
Tmp. Smx	Tri methoprim- sulfamethoxazole
TSI	Triple sugar iron
VP	Vogas proskaur
WBCS	White blood cells
WHO	Word Health Organization

١-١ INTRODUTION:

Appendix is an organ connected to the digestive system and usually referred to as functionless organ in human. It is now well recognized that this organ may play a role in immunity (Zuercher *et al*., ٢٠٠٢).The position of this organ lies in the right lower part of the abdomen. In spite of the minor significance of this organ, it is susceptible for infections as other organs such as hepatitis, pneumonitis, cholecystitis, tonsillitis and encephalitis (Schwarts *et al*., ١٩٩٦). Appendicitis has been reported world wide. In USA, it was estimated as ٢٥٠,٠٠٠ case per / year (Addiss *et al*., ١٩٩٠). In Britain

the infection rate was estimated at an average of ٧٠,٠٠٠ case per/ year (Rao *et al*., ١٩٩٨). According the (WHO, ٢٠٠٤). The mortality rates, all around the world are summarized in table (١-١)

There are no accuratel records about appendicitis in Iraq. The general Cases being registered in Najaf General Hospital were ٥٣٠ case per/ year; while in Kufa hospital were ٤٥٠ case per/ year.

Appendix can be infected by a variety of microbes (parasite, viral and bacterial).A surgical operation should be done to remove this organ and to protect the body and maintain its health from complications.

Table (١-١) Country and mortality rate according to WHO, (٢٠٠٤)

Country	Mortality rate per ١ millon people
Banamas	٦.٦٢ deaths
Cuba	٥.١ deaths
Malta	٥ deaths
Denmark	٤.٩ deaths
Eucuador	٣.٨ deaths
Nicargua	٣.٤ deaths
Costarica	٢.٩ deaths
Mexico	٢.٥ deaths
Brazil	٢.٤ deaths
Finland	٢.٢ deaths
Germany	١.٧ deaths
Argentina	١.٣ deaths

South Africa	**١.٢ deaths**
Spain	**١.١ deaths**
Australia	**٠.٦٩ deaths**
Korea	**٠.٦٤ deaths**
Japan	**٠.٥٣ deaths**
Kuwait	**٠.٤٢ deaths**
Egypt	**٠.١٤ deaths**
United Kingdom	**٠.٠٣ deaths**

The great danger following the appendicitis is the rupture of the appendix, leading to release the causative pathogens in to abdominal cavity resulting serious complication (Damjarov *et al*., ١٩٩٨).Such complications could be avoided by abdominal surgical operation particularly in developing countries. It is worthily mentioned that this human organ has been pointous as a concern of bacteriologists. However most studies focused on the diagnosis of the appendicitis. Other studies deal with parasitic infection as it is a reason of appendicitis, but it seems the rare studies have been done regarding the bacteriological infection. Therefore this study was suggested to full fill the following aims:

١-Isolation and identification of bacterial profiles associated with appendicitis

٢-Comparison of bacterial isolates to determine the common

causes of appendicitis.

٣-Detection the susceptibility of isolated bacteria to antibiotics.

١-٢.LITERATURES REVIEW

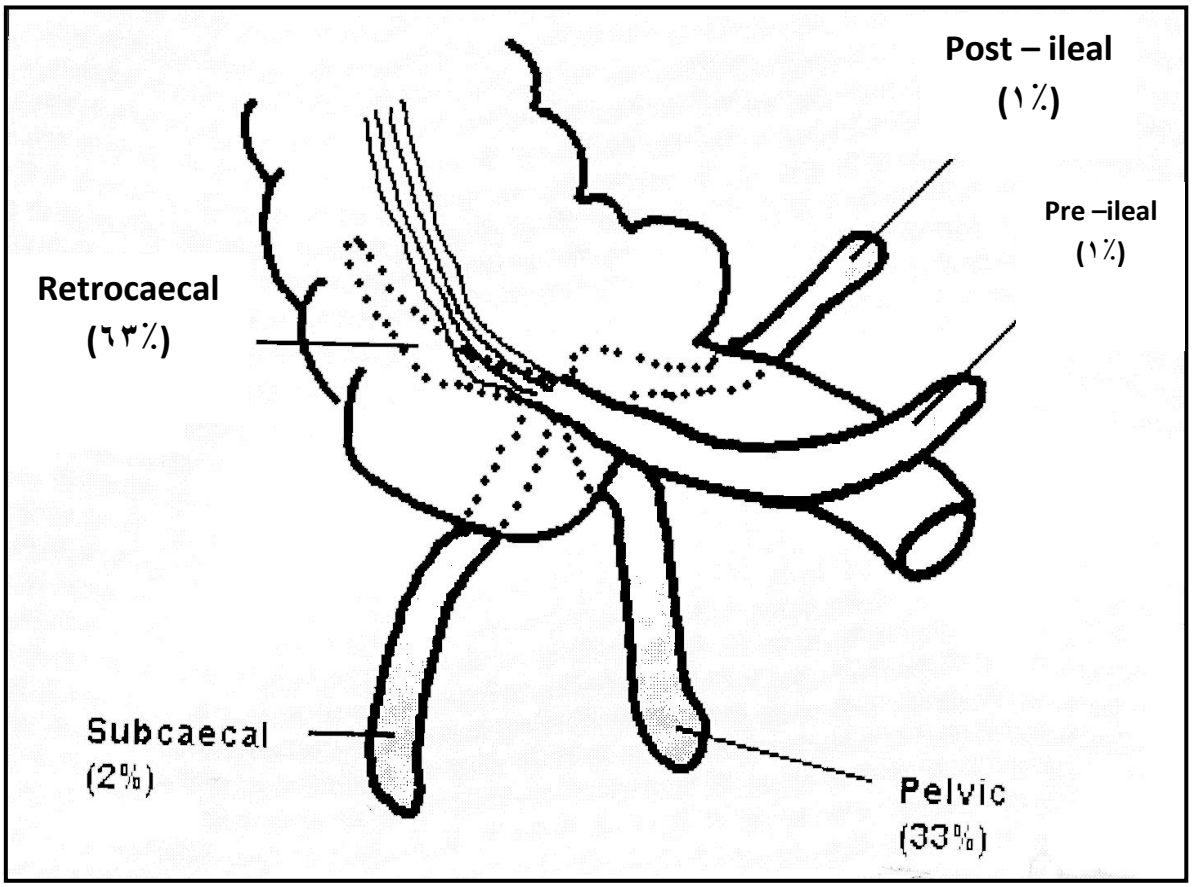
١.٢.١. Appendix

١.٢.١.١. Anatomy

The appendix first becomes visible during embryologic development in the eighth week of fetal life as a protuberance off the terminal portion of the cecum. During both antenatal and postnatal development, the growth rate of the cecum exceeds that of the appendix. Displacing the appendix medially toward the ileocecal valve. The relationship of the base of the appendix to the cecum remains constant, whereas the tip can be found in a retrocecal, pelvic, subcecal, preileal, or right pericolic position. These

anatomic considerations have significant clinical importance in the context of acute appendicitis. (Storer *et al*., ١٩٨٨; Schwartz *et al*., ١٩٩٦).

The appendixes vary in length from less that ١cm to greater than ٣٠ cm. Most appendices are ٤ to ١٠ cm in length. Finger like structure or narrow tubular pouch arising from caecum first part of the large intestine. The appendix is normally found in the right lower part of the abdomen, removed without detrimental effect .The appendix ran be found any where in the abdomen. It may find in retrocaceal in a percentage of ٦٣٪,pelvie ٣٣٪,subcaecal ٢٪,postileal and preileal ١٪ (figure ١-١) (Dohert and Lewis,١٩٨٩; Shelton *et al*.,٢٠٠٣).



(٢٪)

Pelvic
(٣٣٪)

(Fig. ١-١)Diagrame illustrates the location of appendix (Shelton *et al.,* ٢٠٠٣).

١.٢.١.٢. Appendix Function

For many years the appendix was erroneously viewed as a vestigial organ with no know function. It is now well recognized that the appendix is an immumologic organ which actively participates in the secretion of immunoglobulins, particularly IgA (Zuercher *et al*., ٢٠٠٢). Though the appendix is an integral component of the gut associated lymphoid tissue (GALT) system (Craig *et al.*١٩٩٤) lymphoid tissue first appears in appendix about ٢ weeks after birth. The amount of lymphoid tissue increases throughout purberty. Remain steady for the next decade and there begins a steady decrease with age. After the age ٦٠ virtually no lymphoid tissue

remain with in the appendix and complete obliteration of the appendiceal lumen is common (Schwartz *et al*., ١٩٩٦; Wagner *et al*; ١٩٩٦).

The appendix attachment to the intestine when the appendix is blocked it becomes inflamed and results in the condition know as appendicitis. If the blockage continues the inflamed tissue become infected with bacteria and begins to necrotize from alack of blood supply which finally result in appendix perforation (Malone and Shetty, ١٩٩٧; Williams and MarGs., ٢٠٠٣).

The appendixe was regarded as inflamed organ if it has one or more of the following features (Adams *et al*., ١٩٨٨; Davis *et al*., ١٩٩٠).

١- Peritoneum: congestion of the serosal vessels and inflammatory exudates commonly fibrinous or frankly purulent.

٢- Wall: oedema, congestion and swelling of the muscular wall.

٣- Lumen: contain an obstructing faecalith and is distended by purulent material.

٤- Mucosa: hyperaemic swallow and after ulcerated or completely necrotic.

١.٢.٢. Historical Notes on Appendicitis :

Until the end of the ١٩th century appendicitis remained unrecognized, before this time it was comparatively rare disease .but these can be no doubt that it extended even in remote time .for an

acutely in flamed, perforated appendix was found preserved in the mummy or young royal of Egypt (Rains and Capper, ١٩٨٨). According to Mcmurrich in ١٩٣٠ Leonardo davinci in Italia was the first who described and illustrated the appendix on ١٤٤٢. In ١٨٤٨ Henry Hancock presented a paper entitled the infection of the appendix, the condition which is cured by operation .He was the first surgeon to treat peritonitis due to perforated appendix successfully.In ١٨٩٨ The infection aetiology of appendicitis was first proposed by Veillon and Zuber. They emphasized the presence of anaerobes as the case of peritonitis and abscess in appendicitis. Gram negative anaerobic bacilli were the dominate pathogen in ٢١ cases out of ٢٢ suppuration appendicitis (Veillon & Zuber, ١٨٩٨). Dudgeon & Sargent, (١٩٠٥) stated that only one isolate of *Clostridium perfringes* was detected among ٢٧٠ cases. *E-coli,* Streptococci *and Staphylococcus*. Were also isolated as gram negative anaerobic bacilli from ٩٦ case of peritonitis associated with acute perforated appendicitis. In (١٩٠١) Metchuickoff reported three cases of appendicitis due to the presence of *Ascaris lumbricoids* – studies on the incidence of appendical enterobiasis and the possible causal relationship of organism to appenditic have appeared with increasing frequency since ١٩٠٠(Altemeier, ١٩٣٨; Boulose and Cowie, ١٩٧٣).

١.٢.٣. Appendicitis

١.٢.٣.١ Acute Appendicitis

Acute appendicitis is one of the most common causes of abdominal pain often requiring surgical intervention to avoid any suspected complications (Elhag *et al*., ١٩٨٦). Approximately ٧٪ of the population suffer from acute appendicitis during their life time (Wagner *et al*., ١٩٩٦). The etiological factors for acute appendicitis may widely vary (Elhag *et al*., ١٩٨٦; Al-Joubori, ١٩٩٤). The most important factor is the obstruction of lumen, the commonest cause of obstruction was reported to be a fecolith (Majed and Al Bakri, ١٩٨٤). But could be a foreign body (Anderson and Bergdahi, ١٩٧٨; Green *et al*., ١٩٩٤). Appendicitis occurs when the diet is reduced in bulk with diminished cellulose and high protein is taken (Burkitt, ١٩٧١; Gibbons and Houte, ١٩٧٥).

١.٢.٣.٢. Chronic Appendicitis

Although the existence of chromic appendicitis is controversial, most surgenous have managed patients with recurrent abdominal pain that resolved with appendectomy. In some cases the history consists of intermittent right lower quadrant abdominal pain and in fact the appendical pathology demonstrates chronic changes consistent with recurrent inflammation and fibrosis. Some surgeons have suggested that identification of a non filling or partially filling appendix on contrast enema or computer tomographic as well as the failure of contrast to drain from the appendix after few days following contrast administration, is suggestive of chronic or

recurrent appendicitis in the patient with chronic abdominal pain. Although one should perform a through evaluation of the patient with chronic abdominal pain prior to operative inter veration (Drusano *et al*; Weiner *et al*., ١٩٩٥; Schales *et al*., ١٩٩٧).

١.٢.٤ Incidence

١.٢.٤.١. Age Incidence

Appendicitis is rarely encountered before the age of two years. It is unusual encountered the age of one year and becomes increasingly common during childhood and adolescence. The maximum incidence is between the ٢٠ to ٣٠ years, there after there is a gradual decline but no age is exempt (Dohert *et al*., ١٩٨٩; Rain and Capper, ١٩٨٨).

١.٢.٤.٢ Sex Incidence

Sex ratio in acute appendicitis is about ١:١ prior to puberty the frequency in males increases so that the male/female ratio is about ٢:١ between the age of ١٥ to ٢٥ years after which the male incidence gradually declines until the sex related incidence are again equal (Storer *et al*., ١٩٨٨; Benko *et al*., ١٩٩٦).The incidence of

appeudicitis is approximately١.٤ time greater in men than in women while the incidence of primary appendendectomy is approximately equal in both sexes (Dohert and Lewis, ١٩٨٩; Kosloske, ٢٠٠٤).

Primatesta and Coldacre (١٩٩٤) studied the demographic and temporal profiles of emergency appendectomy for age and sex specific admission rates were studied subdividing appendectomy in to three main categories.

١. Emergency appendectomy for acute appendicitis was more common in males that in females peaked in the ١٠-١٩ year age group and declined overtime.

٢.Emergeney appendectomy as the main operation with out appendicitis recorded a diagnosis was more common in women than man (Female male ratio ١.٩:١) peaked at age ١٥-١٧ years and did not decline overtime.

٣. Incidental appendectomy with other operations but with out appendicitis was much common in women (female male ratio ٣:١). Peaked al older ages than the first two groups and declined significantly overtime.

١.٢.٥. Etiology of appendicitis

Although inflammatory disease of the appendix has been recognized as such for more then ١٠٠ years it's aetiology remains a subject of controversy since early of this century consumption of

low fiber diet has been implicated .More recently, this theory has been challenged and the role of infection emphasized (Barker, ١٩٨٥; Larner, ١٩٨٨).

١.٢.٥.١. Obstruction

The presumed etiology is obstruction most commonly afecalith with parasite seen in other parts of the world. Obstruction of the lumen is the dominate causal factor in acute appendicitis .Fecaliths are the usual cause of appendiceal obstruction less common are hypertrophy of lymphoid tissue in spissated barium from previous x-ray studies. Vegetable and fruit seeds and intestinal worm particularly Ascaris also play role in obstruction (Butter, ١٩٨١; Lee *et al*, ١٩٨٥; Blandino *et al*., ١٩٩٠).

Frequency of obstruction rises with the severity of the inflammatory process pecaliths were found in about ٤٠٪ of cases of simple acute appendicitis and about ٦٥٪ of cases of gangreous appendicitis with out rapture while about ٩٪ of cases of gangrenous appendicitis with rapture (Rains & Capper, ١٩٨٨). Schmidt & Robert (١٩٨٥) reported that through appendicitis resulted due to mucosal injury of appendix or destruction of tissue by mechanical or chemical means.

١.٢.٥.٢- Bacterial Appendicitis.

Bacteria seem to play a major role in appendicitis. It occurs when bacteria in vase and grow in the blocked appendix causing it to become swollen and infected, If the infection progress the swelling can cut off the blood supply to the appendix which result in killing the tissue, the wall of the appendix may rupture and infection spills into abdominal cavity causing peritonitis (Shelton *et al*.,٢٠٠٣). A variety of bacterial species have been reported to play a major role in the appendicitis .Aerobic and anaerobic bacteria were detected in appendicitis cases and the commonest anaerobe was *Bacteroid fragilis* (Elhag *et al*., ١٩٨٦; Gilbert *et al*., ٢٠٠٢). The significance of beta haemolytic streptococci in appendicitis has been studied by Jakobsen *et al*.(١٩٨٧) who pointed out that only six cases were identified as streptococcal causes .Okoro (١٩٨٨) stated that *Yersinia enterocolitica* accounted for ١٣.٨% of the microorganisms impliated in appendicitis . A retrospective study on the microbiology of abdominal pus from acute appendicitis or peritonitis was carried out by Guasco *et al*., (١٩٩١) they found that ٤٥ (٨٤.٤٪) specimens were positive for bacteriological examination of which (٧٦.٣%) were polymicrobical the most represented species were *E.coli* (٢٨.٤٪), *Bacteroid fragilis* (٧٨٪), *Streptococcus milleri* (٧.٨٪) and *Ps. aeruginosa* (٣.٩٪) The polymicrobial mostly represented by Bacteroidaceae *spp,* Enterobacteriaceae *spp and* Streptococens *spp.* A study on inflamed appendices revealed mixed infections and the most common organisms were *E.coli,* Enterococci, anaerobic

streptocci together with *Clostridun prfringenes* and *Bacteroides* (Rains & Capper ١٩٨٨). Felner & Dowell (١٩٧١) reported that *Bacteroides* species accounted for ١٠٪ of the appendiceal lesion while Leigh *et al*., (١٩٧٤) isolated *Bacteroides spp* from appendectomy cases in a percentage of ٧٨٪ where as other Gram-negative bacilli represented by *E. coli* and *Klebsiella* were present in a percentage of ٢٩٪ and ٢٧٪ respectively. Gram positive cocci were less frequently.

A bacteriological study for ١١٠ emergency appendectomies in children was reported by Barker, (١٩٨٥). They suggested that *Bacteriodes* considered as an important pathogen in appendicitis. However anaerobic bacteria seemed to be increasingly colonized the appendix and the ileum in cases of appendicitis (Bennion *et al*., ١٩٩٠; Park homenlco *et al*., ١٩٩١) studied the characteristic of viral-bacterial lesion in the appendix of children with appendicitis. They obtained antigen of influenza viruses A, B, C, Entero, Adeno, and paramyxo viruses among which influenza c was significantly more frequent (٦٤.١٪); viral infection was the majority of cases (٧٩.٥٪). It was found in association with opportunistic flora more frequently with *E. coli* and less *Ps. aeroginusa* and *Klebsiella*.

Baron *et al*., (١٩٩٢) reported that bacteria recovered from appendiceal specimens of patients with acute appendicitis were smaller in number and fewer in species in culture compared with

specimens from patients with more complicated disease. Some appendicitis was found to be caused by *Salmonella spp*. (Kazlow *et al*., ١٩٩١). Moreover Parkhomenko(١٩٩٨) isolated *Citrobacter frundii* and *Yersinia enterocolitica* from some appendicitis cases.

١-٢-٥-٣ Viral Appendicitis:

Appendicitis may also subject for infection by a group of viruses such adeno viruses, cytomegalo viruses (Lamps, ٢٠٠٤). In such cases appendix becomes accessible by bacteria which invade the appendix rapidly to become inflamed and filled with pus (Lamps, ٢٠٠٤).

١-٢-٥-٤ Parasitic Appendicitis

Parasites have also been reported to be implicated in appendix infection .*Trichuris trichiura. Ascaris lumbricoides* .*Entamoebia histolytica, Taenia saginata, Schitosoma haematopium and Strongyloides stercoralis* were isolated and identified from infected appendix (Calchi *et al*., ١٩٩٦; Rivero *et al*., ١٩٩٦), but the common parasite was *Enterobius vermicularis* (Chang and Fuh, ١٩٨٧; Dohistrom and Macrthw, ١٩٩٤).

١-٢-٦ Risk Factors for Acute Appendicitis

According to the available knowledge, there is no direct factor causing appendicitis. Even though factors seem to be associated in some way with appendicitis, or make the chance of infection higher

but these are not always lead to cause the infection. (Shsrm and Gupta, ٢٠٠٤). Though the following conditions have been recommended to participate with acute appendicitis in various sources include low fiber diet, refined carbohydrate, amebiasis, bacterial gastroenterities and viral gastroenteritis.

١-٢-٦-١. Diet

A confirmed evidence being concluded from geographical and economical studies revealed that the change from a high to low residue diet is largely associated with appendicitis (Burkitt, ١٩٧١), short (١٩٢٠) showed that if the individuals from African areas migrated to urban areas where appendicitis is common they soon become susceptible to the infection.

This may due to departure from simple diet which rich in cellulose to one relatively rich in meat, but this cannot be the whole explanations, since acute appendicitis may occur in life long vegetarians and even in babies of the breast feeding (Rains & capper, ١٩٨٨).

The reduction of dietary cellulose not only slows fecal transit but also results in firmer and more tenacious stools which are considerably reduced in bulk and predispose to fecalith formation.

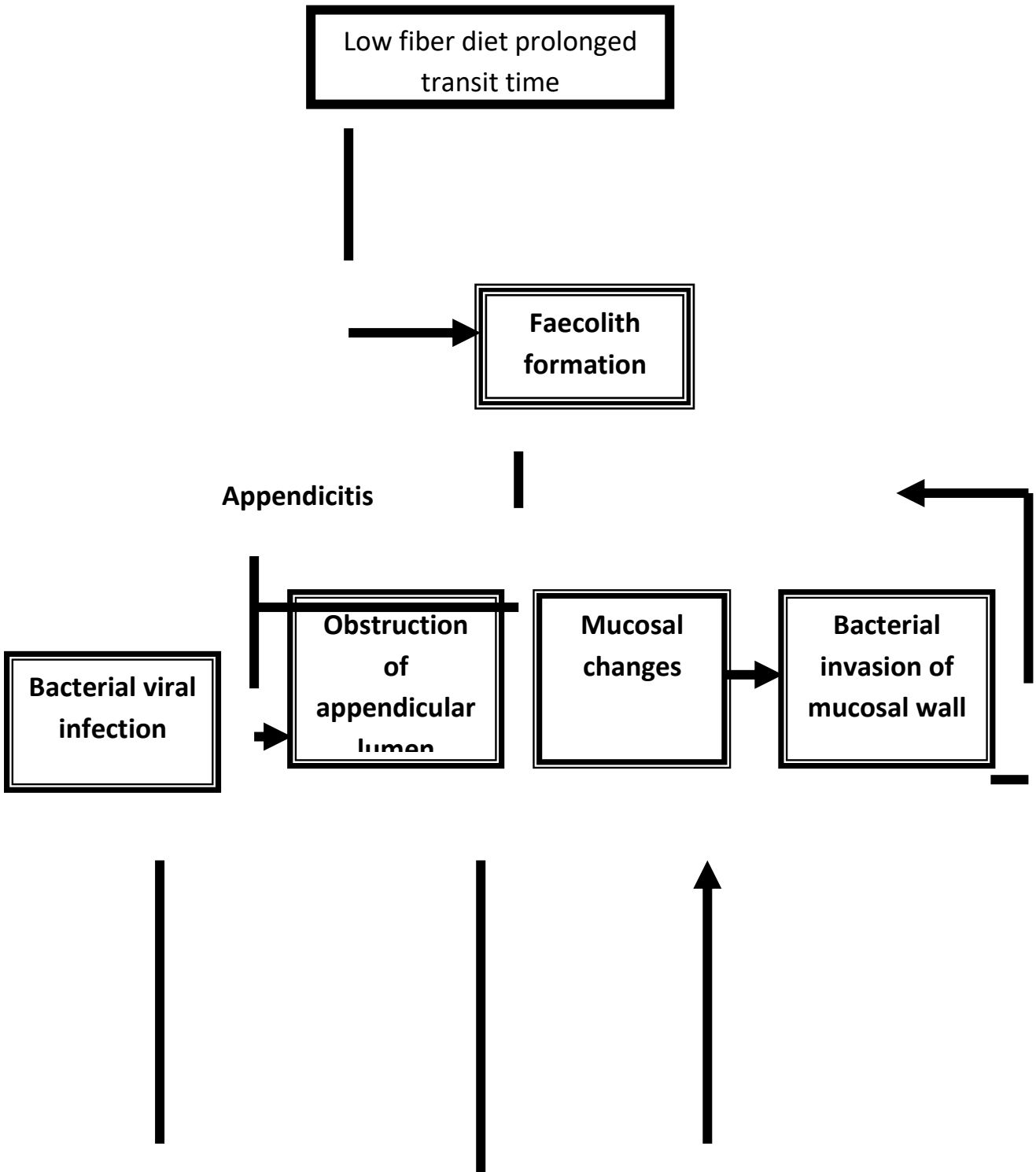
This is in turn lead to high intra luminal pressure (Painter, ١٩٦٩). The urban blacks hare been reported to be relatively free of appendicitis (Bruner *et al*., ١٩٧٠; Lau *et al*., ١٩٨٤; Segal and Walker, ١٩٨٦).

١-٢-٦-٢ Genetic Factors

Larner (١٩٨٨) described a family pedigree in which ٥٠٪ of the members were operated on for appendicitis. Other studies have demonstrated a high incidence of appendicitis among immediate family members (sibling), parent or both not seen in a control group undergoing abdominal operations other appendectomy.

The explanation for this familial tendency is unclear, but the possible reason may include shared dietary habits (Anderson *et al*., ١٩٧٩),a genetic difference in resistance to bacterial infection (Larner,١٩٨٨),or the inheritance of a fibrous brand of peritoneum which kinks the base of the appendix (Fig.١-٢). So partially occluding the lumen and thereby predisposing to appendicitis (Boulose and Cowiem ١٩٩٣).of the sporadic evidence currently available, all that can be said is that in certain cases some hereditary factors seem to be involved in the aetiologic Larner, (١٩٨٨). In (١٩٩٠) Blandino *et al* pointed out the role of a major recessive gene. A follow up study by the same group studying ٣ generation of families using complex segregation analysis supported a polygenic or multifactorial model.

There are no clear evidences to support a major gene although a rare gene could not be ruled out as the cause of small proportion of cases. Specific studies to address genetic and environmental factor in this serious disease seem worth but for now a positive family history of appendicitis might join other evidence leading to improved clinical recognition of acute appendicitis. (Bast *et al*, ١٩٩٠)



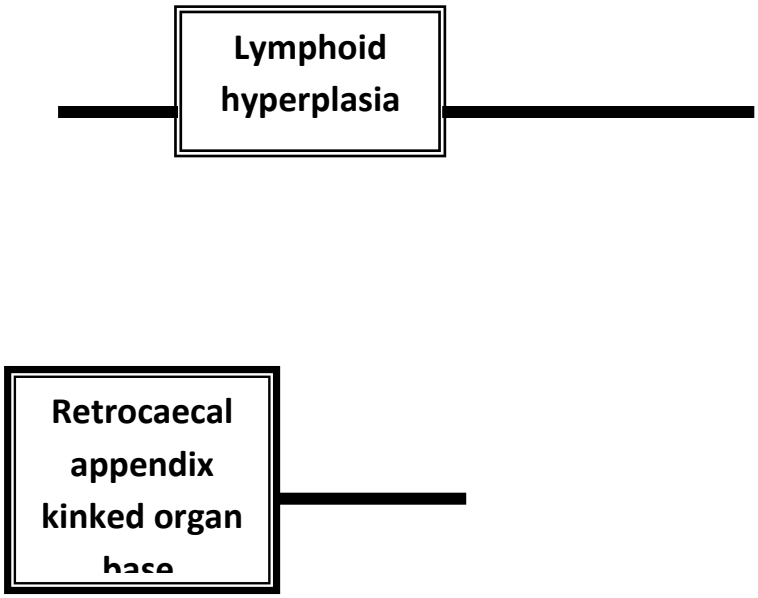


Fig. (١-٢). Different theories for the aetiology of appendicitis (Larner, ١٩٨٨).

١-٢-٧ Pathophysiology

Pathophysiology of acute appendicitis is most likely the result of closed loop obstruction of its lumen related to fecalith or less commonly resulting from hyperplasia of submucosal lymphoid follicles caused by a viral infection or rarely from obstruction due to pin worm or other helminthic infestation. Occasionally obstruction is caused by carcinoid tumor which if it is less than ١cm in size it requires no further therapy following appendectomy with reason

able margins in the presence of obstruction the mucosa continues to secrete some exudates resulting in an accumulation of mucoid material and increasing intraluminal pressure, bacteria located with in the lumen of the appendix proliferate fast. In the presence of stasis and obstruction the flora of the appendix included both aerobic and anaerobic organisms (Lamps, ٢٠٠٤).

However not all luminal obstruction causes acute appendicitis and certainly not all acute appendicitis is caused by obstruction. Following obstruction the appendix continues to secrete into closed lumen causing increased luminal pressure with eventual venous congestion and infarction along the water shed region, eventual perforation is preceded by mucosal ulceration and Tran mural necrosis. Abscess formation or peritonitis (Hart, ١٩٩٧).

١-٢.٨ Investigation

As a result of early diagnosis the case fatality rate has fallen to less than ٠.١٪ for uncomplicated appendicitis (AL-Fahad, ٢٠٠٣).The records for gangrenous and perforated appendicitis are ٠.٦٪ and ٥٪ respectively (Chsheri *et al*., ١٩٧٨; Sandler *et al*., ١٩٨٧). The accurate preoperative diagnosis of acute appendicitis remain a difficult problem, negative appendectomy rate of approximately ٢٠٪ was commonly reported. In young ovulating women the incidence of gynecologic disease make the clinical diagnosis even more difficult and negative appendectomy rate between ٣٤-٤٦٪ have been

reported in such group of patient (Bind and Bahlgrest, ١٩٨٦).However diagnosis of acute appendicitis commonly follows two main investigations, clinical investigation and laboratory investigation. These are briefly presented below.

١-٢-٨-١ Laboratory Investigation

Laboratory investigation is mostly restricted on the white blood cell count (WBCS) since leukocytosis is seen in ٧٥-٨٠٪ of patients with acute appendicitis. Studies consistently show that ٨٠-٨٥٪ of adults with appendicitis have a WBC count density of ١٠,٠٠٠ cell /cum^{٣}. Neutrophils greater that ٧٥٪ occur in ٧٨٪ of patients and ٤٪of patients with appendicitis have a WBC count less than ١٠,٠٠٠ cell/cum^{٣} and neutrophil less than ٧٥%. Moderate leukocytosis ranging from ١٠,٠٠٠-١٨٠٠٠ cell/cum^{٣} accompanied by a moderate polymorphonuclear predominance is the rule in acute uncomplicated appendicitis with normal total and differential while blood cells count, the diagnosis of acute appendicitis in the question through not ruled. (Clark *et al*., ١٩٩٢; Schwartz *et al*., ١٩٩٦).

If white blood cells count is more than ١٨٠٠٠ cell/cum^{٣} or if shift to left is extreme perforated or an acute inflammatory disease of greater magnitude then appendicitis is probable (Shiffoan and Greenes, ١٩٩١; Schwartz *et al*., ١٩٩٦).

١.٢.٨.٢. C. Reactive Protein

In acute phase reactant C- reactive proteins synthesized by liver in response to bacterial infection- serum level begins to rise with ٦ to١٢ hours of acute tissue inflammation. When the symptoms have presented for at least ١٢ hour especially in children, c. reactive protein level was seen to be increased more than ١ mg/l in ٧٢٪ of case .This can be considered as evidence of acute appendicitis (Owen and Willims, ١٩٩٢; Anderson et al., ٢٠٠٤). In addition to the above examinations regarding the diagnosis of appendicitis some other examinations can be recommended such as urine analysis (Arubjornsson, ١٩٨٨; Schwartz *et al*., ١٩٩٦), x ray (Sandler and Pearbery, ١٩٨٧), Barium enema (Alvardo, ١٩٨٦).

١.٢.٨.٣. Other Investigation

١.٢.٨.٤. Clinical Investigation

The diagnosis of acute appendicitis is the classic example of application of clinical skills. Ancillary laboratory and radiological tests are not essential in making the diagnosis. Signs and symptom ranking have been reported but these aids have not really improved the overall accuracy of preoperative diagnosis (Kalan *et al*., ١٩٩٤; Helmer, ٢٠٠٢).

However that following features may be considered during investigations:

- Symptoms include rightileac fossa pain, generalized pain, and fever, loss of appetite, nausea, constipation, vomiting, diarrhea, headache and dysuria.
- Signs: such as generalized tenderness, rebound tenderness, guarding, mass, rovsings sign and psoas sign.

١.٢.٩ Intestinal Fiora

A vast microflora normally present in the gastrointestinal tract. At birth the baby's intestinal tract is sterile within few days, particularly in breast feclinfants, anaerobic lactobacilli and bifidobacteria appear and predominate $١٠^{٩}$-$١٠^{١١}$ cells / gram of feces along with fewer coliform and enterococci with broadening of diet. Coliforms become more numerous and are joined by *Bacteroides spp* and *Clostridium spp*. (Skinner & Carr, ١٩٧٦; Fantin *et al*., ١٩٩٠). In adults the esophageal mucosa contains only the bacteria ingested with food and saliva. Because of low pH of the gastric juice, few bacteria mainly lactobacilli are found in the stomach, the jejunum has a sparse ($١٠^{٤}$-$١٠^{٥}$ cells/ ml of fluid predominantly gram positive bacteria the principle components being lactobacilli and various streptococci. The concentration of the microflora in the ileum increases $١٠^{٤}$- $١٠^{٧}$ cells/ml with the appearance of coliforms and anaerobic organism $١٠^{١١}$ -$١٠^{١٢}$ cells /ml gram of feces, ٩٦- ٩٨٪ of the

colonic flora consists of anaerobic *Bacteroides spp.* mainly *Bacteroid fragilis, anaerobic* streptococci*, Bifidobacterium* and *Fusobacterium*: only ١ - ٤٪ are aerobes and others are facultative anaerobic bacteria such as coliforms and enterococci. Also a small number of *porteus* spp *Pseudomonas aeroginusa, Staphylococci*, *Spirochetes* and other microorganisms are present (Williams, ١٩٧٣; Davis *et al*., ١٩٩٠).

Moderate changes in the diet do not affect the ratios of dominant bacterial species in feces. This situation is altered when gastrointestinal infection takes place (Moore *et al*., ١٩٦٩; Felner and Dowell, ١٩٧١; Vanway *et al*., ١٩٨٢). The dominant bacteria in human intestinal tract are discussed below.

١-٢-٩-١. Aerobic Enteric Bacteria

This is the family of a bacterial group commonly referred to as enteric group, they are Gram negative cocci bacilli normally habitat in the intestinal tract of human. Some of them act as a part of the normal flora and incidentally cause diseases while others are aggressive pathogen for human. They possess a complex antigenic structure and produce a variety of enzymes and toxins with other virulence factor. The members of this group are sharing many

characteristics with each other (Mims *et al*. ٢٠٠٤). Some common species among this group and other related bacteria briefly out lined below.

١.٢.٩.١.١ *E. coli*

Gram negative bacilli, some strains have pili as well as colonization factors antigen (CFA/I, CFA/II, CFA/III). The pili are responsible for adherence so that the adhesion has important role in pathogensis (Matsumoto *et al.*, ١٩٩٠). The capsule of *E. coli* represents the K- antigen which helps in the attachment of bacteria to the epithelial cells of the hosts (Eisentein and Azalenznik, ٢٠٠٠). *E. coli* may produce α and β haemolysin as apart of the virulence factors and some strains are urease producing (Collin and Falkow, ١٩٩٠). (Saxen *et al*.,١٩٩٦) reported that colonization of the gut by virulent E-coli type ١c- fimbriated, P- fimbriated $O_{٢٥}$: $K^{٥}$: $H^{١}$ serotype may lead to a perquisite for the development of appendicitis, The producing of green metallic sheen of colonies EMB the specific diagnostic character for this species. Agar is the specific diagnostic character for this species.

١.٢.٩.١.٢ *Klebsiella*

Klebsiella species are Gram negative bacilli non motile, are specifically characterized by forming long and highly mucoid

colonies. The virulence of *Klebsiella* is not well understood, but its antiphagocytosis capsule plays a role in the infection by preventing phagocytosis. It is thought to be an aerobactin, an iron binding protein and the production of β- lactamase enzyme contributes to pathogencity and resistance to antibiotics. (Thomson *et al*.; Mims, ٢٠٠٤).

١.٢.٩.١.٣. *Proteus* spp

This organism is mainly characterized by its active motility which resulting in what it called swarming phenomena it can cause a disease only when leave the intestinal tract and it produces the urease enzyme. The endotoxin liberated by this organism may play the main role in virulence of bacteria (Gilchrist, ١٩٩٥; Farmer, ٢٠٠٠).

١.٢.٩.١.٤ *Enterobacter* spp

Gram- negative bacilli. They have several virulence factor, have a small capsule and siderophore production which contributes to the infection In addition, they are able to produce some enzymes and endotoxins which have an important role in virulence and resistance to antibiotics (Gilchrist, ١٩٩٥). *Enterobacter* spp can produce antibacterial substance that has antagonistic activity against wide range of bacteria except *Acinetobacter* and *Pseudomonas* (Ya- Ping *et al*., ٢٠٠٣).

١.٢.٩.١.٥ *Salmonella*

Gram negative bacilli, motile by peritrichous flagella. *Salmonella* spp are able to invade the intestinal mucosa and cause intestinal infection which can eventually lead to appear symptoms of typhoid fever. The ٢٢٠٠ know species of *salmonella* are classified according to their surface antigens. The capsular of this organism can cause serious complication in immunosuppressed individuals (Hanberger and Goberndo,١٩٩٩; Farmer *et al*.,٢٠٠٠).

١.٢.٩.١.٦ *Citrobacter freundii*

Gram negative bacilli, motile and invasive. *Citrobacter* infections are associated with high mortality rate. It can cause brain abscess, neonatal sepsis meningitis, blood stream infection and intra abdominal sepsis. *Citrobacter* specis are commonly resistant to extended spectrum cephalosporins (Abbott, ١٩٩٠; Graham and Baud, ١٩٨١; Jones, ٢٠٠١).

١.٢.٩.١.٧ *Pseudomonas aeruginosa*

It is Gram negative, and has several virulence factors very important in pathogenicity. These include capsule or slime layer which associates in adherence and effectively protects the bacteria from phagocytosis. The production of extra cellular protease,

cytotoxins and hemolysin have an important role in virulence in addition to the siderophore which enhances the growth of the organism .The soluble and media diffusible pigments produced by this organism are of important character to diagnose *Ps. aeruginosa* (Macfaddin, ٢٠٠٠).

١.٢.٤.١.٨ *Staphylococcus aureus*

The pathogencity of staphylococci is contributed to hemolysis of the blood, coagulation of plasma and production of extracellular enzymes and toxins (Mims *et al*., ٢٠٠٤) .*Staph aureus* has a polysaccharide capsule and the cell wall composed of peptidoglycan and teichoic acid moieties that protect it from lyses by osmotic condition and aid the bacteria to attach mucosal surface. The virulence of the bacteria resulted by secretion of toxins and enzymes which act on host cells membranes and mediated the cell destruction (Takahasi *et al*., ١٩٩٩).

١.٢.٩.٣. Anaerobic Bacteria

١.٢.٩.٣.١. *Clostridium*

Gram positive obligately anaerobic rod with center or subterminal heat resistant spores (Calo *et al*., ١٩٨٩). Although *Clostridium* species are ubiquitous in nature their principle habitats are the soil and the intestinal tracts of human and animal. The genus *clostridium* includes many species but the most three common species are *Clostridium perfringens ,Clostridium tetani* and *Clostridium botulinum* .The *Clostridium perfringens* is the species most commonly isolated from human clinical specimens excluding feces in the normal adult colon *clostridium perfringens* $١٠^{٣} - ١٠^{٥}$/g. It is encountered in a wide variety of clinical settings ranging from simple contamination of wound to traumatic or nontraumatic myonecrosis, clostridial cellulites, intra abdominal sepsis and gangrenous cholecystitis.

The clostridia form many virulent factors such as spores, many types of toxins and other extracellular products include collagenase and proteases. *Clostridium* species are commonly encountered in a variety of polymicrobial infection involving the abdomen including peritonitis, intraabdominal abscesses and septicemia in patient with obstractive or perforating lesions of the terminal ileum or large bowel (Gorbach *et al*., ١٩٩٨).

١.٢.٩.٣.٢- *Peptostreptococcus* spp.

They are gram positive anaerobic non spore forming cocci. These organisms are the most part components of the normal flora of the skin or mucosal surface of human. As with many other anaerobes they may become opportunistic pathogens when they are allowed to leave their normal habitat and gain access to normally sterile body tissue (Murray *et al.*, ١٩٩٩).

Most (٩٦٪) *peptostreptococcus* isolates are susceptible to B. lactam antibiotics, clindomycin and metronidazole are slightly less active since it was reported that ٨٤٪ - ٨٨٪ of isolates were found to be resistant to these antibiotic respectively (Reig *et al*., ١٩٩٢; James *et al.* ٢٠٠١).

١.٢.٩.٢.٣- Bacteroidaceae

It is obligately anaerobic non spore forming bacilli. The common species among this family is *Bacteroid fragillis* .The members of this family are straight, curve or helical. Some strains are motile. The Bacteroidaceae are prevalent among the indogenous flora of mucosal membranes of human. Anaerobic or mixed infection result from disruption of mucosal surface and introduction of anaerobes into (normally) sterile site (Baron and Mifinegold, ١٩٩٢). Many of these organisms produce virulent factors that contribute to pathogensity. This includes catalase, superoxide dismutase, collagenase,

neuramindase, deoxyribonuclase (DNanse), heparinase, protease and Immoglobulinase (Briselden *et al*., ١٩٩٢). Several strains produce capsule which promotes the abscess formation (Brook *et al*., ١٩٩٨). Enterotoxin production by some strains of *Bacteriods fragilis* has been recently described (Van *et al*., ١٩٩٢; Kenneth *et al*., ٢٠٠٥) *Bacteroides* are resistant to penicillin and ampicillin. The anti pseudomonal penicillins, ticarcillin and mezlocillin are some what more active than penicillins (Sedlock *et al* ١٩٩٠; Cuchural *et al*., ١٩٩٦). Among the metronidazol, cephalosporins and cephamycin, cefoxitin remain very active against *Bacteroids* species (Aldridge *et al*., ١٩٩٧).

١.٢.١٠ Complication of Acute Appendicitis

It has been pointed out that appendicitis and appendectomy may lead to produce some complications from of which the patients remain complain through the life. These complications are outlined below as concluded by (Paulson, ٢٠٠٣):

- Post operative infection.
- Intra abdominal abscess.
- Intestinal obstruction.
- Increase incidence of infertility in female.

١.٢.١١ Management

Appendectomy is seemed to be the suitable chosen management for most patients with acute appendicitis. The surgery often decided laparoscopically, is not complicated and carries little risk unless the appendix has ruptured. Generally the patient can resume normal activities quickly and will have no lingering problems. (Gorecki *et al*., ١٩٩٩) An in flamed appendix some times ruptures within the first ٢٤ hours of symptoms. If the appendix does rupture, infection can spread to the neighboring tissue lining the abdomen (peritoneum) (Shelton, ٢٠٠٣).

The possibility of rupture is highest in young children and older adults. The diagnosis may be delayed for those people because their symptoms may not be as obvious as the symptoms of older children and younger adults. A ruptured appendix can be fatal if it is not managed early (Williams and MarGs, ٢٠٠٣).

Antibiotic could be administered preoperatively to control any local or generalized sepsis that may occur and to reduce the incidence of postoperative infection. The most common antibiotics being recommended for metronidazols the treatment of acute appendicitis are aminoglycosides, cephalosporins and metronidazols (Launrence *et al*., ١٩٩٧; Katazung, ٢٠٠٣), but sensitivity test may help in determine the appropriate antibiotic to be used for treatment .

١.٢.١٢. Antibiotics Used to Treatment of Appendicitis

١.٢.١٢.١. Aminoglycosides

Aminoglycosides antibiotic are in general active against staphylococci and aerobic Gram negative organism including almost all the members and Enterobacteriaceae even though some bacterial species. Emerged mutants resistant to aminoglycosides (Launrence *et al.,* ١٩٩٧; Jones *et al*., ٢٠٠٢).

١-٢-١٢-٢ Cephalosporins

All first three generation of cephalosporin have oral prepartions that have used for treatment of most bacterial infection (Wilhelm and Edson, ١٩٨٧; Forrest *et al*., ١٩٩٣).

First generation cephalosporins act by inhibiting bacterial cell wall synthesis the bactericidal activity is mainly against gram positive bacteria and the administration is either oraly cefadroxil, cephacexin and cephradin or parenteral such as cephalothin,cephazolin and cephradin (martinez *et al*.,١٩٩٥).

Now the usage of first generation cephalosporin. Is limited because of high resistance to it (Matinez *et al*., ١٩٩٥). Second generation cephalosporins have bactericidal activity that inhibts bacterial cell wall synthesis. It has greater activity against anaerobic bacteria and the administration is either oral include cefaclor and cefuroxime) or parenteral second generation of cephalosporin has limited use that (Dumpis *et al*., ٢٠٠٣).

Third generation of cephalosporins have bactericidal action that inhibits cell wall synthesis .they are highly stable in the presence of β-lactamase enzyme and they are effective in wide range of hospital acquired and nosocomical bacterial infections. The administration is either oral cefixime, cefpodoxime and ceftibuten or parenterol cefotaxime, cefotriaxon, cefazidime and ceftizoxime (Katzurg, ٢٠٠٣).

١-٢-١٢-٣ Metronidazol

Metranidozol is antiprotozal drug that also has potent antibacterial activity against anaerobes including *Bacteroides* and *Clostridium* species .It is well absorbed after oral administration widely distributed in tissue and reach serum level of ٤-٦ µg/ml after ٢٥٠ mg Oral close metronidazole was suggested for treatment of anaerobic or mixed intra abdominal infections (Ya-ping *et al.,* ٢٠٠٣; Mims *et al.*, ٢٠٠٤).

١-٢-١٣ Antibiotic Susceptibility Test Methods

Methods of bacteria antibiotic susceptibility test vary as regards accuracy of evaluating bacteria response to antibiotics and determining the boundary between sensitive and resistance against these antibiotics. Quantetative evaluation is frequently used to measure the bacteria susceptibility to antibiotics as it is more accurate and definitive than qualtitative evaluation discovering the bacteria resistance to bacteria susceptibility represented by disk diffusion method (Murray *et al*.,١٩٩٩).Although the last method is not costy and quick, it is some times still unable to show the real level of bacteria susceptibility to antibiotics as it is the case in examining the anaerobic bacteria and the weakly growing bacteria in the solid culture media. Moreover the disk method is affected by some factors such as the growth density the type of bacteria used in the test, the type of antibiotics, concentration of the culture media and its degree of the hydrogenic exponent, beside the incubation period and temperature (WHO,١٩٨٧).The average of antibiotic spread in the culture medium is varies from one antibiotic to another (Murray *et al*.,١٩٩٩).All these factors may decrease the test efficiency using this method still the limitation of the minimum inhibitory concentration MIC of the antibiotic which is the lowest antibiotic concentration that inhibit the bacteria growth after a period of bacteria incubation in a series of dilution antibiotic in the

culture medium(Baron and Jone,١٩٩٤). The method is very important especially to the antibiotic rapidly resisting bacteria to observe the range of the resistance development through noticing the increase in the values of MIC (Murray *et al.,* ١٩٩٩; Collee *et al.,* ١٩٩٦).

MIC test is one of the significant tests used to determine the bacteria susceptibility when more than one antibiotic are mixed (Murray *et al.,* ١٩٩٩).

This test is often used in surveying studies to examine susceptibility to antibiotic on a certain kind of bacteria specifically after determining the values of $MIC_{٥٠}$ or $MIC_{٩٠}$ of the antibiotic. Recently means devices and computer systems have been used to estimate the bacteria growth and to measure susceptibility to antibiotics and charging their results. This field called automated microbiology has witnessed great progress. Therefore, these technicalities have been used in microbiological Laboratories because they are quickly processed and give accurate results.

Results, learning out the old methods of susceptibility tests which are affected by some personal factors as eyesight problems, color blindness, fatigue, exhaustion and some technical mistakes by the people working in this field (Collee *et al.*,١٩٩٦). Sensitivity to antibiotics test by instrumental methods depends on different principles such as the turbidimetric bacterial growth in a liquid

medium or measuring the optical density. Other time if depends on colour and glittering reagents to decide the bacterial growth. Due to the difficulty in determining the bacterial growth in the MIC test. The photometeric has been followed to obtain results compared other methods (Check, ٢٠٠٠). Moreover it was uneasy to do the MIC test in microbiological laboratories test (E) was used. It is one of the recent technologies used during the last years to decide the value of the MIC as soon as possible and accurately (E) test was designed in a way leads to discover antimicrobial resistance testing (ART) quickly and accurately (ART) test is also a means to discover the minimum changes at the level of the antibiotic bacterial susceptibility. Other applications of this test are the determination of the MIC value of both non aerobic and fastidious bacteria because it is not affected by factors as the bacterial growth density and the cells growth level. As the bacterial growth density and the cells growth level results can by read immediately from the test tape .It is also possible to use more than one type for more then one antibiotic in one test (Collee *et al.*, ١٩٩٦; Barron and Jones, ١٩٩٤).

٢.١.١. Patients

From October ٢٠٠٤ to July ٢٠٠٥, a total of ١١٠ acute appendicitis patients who referred to Al- Furate Teaching hospital were investigated. Their ages ranged between ٤-٦٠ years.٦٩ of them were males and ٤١ were females. All patients were under went the following Laboratory investigations: General urine examination (GUE),white blood cell count (WBC) and c-reactive protein .Radiological investigation at abdominal ultrasonography and computerized tomogrraphic (CT) scan being decided by radiologists were also considered.

٢.١.٢. Materials

Many types of instruments and chemical materials in addition to biological material materials were used in this study. The traditional apparatus devices being necessary for the appropriate experiments were carefully used. Culture media being used in this study are shown in table (٢-١). Chemical substances and antibiotics disk are inserted in table (٢-٢) and (٢-٣) respectively. Table (٢-٤) and table (٢-٥) show the selected antibiotics for MIC and API diagnostic kit respectively.

Table (٢-١) List of cultures media

Culture medium	Company (origin)
Blood agar base	Mast diagnostic(USA)
Brain –Heart infusion broth	Mast diagnostic(USA)
Cooked meat broth	B.D.H (UK)
Eosine methylen blue	Mast diagnostic(USA)
Gas generating kit	Al- Raze (Iraq)
Kliglar Iron agar	Difco (USA)
Manitol Salt agar	Oxoid (UK)
MacConkey agar	Mast (UK)
MR- VP broth	Difco (USA)
Mueller. Hintor broth	Difco (USA)
Muneller. Hinton agar	Difco (USA)
Nutrient agar	Mast (USA)
Nutrient broth	Mast (USA)
Peptone water	Biolife (Italy)
Simmon citrate agar	Difco(USA)
Thioglyconate broth	B.D.H (UK)
Urea agar base	Difco(USA)

Table (٢-٢) List of chemical material

Chemical-materials	Company
Absolute Ethanol	BDH
$BaCl_2$	BDH
Crystal viotate	Fluka
Formalin (٤٠٪)	BDH
Glycerol	BDH
HCl	BDH
H_2O_2 (٣٠٪)	Fluka
Iodin	BDH
KI	BDH
KoH	BDH
Kovac's reagent	BDH
Methyl red	Fluka
α -naphthol	BDH
Safranin	Fluka
Tetramethyl- p- paraphenylene diamine dihyrocloride	BDH
Urea. Solution	Fluka

Table (٢-٣) List of Antimicrobial agent

No	Antibiotic	Potency (µg)	Antibiotic	Company
١	Amoxicilln	١٠	Amx	Oxoid
٢	Gentamycin	٣٠	Gm	Oxoid
٣	Amikacin	١٠	Ak	Oxoid
٤	Cefotriaxom	٣٠	CTX	RA
٥	Cephalexin	٣٠	CL	Oxoid
٦	Cefoxitin	١٠	Cxm	Oxoid
٧	Ciprofloxacin	٢٠	CIP	RA
٨	Norfloxacin	٢٠	NF	RA
٩	Chlormphenicol	١٠	C	Oxoid
١٠	Augmentin	٣٠	AC	Oxoid
١١	Refampicin	٣٠	RD	Oxoid
١٢	Vancomycin	٣٠	VA	Oxoid
١٣	Tetracycline	٣٠	TE	Oxoid
١٤	Co- Trimoxazole	٢٥	SXT	RD

Table (٢-٤) Antibiotic Used in MIC Test

Antibiotic	Company (origin)
Ceftriaxone	RA (India)
Cetotaxime	RA (India)
Ciprofloxacin	RA (India)
Amikacin	Oxoid (UK)
Augmentin	Oxoid (UK)

Table (٢-٥) Other materials

Material L	Company
API *staph*	Bio Merieux (franca)
API ٢٠ E and reagents (TDA, UP١, UP٢, NIT)	Bio Merieux (franca)

٢.٢. Reagents

٢.٢.١. Methyl Red Reagent: It was prepared by dissolving ٠.١ gm of methyl red was dissolved in ٣٠٠ ml of ٩٥ % ethanol and then the volume was completed to ٥٠٠ ml with distilled water this reagent was used as indicator in methyl red (MacFaddin, ٢٠٠٠).

٢.٢.٢. Vages-Proskauer Reagent: it included two reagents; A and B. Reagent A: ٥gm of α -naphthol was dissolved in ١٠٠ ml of ٩٩ % ethanol. Reagent B: ٤٠ gm of KOH was dissolved in ١٠٠ ml of distilled water these reagents were used as indicators in voges- proskaur test (Collee *et al*., ١٩٩٦).

٢.٢.٣. Oxidase Reagent: It was prepared by dissolving of ٠.١ gm of tetra -p- paraphenylenediamine dihydrochloride in ١٠ ml of distill water and stored in a dark container this reagent was used as indicator in catalase test (Baron *et al*., ١٩٩٦).

٢.٢.٤. Catalase Reagent: It was prepared by dissolving ٣ml of H_2O_2 to ١٠٠ ml of distill water (Baron *et al*., ١٩٩٦).

٢.٣. Biochemical tests:

٢.٣.١. Catalase test: a colony of the organism was transferred to a drop of ٣٪ H_2O_2 on a microscopes slide the formation of gas bubbles indicate for the positive test (Collee *et al.*, ١٩٩٦).

٢.٣.٢. Oxidase test:

A piece of filter paper was saturated with oxidase reagent. Then the colony to be tested was picked up with a sterile wooden stick and smeared over filter paper the organism under test was spread on this paper .when the colour around the smear turned from rose to deep purple color within ٥- ١٠ second the oxidase test is positive (Collee *et al.*,١٩٩٦).

٢.٣.٣. Coagulase test:

Several colonies of bacteria were transferred with the a loop to test tube containing ٠.٥ ml of rabbit plasma. The tube was covered to prevent evaporation and incubated at ٣٧C for overnight .The test was read by tilting the tube and Observing for clot formation in the plasma. Negative test results in the plasma remained free flowing with no evidence of clot (Collee *et al.*, ١٩٩٦).

٢.٣.٤. Indol test:

Peptone water was inoculated with a young bacterial colonies and incubated at ٣٧c° for ٢٤- ٤٨. Kovacs reagent (٠.٥ ml) was added.

A red color in the alcohol layer indicated a positive reaction (MacFaddin, ٢٠٠٠).

٢.٣.٥. Methyl Red test:

The test was performed on ٥ml of MR-VP broth which inoculated by the organism and incubated at ٣٧c° for ٢٤ h. there ٦-٨ drops of methyl red reagent were added. To the culture, mixed and the result was red immediately the change of color to bright red indicates as a positive reaction (Collee *et al*., ١٩٩٦).

٢.٣.٦. Simmon Citrate Test:

Simmons citrate slant was inoculated with bacterial culture and incubated ٢٤- ٤٨ hours at ٣٧c°.The positive result appears as change in the color of media from green to blue .The unchanging of the color indicates for a negative reaction (Benson, ١٩٩٨).

٢.٣.٧. Urease Test:

It was used to determine the ability of an organism to split urea, forming two molecules of ammonia by the action of the enzyme urease. It was prepared by adding ٢.٣ gram of urea base to ٩٥ ml distilled water, then it was sterilized by autoclaving and cooled to about ٥٠C then ٥ ml of ٢٠٪ solution of sterile urea. Appearance of deep pink color indicates for positive result urea agar slant was

inoculated heavily over the entire slant surface and incubated at ٣٧c° for ٢٤ h. Urease test is positive if the indicator was changed to purple- pink (Benson, ١٩٩٨).

٢.٣.٨. Kliglers Iron Agar Test:

A heavy inoculum was streaked over the surface of the slope and stabbed in to the buttm, incubated at ٣٧c° for ٢٤ h. Results were unformatted as follows:

Slant/ Butt	Color
Alkaline/ Acid	Red/ Yellow
Acid/ Acid	Yellow/ Yellow
Alkaline/ Alkaline	Red/ Red
H_2S	Black precipitate

It was used to detect three primary characteristics of bacterium:
The ability to produce gas from the fermentation of sugars, the production of H_2S gas and the ability to ferment Lactose (Baron and Jones, ١٩٩٤).

٢.٣.٩. Methal- Red Vogas Proskauer:

MR.VP medium was inoculated with the colony of tested bacteria and incubated at ٣٧C° for ٤٨ hours. One ml of ٤٠٪ KOH and ٣ml of ٥٪ solution of α – aphthol were added ٠.٠٦ and α- naphthol.

After ١٥ minutes, the formation of red color is indicative for the presence of acetoain (acetyl methyl carbinoll (Baron *et al*., ١٩٩٦).

٢.٤. Preparation of Culture and Diagnostic Media.

٢.٤.١. Ready- Prepared Media.

Media used in this study (listed in table ٢-١) were prepared in accordance with the manufacturers instructions fixed on their container. All the above media were sterilization in the outoclave at ١٢١C° for min. After sterilization blood agar base was supplemented with ٧.٥٪ defidrinated human blood, and urea agar base was supplemented with ٢٠٪ sterile urea solution all media were used for isolation and diagnosis of bacteria.

٢.٤.٢. Laboratory Prepared Media.

٢.٤.٢.١. Neonycin Blood Agar.

It was used for cultivation of *Clostridinm perfringes*. It was prepared by adding Neomycin ١٠ mg /١٠٠ ml of blood agar (Brook *et al*., ١٩٩٨).

٢.٤.٢.٢. Pepton Glucose Broth.

It was used as transport medium for the samples. It was prepared by dissolving ٥ gram peptone glucose into ١٠٠ ml distal water (Fredriksen *et al*., ١٩٩٦).

٢.٤.٢.٣. Kanamycin - Vancomycin Blood Agar:

It was used for cultivation of *Bacteroids* spp. It was prepared by kanamycin ١٠٠ mg and vancomycin ٧.٥ gm/١٠٠٠ ml of blood agar (Brook *et al*., ١٩٩٨).

٢.٥. Culture Media:

٢.٥.١. MacConky Agar:

It was used for cultivation of grow negative bacteria and to differentiate the lactose fermenter and non Lactose fermenter gram negative bacilli (Baron *et al*., ١٩٩٤).

٢.٥.٢. Nutrient Agar:

It is general medium. It was used for isolation of bacteria from their sources and for studies their morphological characteristics (Collee *et al*., ١٩٩٦).

٢.٥.٣ Mannitol Salt Agar

It was used as selective and differential medium for *staphylococci* (Collee *et al*., ١٩٩٦).

٢.٥.٤. Eosine Methylene Blue (EMB) Agar

It was used as a differential medium for *E. coli* (Brooks *et al*., ١٩٩٨).

٢.٥.٥. Blood Agar

It was used for cultivation of fastidious microorganism and determining the hemolytic activity (Baron *et al*., ١٩٩٤).This medium was prepared by adding ٥%human blood to autoclaved blood agar base (Collee *et al*., ١٩٩٦).

٢.٥.٦. Gas Generating Kit

It was used for cultivation of anaerobic Microbes (Collee. *et al*., ١٩٩٦).

٢.٥.٧. Nutrient Broth

It was used in general experiments and maintain the bacterial isolates.

٢.٥.٨. Cooked Meat Broth (CMB).

It was used for cultivating of anaerobes (Baron *et al*., ١٩٩٤).

٢.٥.٩. Muller-Hinton Agar:

It was used to determine the antimicrobial sensitivity patterns (Bauer *et al*., ١٩٦٦).

٢.٥.١٠. Thioglyconate Broth:

It was used for supporting growth of anaerobes (Baron *et al*., ١٩٩٦).

٢.٥.١١. McFarland Standard Solution (tube no ٠.٥)

It was used for antimicrobials susceptibility testing. It was prepared according to the method suggested by Collee *et al*, (١٩٩٦).

as follows -$BaCl_2$ ١٪ ٠.٠٥٪ml

-H_2SO_4 ١٠٪ ٩.٩٥ ml

٢.٦. Specimens Collection

One hundred ten excised appendixes were collected from ١١٠ patients who were referred to AL-Furate AL-Awsate Teaching Hospital for (suspected)) acute appendicitis through a period of ten months (from October ٢٠٠٤ to July ٢٠٠٥).The collected specimens were transported straight a way to Laboratory for bacteriological analysis as it is illustrated below.

٢.٧. Sterilization of the Outer Surface of the Appendix.

The outer surface of appendix was sterilized by three methods, ethyl alcohol ٧٠٪, iodine solution and heat cauterization (Fredriksen *et al*., ١٩٩٦).

٢.٧.١. Ethyl Alchol ٧٠٪

The appendix was totally immerged in beaker contained ethyl alcohol ٧٠٪ and immediately cultured on the proper media under aerobic and anaerobic conditions (Fredriksen *et al*., ١٩٩٦).

٢.٧.٢. Iodin Solution ١٠٪.

In this method, the appendix was totally immerged in the iodine solution (١٠٪) for ٥ minutes. The excessive solution was then absorbed by sterile filter paper. The tissue was then sectioned by sterile lancet and a swab from the inner part was cultured aerobically on blood agar, MacConky agar and anaerobically on kanamycin-vancomycin blood agar (KvA) and neomycin blood agar (NBA).

٢.٧.٣. Heat Sterilization.

In this method, a metal spatula was used. The spatula was flamed first and then appendix was cauterized by it. The appendix which is supposed to be sterile was then anatomized by sharp Lancet and a swab from the inner tissue was cultured aerobic and anaerobic on the appropriate culture media.

٢.٨. Identification of Bacteria:

For identification and diagnosis of bacterial isolates, the following criteria were considered, colonial, cellular morphology, and biochemical reaction. Diagnosis of some isolates was further confirmed by application of API-system. The results of tests were

compared with standard result according to Bergy's manual for determinative bacteriology (Holt *et al*., ١٩٩٤; Collee *et al*., ١٩٩٦; MacFaddin, ٢٠٠٠; Baron *et al*., ١٩٩٦).

٢.٩. Antibiotic Sensitivity Test.

٢.٩.١. Disk Diffusion Technique.

It was performed following Kirby-Bauer method by using a pure culture of previously identified bacterial isolates .The inocula to be used in this test was prepared by suspending selected colonies grown on blood agar plate in ٥ml of broth to a cells density (٤x١٠^{٦} cell/ml)equal to the turbidity of McFarland tube No ٠.٥. A sterile cotton swab was used to spread a proper inoculums on the standardized Muller Hinton plate .The antibiotic discs were placed on the surface of the medium at evenly spaced intervals with flamed forceps .After ٢٤ hrs incubation at ٣٧ C$^{\circ}$ the antibiotics inhibition zones were measured incompirison with standard results measurement of NCCLS (١٩٩٩) to determine the susceptibility of the tested organisms to each antibiotic.

٢.٩.٢. Determination of Minimum Inhibitory Concentration (MIC).

The MICs tests were determined according to the method suggested by (Barry, ١٩٧٦; Muray *et al.,* ١٩٩٩) as follows.

٢.٩.٢.١. Preparation of Bacterial Suspension.

Bacterial suspension was prepared from liquid culture (١٨-٢٠) hrs. old for each bacterium(٨ types) the suspension was diluted to ١/١٠ in BHI broth for Gram-negative bacilli and ١/٤ for Gram positive ١٠^{٦} cfu/ml (Murray ×cocci for obtaining cell density approximately ٥ *et al.,* ١٩٩٩).

٢.٩.٢.٢. Preparation of Antibiotic Solution

Determination of minimum inhibition concentration was studies against the initial dilutions of the antibiotic used in this study were prepared from the original stock solution by using the two folds technique for five antibiotics.

٢.٩.٢.٣. MIC Test.

By using a plastic microdilution tray, ٥٠ µl from the required concentration of the Muller- Hinton broth were inoculated by ٥٠ µl from the required bacterial suspension then ٥٠ µl from antibiotic solution were added to the first well and other serial dilutions were made in order to obtain double concentration for all wells .A control was also prepared by ٥ ml of each bacterial suspension, antibiotic solution and muller- Hinton broth. A control positive containing of broth without antimicrobe, a control negative inoculated and helped in the refrigerator over night to serve as a negative control. The tray

was closed with its special lid and incubated at ٣٧c for ١٦- ١٨ hr, the concentration which gives no bacterial growth inhibition of bacteria ١٠٠٪, is regarded as MIC.

٢.٩. Statistical Analysis

The data were analyzed statistically using Chi-square(X^2) test and Z-test at the ١٪ level (Daniel, ١٩٧٨).

٣.١. Samples Collection

The study concerned first with the detection of the appropriate medium that can inhance the growth of bacteria being present in excised appendix even in few numbers. Consequently, three types of culture media were suggested. Those were BHI-broth, nutrient broth supplemented with ٠.٩٪ NaCl and peptone water supplemented with ٥٪ glucose solution (PGM). Experientially, the medium (PGM) revealed quite satisfactory results compared with other two media, since it remarkably promoted the growth of all bacterial types. This result was in agreement with(Hussien, ١٩٩٩). Accordingly PGM was chosen as preliminary supportive medium for prepare the infective bacteria in further necessary experiments.

٣.٢ . Sterilization of Appendix

As with culture media, previously described, three methods were tried for sterilization of the outer surface of appendix in order to, investigate the samples properly with out any suspected contamination being gained during the process of appendectomy. Those methods were application of ethyl alcohol ٧٠٪, application of iodine ١٠٪ and heated spatula (cauterization).The results revealed that cauterization was the most effective and useful method for the sterilization of appendix. The results of this experiment were in agreement with that obtained by Gibbous & Houte (١٩٧٥), Beachey (١٩٨١), Brodsky *et al* (١٩٩١) and Hussien (١٩٩٩).

٣.٣ . Clinical Features Related to Acute Appendicitis

The subjects included in were complaining from various symptoms are shown in table (٣-١), ٧٠(٦٣.٦٪) showed right iliac fossa pain, increased fever was seen in ٣٥(٣١.٨%) patients, while ٣١(٢٨.١٪) patient were with nausea. Other features were as follows; headache ١٧(١٥.٥%), constipation ١٦(١٤.٥٪), generalized pain ١٤(١٢.٧٪), diarrhea ١١(١٠٪), loss of appetite ٦(٥.٥%), vomiting ٦(٥.٥٪) and dysuria ٢ (١.٨٪). From the results described above one can concluded that the right iliac fosse pain is the commonest symptom which may draw attention to the case as suspected appendicitis. Those results were in accordance with related study

Kosloske (٢٠٠٤) who pointed out that the right iliac fosse pain was the common feature of appendicitis.

Table (٣-١) Distribution of the acute appendicitis according to the clinical features (n=١١٠)

Clinical feature	No. (%)
Right iliac fossa pain	٧٠ (٦٣.٦)
Fever	٣٥ (٣١.٨)
Nausea	٣١ (٢٨.١)
Headache	١٧ (١٥.٥)
Constipation	١٦ (١٤.٥)
Generalized pain	١٤ (١٢.٧)
Diarrhea	١١ (١٠)

Loss of appetite	**٦ (٥.٥)**
Vomitting	**٦(٥.٥)**
Dysuria	**٢ (١.٨)**

٣.٤ Risk Factors

٣.٤.١ Age Related Disease

The distributions of patients with acute appendicitis according to their ages are shown in table (٣-٢). The results revealed a significant differences upon age factor [cal. $X^{٢}$ = ١٨.٨, tab x= ٧.٨١, p < (٠.٠٥)] since the results indicated that the peak of incidences observed in the age group (١١-٢٠) years old, that the frequency of infection accounted for ٥٧(٥١.٨٪) patients, ٣٩(٣٥.٤٪) of them were males and ١٨(١٦.٣%) were females. The results indicate that there was an

increase in the incidences with the advancement of age, since the lowest rate ٢(١.٨٪) was seen within the age group of (٥١-٦٠) old.

The patients of ١١-٢٠ years old being more susceptible for appendicitis may ascribed to the nature of physiological and anatomial factors of appendix tissue(Jones, ٢٠٠٢), since lymphoid tissue is the most susceptible for infection gradually increase up to the ٢٠ years of age. The lymphoid tissue begins to decrease with advancement of the age up to ٦٠ years old when this tissue is totally disappear. In addition to the configuration of the appendix at the age of group of (١١-٢٠) years due to increase infection with acute appendicitis (Jones, ٢٠٠١).

Table (٣-٢) Distribution of acute appendicitis according to the age and sex

Age(year)	No of patient (%)	No of males (%)	No of females (%)
١-١٠	٣ (٢.٧)	١ (٠.٩)	٢ (١.٨)
١١-٢٠	٥٧ (٥١.٨)	٣٩ (٣٥.٤)	١٨ (١٦.٣)
٢١-٣٠	٣٣ (٣٠)	٢٠ (١٨.١)	١٣ (١١.٨)

٣١-٤٠	٩ (٨.١)	٥ (٤.٥)	٤ (٣.٦)
٤١-٥٠	٦ (٥.٤)	٣ (٢.٧)	٣ (٢.٧)
٥١-٦٠	٢ (١.٨)	١ (٠.٩)	١ (٠.٩)
Total	١١٠	٦٩(٦٢.٧)	٤١(٣٧.٣)

٣.٤.٢. Sex Related Disease

The occurrence of acute appendicitis was found to be higher in males than females in (Table ٣-٢). The results indicated that males ٠.٠٩) more <are significantly (Cal. Z=٢.١٢٦, tab Z=٠.٩٨٤, p

susceptible ٦٩(٦٢.٧%) than females ٤١(٣٧.٣%) with ratio of male to female accounted for ١.٧:١. The same results were being reported by Addiss *et al*.,(١٩٩٠) and al Fahad (٢٠٠٣) since, it has been found the diagnosis of acute appendicitis in males is more reliable compared with females that, the females remain a difficult to diagnose and misdiagnosis may result due to other diseases such as gynecologic diseases.

٣.٤.٣. Seasonal Variations and Acute Appendicitis

Table (٣-٣) illustrates the occurrence of appendicitis according to the seasonal variations. The results indicated that the prevalence were considerably increased during the hot season, begging through may which account ١٤(١٢.٧٪) cases then June ١٨(١٦.٤%) cases and July ٢٦(٢٣.٦٪), whereas a remarkable decreased incidences were seen during the cold season represent by January, ٤(٣.٦٪) cases and February ٥(٤.٥) cases. Rothrock *et al*., (٢٠٠٠) reported closely similar results but the explanation for such results are rare. However this study may conclude that and extreme change in temperatures may have a role in an increasing the incidences during the warm season in addition to other factors such as type of diet and water (for drinking and swimming) may cause the occurring of bacterial and viral infections which may lead to appendicitis (LuckMann and Davis, ١٩٩١; Rothrock *et al*., ٢٠٠٠).

Table (٣-٣) Seasonal Variations of Acute Appendicitis

Month		NO. Of patient (%)
	October	١٠(٩.١)
٢٠٠٤	November	٧(٦.٤)
	December	٦(٥.٥)
	January	٤(٣.٦)
	February	٥(٤.٥)
	March	٩(٨.٢)
April	٢٠٠٥	١١(١٠)
	May	١٤(١٢.٧)
	June	١٨(١٦.٤)
	July	٢٦(٢٣.٦)
Total (%)		١١٠(١٠٠٪)

٣.٥. Isolation and Identification

In this study a total of eleven bacterial genera were found to be implicated in acute appendicitis. Three genera were Gram positive

(Table ٣-٤) and eight genera were Gram negative (table ٣-٥). Those isolates were isolated and identified according to the cellular morphology, cultural characteristics and biochemical tests for each organism and compared with the referential results recommended by Holt *et al*., (١٩٩٤)Baron *et al*.,(١٩٩٦); Benson(١٩٩٨) and McFadden (٢٠٠٠).

٣.٦. Isolation Bacteria From Acute Appendicitis

Table (٣-٦) shows that out of ١١٠specimens (appendix swabs), of which ٩٠(٨١.٨٪) specimens yielded positive results for bacterial growth, while ٢٠(١٨.٢٪) specimens yielded no growth. The same results seen by Lau- WY *et al*., (١٩٩٤) and Cuss& Richard, (٢٠٠٠) who stated that most of bacterial cultures for suspected appendicitis were positive. In this study, a total of eleven bacterial appendicitis. Eight genera were Gram- negative and ٣ genera were Gram- positive as shown in table (٣-٧), Gram- negative bacteria were the common

cause of appendicitis compared with that of Gram positive, they were ١٠٧(٩٦.٤٪) isolates and ٤(٣.٦٪) isolates respectively. Moreover ٢١(١٨.٩٪) cases revealed mixed growth (more than one caustive agent).The most frequent pathogen was *E. coli* which accounted for ٣٦(٣٢.٤٪) isolates, followed by *Bacteroides* spp isolates (٢١, ١٩ %).

Table (٣-٦) Results and Swab Culture in Males and Females Suffering From Appendicitis

Cultural- result	No of cases	No.(%) of	
		Males	Females
Positive	٩٠(٨١.٨)	٦٥(٥٩.١)	٢٥(٢٢.٧)
Negative	٢٠(١٨.٢)	٤(٣.٦)	١٦(١٤.٦)
Total	١١٠(١٠٠)	٦٩(٦٢.٧)	٤١(٣٧.٣)

Table (٣-٧) Types and Frequency of Bacteria Isolated From In ٩٠ Patients Suffering From Appendicitis.

Type of bacteria	No. (%) of Bacteria
Gram- negative n=١٠٧	
Esherichia coli	٣٦(٣٢.٤)
Bacteriodes spp	٢١(١٨.٩)
Klebsiella pneumoniae	١٨(١٦.٢)
Pseudomonas aeruginosa	١١(٩.٩)
Citrobacter frundii	٧(٦.٣)

Salmonella typhi	**٥(٤.٥)**
Proteus mirabilis	**٥(٤.٥)**
Enterobacter aerogenes	**٤(٣.٦)**
Gram positive n=٤	
Peptostreptococcus sp	**٢(١.٨)**
Clostridium perfringnes	**١(٠.٩)**
Staphylococcus aureus	**١(٠.٩)**
Mixed growth	**٢١(١٨.٩)**
Total	**١٣١**

١٨(١٦.٢%) isolates of *Klebsiella pneumoniae*, ١١(١٠٪) isolates of *Pseudomonas aeruginosa*, ٧(٦.٣٪) isolates of *Citrobacter frundii*, ٥(٤.٥٪) isolates of *Salmonella typhi*, ٥(٤.٥٪) of *Proteus mirabilis* and ٤(٣.٦٪) isolates of *Enterobacter aerogenes*.

Peptostreptococcus spp was the most frequent microorganism among Gram-positive bacteria which accounted as ٢(١.٨٪). Whereas each of *staphylococcus aureus* and *Clostridium perfringnes* were accounted as ١(٠.٩٪) as presented in table (٣-٨).

The results being found were in accordance with other results being reported by Elhag *et al*., (١٩٨٦) and Baste *et al*., (١٩٩٩). Those results were accepted and suspected since *E. coli* is the common

organism present in intestine fastly proliferates and quickly adheres to the tissue surfaces (Baron *et al*., ١٩٩٢).

The adhesion of microorganism to the epithelial cells of the tissue is the first stage of infection followed by the invasive stage. Moreover *E. coli* has others virulence factors such as host cell surface- modifying factors, toxins, hemolysin and cytotoxin necrotizing factor type I (CNFI). (Martirosian *et al*., ٢٠٠١).

Bacteroides has been also described to possess several factors capable of participating in intrabduminal infection. The *Bacteroides* Contribute to develop an infections in three ways, stimulation of abscess formation, reduction of phagocytes by polymorphnuclear leukocytes (PMNS) because of the capsule of *Bacteroidis* and inactivation of antibiotics by B-lactamase production(Kenneth *et al*., ٢٠٠٣).

Klebsiella pneumonia isolates were ١٨(١٦.٣٪). This organism has the Capsule which plays an important role during the initial steps of the pathogensis by interacting with mucus producing cells, the colonization of mucus membranes by bacteria is liked to an adhesion process involving specific adhesions on the bacterial surface. In addition to several pilli involved in adhesion to epithelial cell of intestine (Podschun and Sahly ١٩٩١; mims, *et al.,* ٢٠٠٤).

The explanation for detection of *Pseudomonas aeruginosa* in appendix as caustive agent of appendicitis may be attributed to the abilty of this organism to adhere and to colonize the epithelial tissues probably by pili and by the algene (slime) layer surrounding the cells of this bacterium(Zhu *et al*.,٢٠٠٤). Further more *Pseudomonas aeruginosa* possesses other virulence factors (enzymes and toxins, which enable it to cause infection. Other Gram- negative *Cilrobacter frundii*, *Salmonella typhi*, *Proteus mirabilis* and *Enterobacter aerogenes* were also detected in appendicitis although in low frequencies compared with other Gram negative (table, ٣-٧).

However, the implication of these bacteria in appendicitis is suspected, since they belong to the enteric group and frequently present in intestine and all have virulence factors enabling them to cause disease Abbott *et al*., ١٩٩٩). In this study, some of Gram positive member also represented by *Staphylococcus aureus*, *peptostreptococcus* sp and *Clostridium perfringes* were also isolated and identified from appendicitis cases, but in low frequencies in relative with that of Gram negative.

Members (Table ٣-٨) Gram positive appendicitis are rarely reported at the present time. This May due to adhesive and colonizer factor being less in gram positive compared with that of Gram negative- moreover most of Gram positive bacteria are fastidious require for special growth factors (amino acids, vitamin, etc) and

growth condition (O_2, CO_2 ...etc) (Mims *et al.*, ٢٠٠٤). However qualitatively, Gram positive infections are more serious and the detection of *Clostridium perfringnes* in appendicitis by this study may can be the announcement that even the strect obligate anaerobic necrotizer gas gangrene producer *Clostridium perfringes* can implicate in appendix infection.

٣.٧. Mixed Bacterial Growth

In this study, anaerobes, Gram negative and Gram positive were frequently isolated from mixed infection with facultative aerobic bacteria and presented in ٢١ isolates (١٨.٩٪). Table (٣-٨). This pattern of mixed culture between aerobic and anaerobic bacteria may be attributed to the synergistic relationship between aerobes and anaerobes, in which the aerobic bacteria grow first, exhausting oxygen and producing intermediates products may help in growing of the anaerobic bacteria .(James *et al.,*٢٠٠١).

Table: ٣-٨ Appendicitis by Mixed Bacterial Types.

Type of Bacteria	NO. (%)
E .coli ***Bacteroides* sp**	٧(٣٣.٣)
Klebsiella pneumoniae ***Bacteroides* spp**	٤(١٩)

E. coli ***Citrobacter frundii***	٣(١٤.٢)
Klebsiella pneumoniae ***E. coli***	٢(٩.٥)
E. coli ***Enterobacter aerogenes***	٢(٩.٥)
Proteus mirabilis ***Psendomones aeruginosa***	١(٤.٨)
E. coli ***Peptostreptococcus* spp**	١(٤.٨)
Pseudomonas aeruginosa ***Clostridium perfringnes***	١(٤.٨)
Total	٢١(١٠٠)

٣.٨. Susceptibility to Antimicrobial Agents

The susceptibility of all isolates being detected in this study to ten (١٠) traditional antimicrobial agents was determined are shown in table (٣-١٠). The results indicated that *Psendomonas aeruginosa* was the most important bacteria resistant for these antibiotics. All ١١ isolates(١٠٠٪) exhibited considerable resistance toward amoxicillin, Gentamycin, Cefotaxime, Cefixme, trimethoprim, vancomycin, norfloxacin and tetracyclin, while ١٠ isolates (٩٠.٩٪)

were resistance to ciprofloxacin and ٩ isolates (٨١.٨%) resistance to amikacin. ٢١ isolates (١٠٠٪) of *Bacteroides* sp resistance to trimethoprim, vancomycin, norcloxacin and tetracycline, while ١٩ isolates (٩٠.٤%) showed resistance to amoxicillin, Gentamycin and amikacin. However isolates ٥ cases (٢٣.٨٪) exhibited resistance to ciprofloxacin, cefotaxime and cefixme, ٥ isolates (١٠٠٪) of *Salmonella typhi* resistance for Gramycin, amikacin, cefotaxime, cefixme, vancomycin norfloxacin and tetracycline, while ٤ isolates (٨٠٪) were resistance to amoxycillin and trimithoprim and only ١ isolates (٢٠٪) resisted ciprofoxacin. ١٨ cases of *Klebsiella pneumonia* were resistant to vancomycin and norfloxacin, while ١٧ isolates (٩٤.٤٪) resistance to amoxicillin, trimetohprim and tetracycline. In addition to ٧ isolates (٣٨.٨%) were resistance to Gentamycin and amikacin and ٥ isolates (٢٧.٧%) were resistant to ciprofloxacin, cefotaxime and cefixie. Five isolates (١٠٠٪) of *Proteus mirabilis* were resistant of vancomycin and tetracycline, while ٣ isolates (٦٠٪) exhibited resistance for amoxicillin, Gentamycin, amikacin, trimethoprim and norfloxacin in addition to ١ isolates (٢٠٪) resistance to ciprofloxacin, cefotaxime, most isolate of *E. coli* revealed high sensitive to most of tested antibiotics. Such results were true for *Entorobacter. aeruginosa* as well are ٢ isolates (٥٠%) resistance for trimethoprim and norfloxacin while ٣ iaolates (٧٥%) resistance to amoxycillin, Gentamycin, amikacin, ciprofloxacin, cefotaxime and cefixme, ١ isolates (١٠٠٪) of *Clostridium perfringes*

resistance for amoxicillin, Gentamycin, amikacin, trimethoprim, norfloxacin and tetracycline. The ١ isolates of *S. aureus* was resistant to trimethoprim, vancomycin and amoxycillin. The results showed that *Pseudomonas aeruginosa* was the most pathogen resistant toward the most tested antibiotics. Since it resisted amoxicin, Garamycin, cefotaxime, cefixme, trimethoprim, vancomycin, norfloxacin and tetracycline, in aremakable level. Resistance mediated by *Pseudomonas aernginosa* can by attributed both to an inducible, chromosomally mediated β lactamase that render board spectrum cephalosporins to inactive, and to plasmid mediated beta lactamase that can lead to resistance to several penicillins and older cephalosporins (Cross and Campbell, ١٩٩٩). In addition other factors may enable *Pseudomonas aeruginosa* to resist antimicrobial agents such as, altered and membrane site, nature of cell wall and alginate layer surrounding the bacterial cell (Mandell, ١٩٩٩; Norrby, ١٩٩٧). Moreover *Pseudomonas aeruginosa* having lipopolysacchride (LPS) permeability barrier connected with complex proteins in their outer membrane, which are preventable passing of antimicrobial agents to the inside of the bacterial cell (Moore *et al*.,١٩٦٩; Jones *et al*.,٢٠٠١). Gram negative bacilli (*klebsiella pneumoniae* and *Citrobacter frundii*) have broad spectrum resistance for antimicrobial agents. The production of β lactamase is the main mechanism for this resistance (Yamagnchi *et al*., ١٩٩٩; Ya *et al*., ١٩٩٩; Robert, ١٩٩٩).

Additionally other mechanisms may enable the bacteria to decrease the permeability of cell membrane (Katzung, ٢٠٠٣; Mims *et al*., ٢٠٠٤).This may be true for *Protous mirabilis* and *E. coli*, since they showed relative resistance for many of the antimicrobial agents.

The high sensitivity of *Staphelococcus aureus* to most antimicrobial agents (except trimethoprim and tetracycline) is an expected result to these bacteria, since *S. aureus* belong to the Gram positive bacteria which has been characterized to be sensitive to most common antibiotics in comparison with Gram negative bacteria due to the difference in the outer membrane structure which appears to be permeable to most antibiotics in gram positive bacteria than gram negative bacteria (Brook *et al*., ١٩٩٨).

٣.٩ Determination of Minimum Inhibitory Concentration (MIC)

In this study, five antimicrobial agents were used to determine the MIC against bacterial isolates. Those antimicrobial agents were cefotaxime, amikacin, amoxiclave, cefotriaxon and ciprofloxacin as shown in table (٣-١٠). The results were recorded according to NCCLS (١٩٩٩). It was found that amoxiclave and amikacin were most effective antibiotics against *E. coli*, since the MIC ranged from ٠.٥ - ٤٦ µg/ml for amoxiclave and ٠.٥ - ٨ µg/ml for ciproxacin. *Proteus mirabilis* exhibited more sensitive for cefotriaxon and ciprofloxacin, since the MIC ranged from ٢-٣٢ µg/ml for cefotriaxon and ١-٤ µg/ml for ciproxacin.

Klebsiella pneumoniae revealed high sensitivity for cefotaxime and ciprofloxacillin, since their MIC ranged from ٨-١٢٨ ug/ml and ١-٨ug/ml respectively. *Enterobacter aerogenes* was highly sensitive to ceftriaxon, cefotaxime and ciprofloxacin, since their MIC ranged from ٠.١٢٥- ٤ µg/ml, ٠.١٢٥-٤ µg/ml and ٠.١٢٥ - ٠.٥ µg/ml respectively.

Citrobacter frundii exhibited more sensitivity for amoxiclave and cefotaxime, since three MIC ranged from ٠.٢٥- ١٢٨ µg/ml and ٠.٠٠٤- ١٢٨ µg/ml respecfively, while ciprofloxacillin and cefotriaxon were the most effective against *Salmonella typhi*, since their MIC ranged from (٠.٥-٣٢ and ٠.٥-٢ µg/ml respectively.

Pseudomonas aeruginosa was the highest resistant for most antibiotics, except the ciprofloxacin which was the more effective

against this bacteria (Jones *et al*., ٢٠٠٢), since the MIC of this antibiotic ranged from (٠.٥-٨ µg / ml) for ciprofloxacin. The result of this method which in accordance the result of disk diffusion method used in this study.

Conclusion

١- **Appendicitis can be resulted due to bacterial infection of appendix.**

٢- **Gram positive & negative (aerobic & anaerobic) were implicated in appendicitis.**

٣- **Gram negative bacteria were more common in appendicitis than Gram positive.**

٤- ***E. coli* constitutes the predominant bacteria in appendicitis.**

٥- **The age group ranges from ١١ to ٢٠ years are more susceptible for appendicitis.**

٦- **The occurrence of appendicitis increases during the warm season.**

٧- **Women are some times misdiagnosed as appendicitis leading to unnecessary appendectomy.**

Recommendations

١- Achievement of further studies to detect serologically the early diagnosis of appendicitis.

٢- Achievement of further studies to detect the role of diet type and nature in appendicitis occurrence

٣- Careful management with women complaining from symptoms suspected for appendicitis

٤- Search for other member of quinolones and new generation cephalosporins that are used to prevent complication.

٥- Working on viral appendicitis.

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Table (٣-٤) Biochemical tests used for the diagnosis of Gram- positive bacteria

Tests / Types of Bacteria (No.)	Aerobic growth	Anaerobic growth	Motility	Oxidase test	Catalase test	Hemolysis	Pigment formation	Fermentation of		Coagulase test
								Glucose	manitol	
S. aureus (١)	+		-	-	+	+	+	+	+	+
Peptostreptoccus sp (٢)	-	+	-	-	-d	-	-			
Cl. Perfringes (١)	-	+	-	-	-	+				

d. different
*** not done**

Table (٣-٥) Biochemical Tests used for the Diagnosis of Gram- negative bacteria

	Aerobic	Anae	Oxide	Indol	Meth	Voges	Citrate	Urease	Catal	Reaction On KIA:	Motility	Hemolysis	Growth on	O – F gluco	Fermentation of

Tests / Type of (No.)										Slant/ bupp	Gas	H ٢S				Glucose	Lactose	Manitol	Arabinose	Rhamnose	Trehiaose
coli (٣٦)	+	+	-	+	+	-	-	-	+	A/ A	+	-	+	+	+	F	+	+	+	+	+	
oniae (١٨)	+	+	-	-	-	+	+	+	+	A/A	+	-	-	-	+	F	+	+	+	+	+	
osa (١١)	+	-	+	-	-	-	+	-	+	K/K	-	-	+	+	+	O	+	-				
Mirabilis (٥)	+	+	-	-	+	-			+	K/A	V	+	+		+	F	+	-	-	-	-	
ct frundii (٧)	+	+	-	-	+	-	+	-	+	K/A		+	+	-	+		+	V	+	+		
ella typhi (٥)	+	-	-	-	+	-	-	-	+	K/A	-	+	+	-	+							
erobacter. genes (٤)	+	+	-					V	+	A/A	-	-	+		+		+					
oides. Spp (٢١)	-	+	-	-				V		A/ ٨	–	V	-	+	-	F	+	+				V

KIA= kligler iron agar K= alkaline A= acid V= variable *= not done

Table (٣-٩) The Numbers and Percentage Resistance of ١١١ Bacterial to ١٠ Antibacterial Agents

Type of bacteria (No)	NO of isolate	AMX (%)	GM (%)	AK (%)	CF (%)	CE (%)	CXM (%)	TMX
E.coli	٣٦	٢٩(٨٠.٥)	١٤ (٣٨.٨)	١١ (٣٠.٥)	١٦ (٤٤.٤)	١٥ (٤١.٦)	١٥ (٤١.٦)	(٦
K. pneumoniae	١٨	١٧ (٩٤.٤)	٧ (٣٨.٨)	٧ (٣٨.٨)	٥ (٢٧.٧)	٥ ٢٧.٧	٥ ٢٧.٧	(٩
P.aeruginosa	١١	١١ (١٠٠)	١١ (١٠٠)	٩ ٨١.٨	١٠ (٩٠.٩)	١١ (١٠٠)	١١ (١٠٠)	(

C. frundii	٧	٦ (٨٥.٧)	٥ (٧١.٤)	٥ (٧١.٤)	٤ (٥٧.١)	٤ (٥٧.١)	٥ (٧١.٤)	(
P. mirabilis	٥	٣ (٦٠)	٣ (٦٠)	٣ (٦٠)	١ (٢٠)	١ (٢٠)	٢ (٤٠)	
S. typhi	٥	٤ (٨٠)	٥ (١٠٠)	٥ (١٠٠)	١ (٢٠)	٥ (١٠٠)	٤ (٨٠)	(
E. aerogenes	٤	٣ (٧٥)	٣ (٧٥)	٣ (٧٥)	O	٠	٣ (٧٥)	
S. aures	١	٠	٠	٠	٠١	٠	٠	(
Bacteroides spp	٢١	١٩ (٩٠.٤)	١٩ (٩٠.٤)	١٩ (٩٠.٤)	٥ (٢٣.٨)	٥ (٢٣.٨)	٥ (٢٣.٨)	(
C.perfringes	١	١ (١٠٠)	١ (١٠٠)	١ (١٠٠)	٠	٠	٠	(
Peptstreptococcus spp	٢	١ (٥٠)	١ (٥٠)	٢ (١٠٠)	١ (٥٠)	١ (٥٠)	٢ (١٠٠)	(
Total (%)	١١١	(٨٤.٦) ٩٤	(٦٢.١) ٦٩	(٥٨.٥) ٦٥	(٣٨.٧) ٤٣	(٤٢.٣) ٤٧	(٤٦.٨) ٥٢	(٨٣.٨

Type of Bacteria	No of Isolates	Antimicrobial agent	Approximate MIC Correlate (µg / ml) S	I	R	Range of MIC (µg / ml)	Susptibility
			No of strain				
P. mirabilis	(٥)	Amoxiclave	≤٤(١)	٨(١)	≥٣٢(٣)		
		Cefotriaxone	<٨(٣)	١٦(١)	>٣٢(١)		
		Cefotaxime	<١(١)	٣٢(١)	≥٦٤(١)		
		Amikacin	≤٤(٤)	١٦(١)	>٣٢(٣)		
		Ciprofloxacin	<١(٤)	٢(١)	≥٤(١)		
E. coli	(٣٦)	Amoxiclave	٠.٥- ٤(٣٢)	١٦(١)	٦٤ (٣)	٠.٥-٦٤	٨٨.٨
		Cefotriaxone	≤٤(٤)	١٦- ٣٢(١٨)	>٣٢(١٤)	١-٣٢	١١.١
		Cefotaxime	≤٤(٤)	≥١٦(١٨)	>٣٢(١٤)	٠.٥-٣٢	١١.١
		Amikacin	١ - ≤٨(١٨) ≤١(١٥)	>١٦(٧) >٢(٥)	>٣٢(١١) ≥٤ (١٦)	١-٦٤ ٠.٥-٨	٥٠ ٤١.٦
K. pneumoniae	(١٨)	Ciprofloxacin					
		Amoxiclave	≤٢(٥)	≥٨(٤)	>٣٢ (٩)	١-٦٤	٢٧.٧
		Cefotriaxone	≤٨(٩)	≥٣٢(٤)	≥٦٤(٥)	٤-١٢٨	٥٠
		Cefotaxime	٨(١٠)	≥١٦(٣)	≥٦٤(٥)	٨-١٢٨	٥٢.٥
		Amikacin	≤١٦(٨)	٣٢(٣)	≥٦٤(٧)	٨-١٢٨	٤٤.٤
		Ciprofloxacin	١-٨(١٠)	≥٢(٢)	≥٤(٦)	١-٨	٥٥.٥
P. aeruginosa	(١١)	Amoxiclave	٤(١)	١٦(١)	≥٣٢(٩)	١-٦٤	٢٠
		Cefotriaxone	≤٨(٢)	١٦(١)	٦٤-	٤-١٢٨	٦٠
		Cefotaxime	≤٨(٢)	١٦(١)	١٢٨(٨)	٤-١٢٨	٦٠
		Amikacin	<١٦(٢)	٣٢(١)	≥٦٤(٨)	٢-٦٤	٢٠
		Ciprofloxacin	<١(٨)	٢(١)	٨>(٨) ٧≥(٢)	٠.٥-٨	٨٠
C. frundii	(٧)	Amoxiclave	<٤(٣)	≥٨(١)	≥٣٢(٣)	٠.٢٥- ١٢٨ ٠.٠٠٤- ١٢٨ ٠.٠٣- ١٢٨ ٠.٠٠٤-٨	٤٢.٨ ٢٨.٥ ٤٢.٨ ١٤.٢ ٢٨.٥
		Cefotriaxone	≤٨(٢)	١٦(١)	≥٦٤(٤)		
		Cefotaxime	≤٨(٣)	———	≥٦٤(٤)		
		Amikacin	≤١٦(١)	٣٢(١)	≥٦٤(٥)		
		Ciprofloxacin	١(٢)	٢(١)	≥٤(٤)		

Table (٣ -١٠) Antimicrobial susceptibility of bacteria isolated from patients with acute appendicitis, MICs of five antibiotics to wards isolated bacteria

S. typhi	(٥)	**Amoxiclave**	<٨(١)	٨(١)	≥١٦(٣)	
		Cefotriaxone	≤٨(٣)	١٦(١)	٣٢(١)	٠
		Cefotaxime	———	٣٢(١)	≥٦٤(٤)	١
		Amikacin	———	———	≥٣٢(٥)	٢
		Ciprofloxacin	<١(٤)	٢(١)	>٢(١)	٠
E. aerogenes	(٤)	**Amoxiclave**	≤٤(٣)	٨(١)	———	١
		Cefotriaxone	≤٤(٤)	———	———	٠.
		Cefotaxime	≤٤(٤)	———	———	٠.
		Amikacin	٨(٢)	———	>٣٢(٢)	٨
		Ciprofloxacin	<١(٤)	———	———	٠.١

- **S= Sensitive**
- **I= Intermediate**
- **R= Resistance**