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College of Medicine

A Bacteriological Study of Maxillary Sinusitis in Babylon Province

A Thesis

Submitted to the Council of the College of Medicine - University of Babylon In Partial Fulfillment of the Requirements For the Degree of Master of Science In Medical Microbiology

By

Taiseer Safa'a Jawad

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بسم الله الرحمن الرحيم

((قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا

إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ))

صدق الله العلي العظيم

سورة البقرة

الآيـة (٣٢)

We certify that this thesis was prepared under our supervision at the University of Babylon as partial Fulfillment of the requirements for the degree of M.Sc. in Medical Microbiology….

Signature of Supervisor
Dr. Habeeb S. Naher
Professor
Department of Microbiology
College of Medicine
University of Babylon
Date: / / ٢٠٠٧

Signature of Supervisor
Dr. Safa'a Sahib
Assis.Professor
Department of Oto. Rhino. Laryngology
College of Medicine
University of Babylon
Date: / / ٢٠٠٧

Recommendations of The Head of Department of Microbiology:-

According to the available recommendations, we forward this thesis for debate by the examination committee.

Signature

Dr. Mohammad Sabri Alsaeed
Assistant Professor
Head of Microbiology Department
College of Medicine
University of Babylon
Date: / / ٢٠٠٧

We , the examiner committee , certify that we have read the thesis entitled (A Bacteriological Study of Maxillary Sinusitis in Babylon Province) and have examined the student (Taiseer Safa'a Jawad) in its contents , and that in our opinion it is accepted as a thesis for the degree of Master of Science in Medical Microbiology

Signature

Dr. Khairy Abdullah Dawood
College of Dternary Medicine
Al-Quadisiya University
(Chairman)

Signature
Assistant Prof.
Dr. Jaafar K. Naama
College of Medicine
Al-Kufa University
(Member)

Signature
Assistant Prof.
Dr. Saad A. Al-Jubory
College of Medicine
Babylon University
(Member)

Signature
Prof.
Dr. Habeeb S. Naher
College of Medicine
University of Babylon
(Member and Supervisor)

Signature
Assistant Prof.
Dr. Safa'a Sahib Naji
College of Medicine
University of Babylon
(Member and Supervisor)

Approved for the College Committee of Graduate Studies

Signature
Assistant Prof.
Dr. Ali Khair Alla Al-Zubaidy
Dean of Coll

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Taiseer

DEDICATION

To

Holy Imam Al- Hujja…

Mohammed Al-Mahdi….

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Abbreviations

Symbol	Description
DW	Distilled water
ENT	Ear nose throat
MRSA	Methecilin Resistant *Staphylococcus aureus*
MSSA	Methecilin Sensetive *Staphylococcus aureus*
NCCLs	National Committee for Clinical Laboratory Standards
URTI	Upper respiratory tract infection

Abstract

A total of ١٠٠ samples were collected from ١٠٠ patients (٦٥ of them were females and ٣٥ were males) who were referred to Hilla Teaching Hospital and special clinics during a period of ten months (from October ٢٠٠٤ to July ٢٠٠٥). The collected samples were investigated for bacterial analysis. These patients were suffering from sinusitis (٥٨ cases were acute sinusitis and other ٤٢ cases were chronic) as being diagnosed by ENT experts (through clinical and radiological examination X-ray and / or CT scan).

The ages of those patients ranged from ١٠ to ٦٠ years .The results indicated that, the most age group being susceptible for both acute and chronic sinusitis was between ٢٠ to ٢٩ years old.

The results of bacterial culture for acute sinusitis were Positive in ٥٠ patients (٨٦.٢٪) versus ٨ (١٣.٨٪) revealed negative bacterial culture, while for chronic sinusitis ٣٥ patients (٨٣.٣٪) had positive culture and ٧ patients (١٦.٧٪) versus negative culture. The most common type of bacteria which was isolated from acute sinusitis was ***Streptococcus pneumonia*** (٢٥٪) followed by ***Moraxella catarrhalis*** (١٨٪), ***Staphylococcus aureus*** (١٦.٧٪), Coagulase negative staphylococci (١٤٪), Viridans group (١٢٪), ***Klebseilla pneumoniae*** (٨٪) and ***Streptococcus pyogenes*** (٦٪).

In chronic sinusitis the dominant bacterial type was ***Staphylococcus aureus*** (٤٢.٨٪) followed by ***Streptococcus pneumoniae*** (٢٣.٤٪), ***Moraxella catarrhalis*** (١٠.٧٪), ***Klebseilla pneumoniae*** (٩٪), ***E.coli*** (٥.٣٪), Viridans group (٥.٣٪) and ***Proteus mirabilis*** (٣.٥٪).

The Methicillin Resistance ***Staphylococcus aureus*** **(**MRSA**)** was detected in both acute and chronic sinusitis which are accounted for ١٤٪ and ٣٧٪ respectively.

The potency (Ampicillin, Amoxicillin, Gentamycin, Cefotaxim, Cefalothin, Ciprofloxacin, Erythromycin, Tetracyclin, Vancomycin, Trimethoprim) of antibiotics against bacterial isolates in this study was investigated. The results of this study showed that ciprofloxacin and cefotaxim were the most effective antibiotics towards the tested bacterial isolates.

Chapter One

Introduction and Literature Review

١-١ *Introduction:*

Sinusitis is one of the most common infections of humans respiratory tract (Gwaltney, ١٩٩٩). The lining of the nose and paranasal sinuses is continuous and inflammation which affects the lining of the nose will spread to variable extent, into the sinuses (Buchem ***et.al.,*** ١٩٩٥).

Sinusitis generally develops as a complication of viral or allergic inflammation of the upper respiratory tract, because the inflammed nasal mucosa block ostial draining from the sinus and the result in stasis in the sinuses encourage development of infection (Calhoun, ١٩٩٢).

Acute sinusitis is a short-term condition that responds well to antibiotic and decongestant. Patients with acute sinusitis may be difficult to diagnose on clinical ground only, since symptoms includes; malaise, fever, purulent discharge, tenderness and facial pain (Ferguson, ١٩٩٥).

Chronic sinusitis is more prolonged than acute sinusitis and it is characterized by at least four recurrences of acute sinusitis, either medication or surgery is a possible treatment for that. Symptoms of chronic sinusitis include facial pain, purulent discharge, rhinorrea and malaise (Finegold ***et.al.,*** ٢٠٠٢). It is important to differentiate between acute and chronic sinusitis on the bases of the duration of symptoms because their etiologies and treatment may differ (Snow ***et.al.,*** ٢٠٠١).

Early identification of sinusitis is medically important because the infection in such closed space carries a risk of complication and may warrant on active therapeutic approach including drainage of infected secretion and administration of antimicrobial agent (Lundberg ***et.al.,*** ١٩٧٩ and Stankiewicz ***et.al.,*** ١٩٩٣). Radiological examination by CT-

scan is the most reliable method of diagnosing of maxillary sinusitis (Lund and Lloyd, ١٩٨٣).

Aspiration is important for the demonstration of secretion in the sinus and for the identification of the specific etiology by culture, which may be useful as a guide for antimicrobial therapy (Bridger, ١٩٨٠; Axelsson and Brorson, ١٩٧٣ and Little ***et.al.,*** ١٩٩٨).

The etiology of acute sinusitis is primarily bacterial ***S. pneumoniae , H. influnzea*** and ***S. aureus*** are the main cause and present over half of the cases (Kortekangas,١٩٦٤; Lystad ***et.al.,***١٩٦٤; Mann ***et.al.,***١٩٨٢; Berg ***et.al.,***١٩٨٨ and Diaz and Bamberger,١٩٩٥). Lately ***M. catarrhalis*** has also been implicated as a pathogen in sinusitis (Wald ***et.al.,*** ١٩٨٤; Chapman ***et.al.,***١٩٨٥), ***S. pyogenes*** is relatively uncommon pathogen in acute maxillary sinusitis, with frequencies ١and /or ٣ % (Wald ***et.al.,***١٩٨١; Gwaltney ***et.al.,***١٩٨١). In chronic sinusitis mostly the same type of bacteria with anaerobic bacteria like (***Bacteriodes, Peptostreptococcus, Fusobacterium and Viellonella***) is well established, but the occurrence of anaerobe bacteria in acute sinusitis is less clear (Radosz ***et.al.,*** ١٩٩٧; Liu ***et.al.,*** ١٩٩٨).

This study was suggested in an attempt to fulfill the following goals :-

١-Detection of the bacterial causes of sinusitis.

٢-Determination of the environmental factors related with sinusitis.

٣-Determination of some risk factors associated with the infection.

٤-Determination of the susceptibility of isolated bacteria to traditional antibiotics.

١-٢ *Literature review:*

In this chapter light will be thrown upon the sinuses through the summarized themes below:-

١-٢-١ *Embryology:-*

The maxillary sinus is present at birth, undergoing further expansion with age. Two main points of rapid growth occur from birth until the age of ٣Years, and then from the age of ٧ years through early adolescence. The sinus then slowly grows until it reaches adult size by the age of ١٨ years (Maresh, ١٩٨٩).

Later in adult life, the sinus continues to pneumatize inferiorly and may expand to contain tooth root. The floor of the maxillary sinus is below the level of the nasal floor (Hengerer, ١٩٨٤).

١-٢-٢ *Anatomy of the Paranasal sinuses :*

There are five Paranasal sinuses (air – filled spaces lined by mucous membrane) on each side of the head. They are ethmoid, maxillary, frontal and sphenoid sinuses (Lazar, ١٩٩٣). Sinuses are divided into two groups, the anterior group and the posterior group. The anterior group comprises the frontal, maxillary and anterior ethmoidal sinuses while the posterior group is comprised of sphenoid and the posterior ethmoid sinuses. They are lined with a pseudostratified columnar (respiratory epithelium) which is continuous with the nasal mucosa (Baily ***et.al***., ١٩٩٣).

The lining provides a mucous secretion which traps bacteria and subsequently extrudes the mucous and bacteria through the sinus ostia to be swallowed or expectorated. Under normal conditions, the sinuses are air filled and communicate directly with the nasal passages through patent ostia (Chow, ١٩٩٨).

١-٢-٣ *The Maxillary Sinus:*

The maxillary sinus occupies the body of the maxilla and is the largest paranasal sinuses, which has pyramidal shape. The maxillary sinus cavity has a volume of ١٥ to ٣٠ ml; the two sinuses are usually equal in size so rarely one sinus is completely absent (Geurkink, ١٩٨٣).

There is a relationship between the teeth and floor of sinus where the root of first, second and the ٣rd premolar and sometimes the root of canine project up into sinus, hence root infection of these teeth is the frequent cause of maxillary sinusitis. The maxillary sinus is lined by ciliated columnar epithelium and covered with a mucus blanket; this epithelium is well supplied with goblet cell (Jack and Gwaltney, ٢٠٠٠).

١-٢-٤ *Physiology of Paranasal sinuses : -*

There are many theories about the function of these sinuses, but there is general agreement to their specific functional role: - (Courtiss ***et.al.***, ١٩٨٤)

١- Air conditioning.

٢- Aid to balance the head.

٣- Vocal resonance.

٤- Thermal insulators.

Moreover sinuses have defensive functions for the respiratory tract represented by:

١-Local defensive functions include specific immunoglobulin mechanisms with secretary IgA, IgG and lymphocyte. Non specific defensive functions reflexes coughing, sneezing mucocilliary system, nasal hair epithelial integrity, lining fluid and antimicrobial substances in lining fluid.

٢-Systemic mechanisms which include specific serum IgA, IgE, and lymphocytes. And non specific serum factors opsonin, complement and mononuclear phagocytes (Dark-Lee, ١٩٩٧).

١-٢-٥ *Infection of Paranasal Sinuses*:

Since the lining of the nose and paranasal sinuses continuous, the inflammation which affects the lining of the nose will spread to variable extent into the sinuses (Norrby, ١٩٨٣).

The inflammation process that is primarily present in the sinuses will in turn extend to the nasal cavity and result in a variable amount of rhinitis; most conditions of the nose therefore affect both the nasal cavity and the sinuses (rhino sinusitis) (Peter, ٢٠٠٠).

rhinosinusitis could be classified into allergic and non-allergic, and the later being infective and non infective has advantage of simplicity, but masking the fact that rhinosinusitis are multifactor etiology with considerable overlap of clinical manifestation (Macky and Bull, ١٩٩٧).

There is a key element which is used for the diagnosis of sinusitis which consist of two groups of symptoms:-

١-The first group is called major symptoms (Facial pain –pressure, facial congestion – fullness, nasal obstruction, nasal discharge –purulent or discolored postnasal drainage, hyposomial-anosmia, fever in acute sinusitis).

٢-The second group is called minor symptoms (headaches, Halitosis, fatigue, dental pain, cough). In general terms the diagnosis of sinusitis = ٢ or ١ major symptom + ٢ minor symptoms (Evans, ١٩٩٨).

Sinusitis can be classified into two groups; acute and chronic sinusitis; this classification depends on the period of the infection and symptom (Pool, ١٩٩٩).

Acute sinusitis: - Bacterial infection of Paranasal sinuses lasting less than ٣٠ days in which symptoms completely are resolved.

Chronic sinusitis: - Episodes of inflammation of the Paranasal sinuses are lasting more than ٩٠ days. Patients have a persistent residual obstruction (Spector ***et.al.***, ١٩٩٨)

١-٢-٥-١ *Acute sinusitis:-*

Acute bacterial sinusitis usually occurs following an upper respiratory tract infection that result in obstruction of osteomeatal complex, impaired mucociliary clearance and over production of fluid secretions (Gooch, ١٩٩٨). Acute infection may be suppurative or non - suppurative. It is one of the most common infections of humans (Osguthorpe and Hadley, ١٩٩٩).

In general it develops as a complication of viral or allergic inflammation of the upper respiratory tract (Brook, ١٩٩٦). Low ***et.al.***, (١٩٩٧) classified acute sinusitis according to its microbial etiology (viral, bacterial, parasitic). The distinguishing of acute viral rhinosinusitis of cold

influenza like illness from cases with secondary bacterial infection is a frequent challenge to the physicians in primary care.

The distinguishing is important because antibiotics should be administered to patients with acute bacterial sinusitis but not to those with viral rhino sinusitis (Dykewicz, ٢٠٠٣).

A- Pathology:

The changes in the mucous membrane in acute sinusitis are those of acute inflammation in nasal tissue increased blood supply with outpouring of serum and polymorphonuclear leukocytes associated with local swelling, redness and edema which is caused by obstruction of veins and lymphatic duct (Davidson ***et.al.***, ١٩٨٩). If the obstruction and edema persist, cell degeneration occurs with cloudy swelling and necrotic changes will take place if interference with circulation becomes prolonged (Stamberger, ١٩٨٦).

Clinically, inflammation of the sinus may be either catarrhal or suppurative; catarrhal inflammation is characterized by hyperemia and excess secretion of mucous, but with few leukocytes and little or no destruction of the mucous membrane (Krishna, ٢٠٠٤).

If the inflammatory reaction is more severe there will be more extensive extraction of leukocyte together with the mucous membrane or with severe infection; the discharge becomes increasingly purulent and less mucoid until, with necrotic mucous membrane, pus alone will be found although all combinations of this catarrhal condition and suppurative sinusitis are found (Wald, ١٩٩٨).

It is an undoubted and important fact that in some cases the acute inflammation quickly produces a suppurative sinusitis and this is particularly true in the maxillary sinus with dental infection. The opposite

case is one in which the acute inflammation is accompanied by a marked swelling and edema of the sinus mucous membrane and in which hyperplasia gives arise to the most prominent sign, moreover the mucous gland becomes hypertrophied and secrets unusual thick and tenacious mucous and the membrane may become so thick as to fill the sinus cavity completely. Polyploidy mucous membrane or polypi is often present (Macky and Bull, ١٩٩٧).

B- Pathogenesis:-

The most common sequence of events leading to infection community acquired is far a viral rhino sinusitis to be complicated by a ٢nd bacterial infection in sinus cavity (Gwaltney, ١٩٩٦).

Sinusitis can be the result of an alteration in ostial size, mucocilliary transport, oxygen exchange or mucosal blood flow; so the most important factor in the pathogenesis of sinusitis is narrowing of the ostia, either by mucosal inflammation or an anatomic abnormality (Wagenmann and Naclerio, ١٩٩٢).

Development of purulence may decrease the oxygen tension to near zero, thus favoring growth of anaerobic bacteria and inhibiting the function of the phagocytic cell (Robert, ١٩٩١).

١-٢-٥-٢ *Chronic sinusitis :*

Chronic sinusitis is a common illness. In many cases it follows an incompletely resolved acute sinusitis, but it may appear insidiously following a cold or a tooth infection (Lanza and Kennedy, ١٩٩٢). There are some factors contributing to sinusitis pathogenesis including anatomic factors, disturbances in mucociliary clearance, microbial pathogen and inflammatory factors (Hamilos, ٢٠٠٠).

A- *Pathophysiology:-*

Wald (١٩٩٨) showed that patients typically experience several episodes of acute sinusitis before developing chronic sinusitis; the resistance of the maxillary ostium is more pronounced in cases of chronic sinusitis than in the normal or acutely inflamed antrum.

The common cold and other respiratory viral syndromes may in turn lead to sinusitis .More commonly they lead to swelling of the lining of the nose and sinuses, blockage of ostia draining the sinus (particularly the ostia of the maxillary sinus), interference with the clearance mechanism and stasis (Turner ***et.al.***, ١٩٩٢). These factors favor secondary infection by the multitude of bacteria already present in the nasal cavity. With chronic infection the sinus ostia may well become totally blocked leading to a negative pressure and low oxygen concentration combined with an impaired blood supply to the nasal mucosa may explain the high frequency of anaerobic organisms found (Fredrich and Braude, ١٩٧٤).

There are some conditions that lead to chronic or recurrent sinusitis as follows :- (Zinreich, ١٩٩٢).

١-Untreated acute sinusitis that results in damage of the mucous membranes.

٢-Chronic medical disorders that cause inflammation in the air ways or persistent thickened stagnant mucus.

٣-Structural abnormalities.

١-٢-٦ *Normal Flora of The Nose :-*

A wide spectrum of organisms colonizes the nose, such as ***Streptococcal*** and ***Staohylococcal*** species. Occasional outbreaks of

disease due to this organism, can be traced to nasal, skin or perianal carriage by personnel (Baron and Finegold, ١٩٩١).

The flora of paranasal sinuses include ***streptococcus pneumonia, Haemophilus influenza, Staphylococcus aureus*** and ***Staphylococcus epidermidis*** **a**nd anaerobes such as ***Bacteroides spp.***, anaerobic gram positive cocci and ***fusobacterium spp.*** , Corynebacteria , Viridans Streptococci , β-haemolytic Streptococci and ***M. Catarrhalis*** (Almadori ***et.al.***, ١٩٨٦). Enterobacteriaceae and non-fermentative Gram-negative group (***Acinetobacter spp. and Pseudomonas spp***.) may colonize the nasopharynx of elderly, immunodificient or malnourished patients particularly when they have received antimicrobial agents. Normal flora are generally non pathogenic and the use of corticosteroid and immune suppressive agents leads to the emergence of opportunistic infection by organisms which are not previously regarded as pathogens (Vandepitte ***et.al.***, ١٩٩١). The most common normal flora which causes sinusitis will be discussed in detail as follows:

١-٢-٦-١ *Gram-positive bacteria*

A- *Streptococcal spp.*:

Streptococcal spp are Gram positive, catalase- negative, facultative an aerobic bacteria, form spherical or ovoid cells that characteristically form pairs or chains during growth (Facklam and Eliot, ١٩٩٥).

- ***Streptococcus pyogenes***:

  β- Hemolytic, bacitracin susceptible contain group A antigen. It's the main human pathogen which causes a wide range of suppurative infections in the respiratory tract and skin life-threating soft tissue infections (Kirby and Ruoff, ١٩٩٥).

Strains of ***Streptococcus pyogenes*** express a large arsenal of virulence factors and hence their pathogenicity and the clinical signs that they induce are very diverse (Boyle, ١٩٩٥).

The adhesion by interaction with host fibronectin, a matrix protein on prokaryotic cells, is considered the principal mechanism by which ***Streptococcus pyogenes*** bind to epithelial cells of respiratory tract and skin (Kilian, ٢٠٠٢).

- **Viridans Streptococci*:-***

Viridans group consists of several species which may be either α- hemolytic with production of green color around their colonies on blood agar or non hemolytic, these bacteria are dominant members of the resident flora of oral cavity and pharynx in all age groups (Douglas ***et.al.,*** ١٩٩٣).

Viridins groups are part of normal flora and comprise a large proportion of the commensal bacteria in occasionally cause Bacteremia or endocardities; however the mechanism of pathogenesis is poorly understood (Jacops ***et.al.,*** ١٩٩٥).

- ***Streptococcus pneumoniae:-***

It is one of the major causes of sinusitis both in adult and children; ***Streptococcus pneumonia*** is a primary cause of bacterial pneumonia, meningitis and otitis media (Tinkel man and silk, ١٩٨٩). The most important virulence factor is the capsular polysaccharide and disease results from the ability of this microbe to invade and multiply in tissue (Monora ***et.al.,*** ١٩٩٧).

The production of pneumolysin and H_2O_2 which acts as toxins by ***S. pneumoniae*** in certain concentration leads to inhibition of cilia beating frequency of the epithelium of the respiratory tract and inhibiting the antimicrobial properties of the neutrophils and opsonic activity of serum (Chilvers ***et.al.***, ٢٠٠٠) .

Streptococcus pneumoniae infection frequently follows the viral infection that may produce mucosal damage, diminish the epithelial ciliary's activity and depress the function of macrophage (Kim ***et.al.,*** ١٩٩٥). In the tissue, ***S. pneumoniae*** multiplies and spreads throughout the lymphatic or direct extension from the local site of infection or through blood stream causing bacterimia (Torzillo ***et.al.,*** ١٩٩٥).

B- *Staphylococcal spp.*:-

They are gram-positive spherical bacteria, non spore forming, aerobic, non motile usually arranged in grape like irregular clusters. The main species are ***Staphylococcus aureus, Staphylococcus epidermidis*** and ***Staphylococcus saprophyticus*** (MacFaddin, ٢٠٠٠).

The pathogenicity of ***Staphylococcus aureus*** contributes to hemolysis of the blood, coagulation of the plasma and production of intracellular enzymes and toxins which act on host cell membrane and mediate the cell destruction (Brooks ***et.al***., ٢٠٠٤).

Infection of maxillary sinusitis with this organism has been described as the staphylococcal beta-toxin and may reduce ciliary's activity and induce sinusitis without occlusion of the natural ostium of maxillary sinus (Kim ***et.al.,*** ٢٠٠٠). Treatment of ***staphylococcus aureus*** infection has become increasingly problematic due to the emergence of multidrug resistant strains (Lowy, ١٩٩٨). More than ٤٠٪ of ***Staphylococcus aureus*** contain plasmids that encode B-lactamase,

the enzyme that degrades many penicillin drug by virtue of change in the penicillin-binding protein in their cell membrane; these strains are commonly known as Methicillin Resistant ***Staphylococcus aureus*** (MRSA) (Herwald, ١٩٩٩).

Staphylococcus epidermidis (coagulase-negative Staphylococci): this type of Staphylococci constitutes a major component of the normal flora of humans; causing nosocomial infections (Kloss and Bannerman, ١٩٩٤).

The virulence factors of coagulase-negative Staphylococci follow initial colonization, a copious amount of extra cellular polysaccharide or slim which may correlate with pathogenicity and bacterial adherence (Heikens ***et.al.,*** ٢٠٠٥).

١-٢-٦-٢ *Gram-negative bacilli*

A- *Enterobacteriaceae:-*

Enterbacteriaceae are large heterogeneous group of gram negative rods whose natural habitat in intestinal of human (Eisenstin and zaleznik, ٢٠٠٠). The gut is considered a primary source for dissemination and transmission of these pathogens to susceptible sites (Zogaj ***et.al.,*** ٢٠٠٣).

- ***Escherichia coli:-***

Escherichia coli is one of most important Enterbacteriaceae species, usually motile, produce polysaccharide capsule; they grow on non selective media, most strain ferment lactose producing large red colony on MacConkey agar and this bacteria is predominant among aerobic commensal bacteria in healthy human intestine (Smith and Scotland, ١٩٩٣).

Escherichia coli has many virulence factors that enable it to cause disease and the most important virulence factor is polysaccharide of somatic antigen O and capsular antigen K which protect the organisms from bactericidal effect of complement phagocytes in the absence of antibody(Chart, ١٩٩٨).

Many strain express haemolysin S and in general strain of ***Escherichia coli*** isolated from extra intestinal infection are more haemolytic than strain isolated from the fasces of healthy human(Chart and Tenkin, ١٩٩٩).

- ***Klepsiella pneumoniae*:-**

Non motile exhibit mucoid growth, they have large polysaccharide capsule; ***Klepsiella pneumoniae*** is opportunistic bacteria among gram-negative bacilli responsible for nosocomial infections (Henry and Richard, ١٩٩٨).

The polysaccharide capsule protects the bacteria by preventing phagocytosis and plays an important role in pathogenesis by interacting to the mucus producing cell (Podschum and Ullman, ١٩٩٨).

- ***Proteus mirabilis*:-**

Proteus mirabilis is a small gram negative, oxidase negative, swarming motility appearing flat with tapered edge on MacConkey agar and not ferments lactose (Farmer, ٢٠٠٠).

This bacteria have many virulence factors like fimbiria, flagella, out membrane protein, lipopolysacharide, capsule antigen and hemolycin which attribute to the pathogenesis of this bacteria (O'Hara ***et.al.,*** ٢٠٠٠).

B- *Haemophilus influenzae*

Haemophilus influenzae is a small, non motile, non spore forming bacterium and a parasite of humans which is found principally in the upper respiratory tract (Monox and Murphy, ٢٠٠٠).

Among ***H. infleuenzae*** strains, there are ٢ broad categories typeable and non-typeable strains are typed based on capsular characteristic. The capsule is composed of sugar-alcohol phosphate (i.e.polyribitol phosphate).complex. Differences in this complex are the basis for separating encapsulated strains into one of six groups: type a, b, c, d, e, r, and f, type b-***inflauzae*** is most commonly encountered in serious infections in human (Moller ***et.al.,*** ١٩٩٥).

Harabuchi ***et.al***.(١٩٩٤) stated that non-typeable strains do not produce a capsule and are most commonly encountered as normal inhabitants of the upper respiratory tract. Non capsulated strain usually cause localized infections such as otitis media, sinusitis, conjunctivitis and exacerbation of chronic bronchitis (Nizet ***et.al.,*** ١٩٩٦). For encapsulated strains of ***H. influenzae*** (is most common) the capsule is antiphagocytic in the absence of specific anticapsular antibodies and highly associated with virulence of other cell envelope factors like pilli, lipopolysacchariedand outer membrane protein also which may facilitate attachment to host cell (Harper and tilse,١٩٩١) .

For non-capsulated strains,pili and other cell surface factors, not fully understood, play a role in attachment to host cell; ***H. influenzae*** produces no demonstrable exotoxine and the role of its somatic antigens in natural disease is not clearly understood. The events that result in entry into the intravascular compartment by ***H. inflaenzae*** serotype b are unclear (Stephenson ***et.al.,*** ١٩٨٥).

١-٢-٦-٣ *Gram-negative cocci*

- ***Moraxella catarrhalis***

M. catarrhalis is gram-negative, aerobic, oxidase positive diplococcic. It was described for the first time in ١٨٩٦. It is considered to be ammeter of the normal flora of upper respiratory tract; it can cause sinusitis, otitis media, bronchitis and pneumonia (Abuhammour ***et.al***., ١٩٩٩).

The way of transmission is by the spread of patients endogenous strain to normally sterile site, person to person; nosocomial spread by contaminated respiratory droplets also can occur (Janda and Knapp, ٢٠٠٣). Librations of endotoxin, histamine and chemotactically active factors can be considered the major pathogenicity factors of ***M. catarrhalis***. The surface of these bacteria contains an outer membrane protein (Karalus and Campagnari, ٢٠٠٠).

The pathogen can protect itself by binding of the C١ a subcomplement of the complement system followed by subsequent formation of a functionally inactive complex with C١and, on the other hand, by inactivation of the terminal lytic complement complex by means of specific protein on the surface of the outer cell wall (Murphy, ١٩٩٦ and Cullman, ١٩٩٧).

١-٢-٧ *Bacterial Causes of Sinusitis :-*

Various studies were done concerning the microbiology of acute maxillary sinusitis: Bjorkwall (١٩٥٠) pointed out that ***S. pneumonia*** was the most common microorganism being isolated from sinusitis which was followed by other ***Streptococcal spp.*** Mounier-kuhn (١٩٥٣) observed similar proportions. Dishock and Franssen (١٩٥٧) demonstrated three times as many cases infected by ***Streptococcus pyogenes*** as by ***S.***

pneumonia and ***H. influenzae***. These findings were confirmed by Gronroos and Palva (١٩٦٢).

Wald ***et.al***.(١٩٨١) mentioned that the most common organisms found in acute maxillary sinusitis were ***S. pneumoniae, S. pyogenes ,staphylococcus aureus , H. influenzae , E. coli*** and ***M. catarrhalis*****.** Hamory ***et.al.***(١٩٧٩) reported that the bacterial causes of acute sinusitis were: ***S. pneumoniae, H. influenzae, M. catarrhalis***, ***S. pyogenes, staphylococcus aureus, pseudomonas aeruginosa*** and ***E. coli***.

Moreover, studies showed that ***H. influenzae*** constitute the highest percentage (٥٠ %), followed by ***S. pneumoniae*** (١٩ %), coagulase-negative staphylococcus (٨ %), ***S. pyogenes*** (٥ %), ***M. catarrhalis*** (٢٪), ***Staphylococcus aureus*** (١ %), Viridanse Streptococcal group (١٪) and negative culture was (٢٤٪) (Hannell ***et.al.,***١٩٨٨). Farhadi ***et.al.***(١٩٨٩) showed that the most common bacteria were ***Staphylococcus aureus*** followed in order by ***S. pneumoniae*** and some members of enteric group were represented by ***E. coli***, ***klebsiella pneumoniae, proteus merabilis, Enterobacter,*** and ***pseudomonas***.

Berg ***et.al.***(١٩٨٨) showed that the pathogens being isolated from patient with untreated acute sinus empyema were ***S. pneumoniae*** being the most prevalent followed by ***H. influenzae, staphylococcus aureus*** and anaerobic ***Bacteriods spp.***, while Brook ***et.al***.(٢٠٠٠) discussed the frequency of potential bacterial pathogens including ***S. pneumoniae, H. influenzae*** and ***M. catarrhalis*** in patients clinically diagnosed with sinusitis.

In chronic sinusitis bacteriological culture showed that beside ***S. pneumoniae, H influenzae and M. Catarrhalis*** additional bacteria are present including anaerobes ***(Bacteriodes spp., peptostreptococcus spp.***) and gram negative bacteria (***pseudomonas aeroginisa, klbsiella pneumoniae, E. coli***). Evan ***etal.,***(١٩٧٥); Laine and Smoker (١٩٩٤) and

Berg ***et.al.***(١٩٨٨) found that ***S. aureus*** and anaerobic bacteria were frequently finding in chronic sinusitis. Hartog ***et.al.***(١٩٩٥) found that the predominant aerobic bacteria were ***S. aureus*** (٤٢٪) and ***S. pneumoniae*** (٣٢٪) while the predominant anaerobic bacteria were ***propionibacterium spp.*** (٨٪) and gram negative rods (٩٪). Brook ***et.al.*** (١٩٩٦) concluded that the predominant bacteria in chronic sinusitis were ***S. aureus*** followed by ***Prevotella spp.***, ***Fusobacterium*** and ***Bacteriodes fraglis***.

Another study done by Bile ***et.al.***(١٩٩٨) found that Coagulase negative staphylococci were the most common isolates (٣٦٪) followed by ***S. aureus*** (٢٥٪), S. viridans (٨.٣٪), ***Corynebacterium*** (٤.٦٪) and anaerobe bacteria (٦.٤٪). Brook and Fraizer(٢٠٠١) found that the predominant aerobic isolates were ***S. aureus*** (١٧) isolates, ***S. pneumoniae*** (١٤) isolates, ***pseudomonas aeroginosa*** (١٢) isolates and ***M. Catarrhalis*** (١٠) isolates; the predominant anaerobes were ***Peptostreptococcus spp.*** (٦١) isolates, ***Prevotella spp.*** (٤٥) isolates, ***Fusobacterium spp.***(١٥) isolates and ***propionibacterium acnes*** ١٤ isolates. Ologe and Nwabisi (٢٠٠٣) recorded that the main cause of chronic sinusitis were ***S. aureus*** (٤٨.١٪), ***E. coli*** (٢٠.٤٪), ***Klebsiella spp***. (٢٠.٤٪) and ***S. pneumoniae*** (٣.٧٪). In (٢٠٠٥) Brook and Frazier found that the predominant aerobic bacteria were ***S. aureus*** followed by ***S. pneumoniae*** and ***M. Catarrhalis*** while anaerobic bacteria were ***Peptostreptococcus spp.*** followed by ***Prevotella spp.***, ***Porphyromonas asaccharolytica*** and ***Fusobacterium*** spp. Recently, Moungthong ***et.al.***(٢٠٠٥) reported that the predominant orgamisms among sinusitis were ***pseudomonas aeroginosa***, ***Staphylococcus spp.***, and ***Streptococcus spp.*** also ***H. influenzae*** consequently.

The causative agents of sinusitis are widely variable. This may be related to the environmental, climatic, familial or other factors. Hence the suggested recommendation is to study sinusitis locally.

١-٢-٨ *Treatment:-*

The goal of treatment for both acute and chronic sinusitis is drainage of the congested sinus and elimination of the pathogenic bacteria (Benninger ***et.al.,*** ١٩٩٧). Studies using pre and post treatment sinus aspirate culture have shown that the antimicrobials with appropriate spectra and given in adequate doses and with an adequate duration are effective in eradicating or substantially reducing bacterial titer in the sinus(Gwaltney ***et.al.,***١٩٩٢); but pre treatment sinus aspiration may not yield bacterial pathogen in approximately ٣٠٪ to ٤٠٪of patients with acute sinusitis; for this the initial choice of antibiotic should be selected on an empirical basis(Chow ***et.al.,***١٩٩٢).

A broad spectrum antibiotic is typically chosen to cover the usual sinus pathogen (***H. influenzae, S. pneumoniae, M. catarrhalis, S. aureus***) like amoxicillin or tetracycline ,erythromycin as an initial treatment for management of sinusitis (Temple and Nahata, ٢٠٠٠). There is an increasing incidence of resistance to β-lactam drug, cephalosporin providing more powerful alternative for non responders (Shikani, ١٩٩٧). If drug therapy fails to unblock the sinuses or other complications such as structural abnormalities so in this cases surgery may be required (Lanza and Kennedy, ١٩٩٢).

Chapter Two
Materials & Methods

٢-١ *Materials:-*

Many types of instruments and chemical materials in addition to biological materials were used in this study for completing the research work. These materials were taken from different sources and companies which are listed in the following tables.

٢-١-١ *Cultures media:-*

Table ٢-١ shows the list of culture media which were used for isolating and identifying the isolated bacteria.

Table ٢-١: The Culture Media

S.No	Culture media	company
١.	Nutrient agar	Mast Lab. (Uk)
٢.	nutrient broth	Mast Lab. (Uk)
٣.	brain heart infusion broth	Mast Lab. (Uk)
٤.	brain heart infusion agar	Mast Lab. (Uk)
٥.	MacConcky agar	Mast Lab. (Uk)
٦.	blood agar	Mast Lab. (Uk)
٧.	Muller-Hinton agar	Mast Lab. (Uk)
٨.	Agar-agar media	Mast Lab. (Uk)
٩.	Peptone water medium	Mast Lab. (Uk)
١٠	Simmon Citrate agar	Difco-Michigan
١١	Kliglar iron agar	Difco-Michigan
١٢	MR-VP broth	Difco-Michigan
١٣	Meet extract	Difco-Michigan
١٤	Urea base agar	Difco-Michigan
١٥	EMB agar	Difco-Michigan

٢-١-٢ *Chemical materials:-*

Table ٢-٢ shows chemical materials used in this study for identifying different types of bacteria.

Table ٢-٢ Chemical Materials Used in This Study.

S.No	Company	Chemical materials
١	Crystal violet	Fluka (UK)
٢	Safranin	Fluka (UK)
٣	Tetramethyl-p Phenylene-diamine dihydrochloride (oxidase test)	Fluka (UK)
٤	Nacl	Fluka (UK)
٥	$H_٢So_٤$	Fluka (UK)
٦	Urea	Fluka (UK)
٧	Kovac's reagent	Fluka (UK)
٨	Phenol red	B.D.H (UK)
٩	Bromo phenol blue	B.D.H (UK)
١٠	Iodine	B.D.H (UK)
١١	KOH	B.D.H (UK)
١٢	Barium chloride	B.D.H (UK)
١٣	α-napththol amine	B.D.H (UK)
١٤	Glycerol	B.D.H (UK)
١٥	Absolute Ethanol	B.D.H (UK)
١٦	Hcl	B.D.H (UK)
١٧	Toluidine blue	B.D.H (UK)
١٨	Acetic acid (٥m)	B.D.H (UK)
١٩	Amyle-alcohole	B.D.H (UK)
٢٠	$kNo_٣$	B.D.H (UK)
٢١	$H_٢O_٢$ (hydrogen peroxide)	Oxiode

٢-١-٣ *Charbohydrate Materials:-*

Table ٢-٣ shows carbohydrate materials used in the sugar fermentation test.

Table ٢-٣ Carbohydrate Materials Used in This Study

S.No	Materials	Company
١	Sucrose	Difco (USA)
٢	manitol	Difco (USA)
٣	Lactose	Difco(USA)
٤	Maltose	Difco(USA)
٥	Rhamnose	Fluka (UK)
٦	Trehlose	Fluka (UK)
٧	Starch	Sigma
٨	Arabinose	B. D.H (UK)

٢-١-٤ *Diagnostic Disks:-*

١. Bacitracin (١٠ IU) (Himedia, India)

٢. Optochin (ethyl hydrocuprein hydrochloride ٥µg) (Himedia, India)

٢-١-٥ *Antibiotic Sensitivity Disks:-*

The antimicrobial sensitivity disks used in this study were made by Bioanalyse, Oxoid Company and listed in Table ٢.٥. They were used for detecting the sensitivity of isolated bacteria for these antibiotics. The results of this study were recorded according to standard guidelines recommended by National Committee for Clinical Laboratory Standards (NCCLS, ٢٠٠٠).

Table ٣-٣ Antimicrobial Discs Used in This Study

Antimicrobial agent	Symbol	Concentration (µg)/disk	Diameters of inhibition zones (mm)		
			resistance	intermediate	sensitive
Ampicillin	Am	١٠	≤١١	١٢-٢١	≥٢٢
Amoxicillin	Amx	٢٥	≤ ١٩	----------	≥٢٠
Trimethoprim	TMP	٣٠	≤١٠	١١-١٥	≥١٦
Erythromycin	E	١٥	≤١٣	١٤-٢٢	≥٢٣
Tetracycline	TE	٣٠	≤١٤	١٥-١٨	≥١٩
Cefotaxime	CTX	٣٠	≤١٤	١٥-٢٢	≥٢٣
Ciprofloxacin	Cip	٥	≤١٥	١٦-٢٠	≥٢١
Cephalothin	KF	٣٠	≤١٤	١٥-١٧	≥١٨
Gentamicin	GN	١٠	≤١٣	١٤-١٦	≥١٧
Vancomicin	VA	٣٠	≤٩	١٠-١١	≥١٢
Oxacillin	OX	١	≤١٣	----------	≥١٥

٢-١-٦ *Preparation of The Culture Media:-*

The general culture media described below were prepared by the routine methods and used in appropriate experiments:-

٢-١-٦-١ *Blood Agar Medium:*

Blood agar medium has been prepared according to Macfaddin (٢٠٠٠) by dissolving ٤٠ gm blood agar base in ١٠٠٠ ml D.W. and autoclaved at ١٢١C° for ١٥ min, then cold to ٥٠ C° and ٨٪ of human blood was added. This medium was used to cultivate bacterial strain and to determine their ability to blood haemolysis.

٢-١-٦-٢ *Chocolate Agar Medium:*

Chocolate agar medium has been prepared by dissolving ٤٠ gm of blood agar base in ١٠٠٠ ml D.W. and sterilized by autoclaving. Then ٨٪ of human

blood was added to the medium after cooling to ٨٠ C°. This medium was especially used for isolation and cultivation of bacteria that need ٥-١٠٪ CO٢ tension (Baron and Finegold, ١٩٩٦).

٢-١-٦-٣ *MacConkey Agar Medium:*

MacConkey agar medium has been prepared according to the method recommended by the manufacturing company and it is used for the primary isolation of most Gram- negative bacteria and differentiation of lactose fermentative from the non lactose fermentative (Collee ***et.al.,*** ١٩٩٦).

٢-١-٦-٤ *Nutrient Agar Medium:*

Nutrient agar medium has been prepared according to the manufacturing company. It has been used for general experiment isolate culture, cultivation and activation of bacterial isolates when it is necessary (Macfaddin, ٢٠٠٠).

٢-١-٦-٥ *Mannitol Salt Agar Media*

This media has been used as a selective media for the isolation of Staphylococci and differentiation of ***Staphylococcus aureus*** (Macfaddin, ٢٠٠٠).

٢-١-٦-٦ *Muller- Hinton Agar*

Muller- Hinton agar has been prepared according to the method recommended by (Cruikshank ***et.al.,*** ١٩٧٥) and it is used in anti-microbial susceptibility testing.

٢-١-٦-٧ *Brain Heart Infusion Broth*

This medium has been used for the enhancement of bacterial growth and reactivation of bacterial isolates when it is necessary (Macfaddin, ٢٠٠٠).

٢-١-٦-٨ *Brain Heart Infusion Agar*

This medium has been used as a storage medium after the addition of ٥٪ glycerol it is prepared according to (Cruikshank ***et.al.,*** ١٩٧٥).

٢-١-٦-٩ *Gelatin agar media*

Gelatin agar media used for the detection of bacterial ability to proteolytic or liquefy gelatin. It was prepared by adding ٤.٤ % of gelatin to the nutrient agar medium (Macfaddin, ٢٠٠٠).

٢-١-٦-١٠ *Kligler Iron Agar*

Kligler Iron agar has been used for determining glucose and lactose fermentation and possible hydrogen sulfide H٢S production as a first step in the identification of Gram- negative bacilli (Macfaddin, ٢٠٠٠)

٢-١-٦-١١ *Pepton Water Media*

Pepton Water Media was prepared and used to detect the ability of bacteria to produce Indole according to the method described by (Baron and Finegold, ١٩٩٦).

٢-١-٦-١٢ *MR-VP Medium*

MR-VP medium has been prepared and used to detect the partial and complete hydrolysis of glucose according to Macfaddin (٢٠٠٠).

٢-١-٦-١٣ *Simmons' Citrate Medium*

Simman's Citrate Medium has been used for determining the ability of bacteria to utilize citrate as the sole carbon source (Macfaddin, ٢٠٠٠).

٢-١-٦-١٤ *Nitrate Reduction Medium*

Nitrate reduction medium has been prepared by dissolving a mixture of ٠.٢gm of potassium nitrate (KNo_3) and ٥gm peptan in ٩٥٠ and ml of D.W. then the medium was dispensed in test tubes, ٥ ml in each one and autoclaved. It was used for detection the ability of bacteria to reduce nitrate into nitrite as recommended by Baron and Finegold (١٩٩٦).

٢-١-٦-١٥ *Sugar Fermentation Medium*

Sugar fermentation medium has been used for determining the ability of an organism for sugar fermentation and gas production. The medium was prepared according to Macfaddin (٢٠٠٠) as follows:-

a- *Medium Base:-*

Medium Base was prepared by dissolving ١٠gm peptone, ١ gm meet extract, ٥ gm sodium chloride and ٠.٠٨ gm phenol red in one liter of D.W. the ph was adjusted to ٧.٤; the medium was dispensed in tubes in ٥ ml amounts. Durham tube was inverted in each tube and then sterilized by autoclave.

b- *Sugar Solution:-*

Sugar solution was prepared by dissolving ١gm of required sugar in ١٠٠ ml D.W and sterilized by chloroform (Macfaddin, ٢٠٠٠). Then ٠.١ ml of this solution was added to each test tube mentioned in (a) above.

٢-١-٦-١٦ *Eosin Methylene Blue (EMB) Agar:-*

Eosin Methylene Blue has been prepared according to Collee ***et.al.*** (١٩٩٦) and used to differentiate ***E. coli*** which exhibits a characterized greenish metallic sheen colonies medium.

٢-١-٦-١٧ *Urea agar medium (Biolife)*

The base medium was prepared according to manufacturing company, autoclaved and cooled to ٥٠C˚ then added ٥ml of ٢٠٪ filtration sterilized urea solution to ٩٥ml of media in which this media insured that their pH was (٦.٨-٦.٩) and tubed the medium as deep slopes. This medium was used for detecting bacterial ability to produce urease enzyme (MacFaddin, ٢٠٠٠).

٢-١-٦-١٨ *Motility medium*

This medium was prepared according to McFadden(٢٠٠٠)by adding ٤gm of agar-agar to ١٠٠ ml of nutrient broth in ١٠٠٠ ml of distilled water then sterilized by autoclave at ١٢١^{o}C \ for ١٥ minutes then it was distributed in tubes; it was used to detect bacterial motility (MacFaddin, ٢٠٠٠).

٢-١-٧ *Reagent and Solutions:-*

٢-١-٧-١ *Oxidase Preparation:-*

This reagent was prepared by dissolving ١ gm of (tetramethyl-paraphenylene-diamine-dihydrochloride) in ١٠٠ ml of D.W and immediately used (Baron and Finegold, ١٩٩١).

٢-١-٧-٢ *Catalase Preparation :-*

This reagent was prepared in (٣٠٪) using $H_٢O_٢$ dilute by D.W and stored in a dark container (Baron and Finegold, ١٩٩١).

٢-١-٧-٣ *Methyl Red Reagent:-*

Methyl Red Reagent was prepared by dissolving ٠.١ gm of methyl red in ٣٠٠ ml of ٩٥٪ ethanol and then the volume was completed to ٥٠٠ ml by D.W(Macfaddin, ٢٠٠٠).It was used to detect the complete glucose hydrolysis .

٢-١-٧-٤ *Voges-Proskaur Reagent:-*

It is composed of two solutions as below:-

a- α-Naphthol-reagent was dissolved in ١٠٠ ml of ٩٩٪ ethanol.

b- Potassium Hydroxide (KOH) solution: - ٤٠ gm of KOH was dissolved in ١٠٠ ml of D.W. and it was used to detect the partial glucose hydrolysis (Collee ***et.al.,*** ١٩٩٦).

٢-١-٧-٥ *Indication of Nitrate Reduction:-*

It is composed of two solutions:-

a- Sulfanic acid solution: - prepared by dissolving ٨ gm of Sulfanic acid in ١٠٠ ml of acetic acid(٥m).

b- α - Naphthylamine Solution: - prepared by dissolving ٦ ml of α - naphthylamine in ١٠٠ ml of acetic acid (Collee ***et.al***., ١٩٩٦).

٢-١-٧-٦ *Gram Stain Solutions:-*

The reagents of this staining method were routinely prepared according to Talib (١٩٩٦).

٢-١-٧-٧ *Covac's Reagent:-*

It was prepared by dissolving ٥ gm of P-dimethylamine benzylaldehyde in ٧٥ of amyle-alcohole and was added ٣٥ ml of concentrate HCl acid. It was used to detect the Indol production (Baron and Finegold, ١٩٩١).

٢-١-٧-٨ *McFarland standard solution:*

McFarland standard solution was used in antimicrobial susceptibility test, tube No. (٠.٥) was used which prepared by adding ٠.٠٥ ml of $BaCl_{٢}.٢H_{٢}O$ con. (١.١٧٥٪) to ٩.٩٥ ml of $H_{٢}So_{٤}$ con. (١٪). The

tube No.(٠.٥) of McFarland standard tubes was used to compare the bacterial cell in suspension which given a cell density ١.٥x١٠^{٨} cell/ml. (Baron ***et.al.,*** ١٩٩٦).

٢-١-٧-٩ *Frazier's reagent*

This reagent was prepared according to the method being recommended by (Collee ***et.al.***, ١٩٩٦). It was prepared by dissolving ٥ gm $HgCl_{٢}$ in ٢٠ ml of concentrated HCL (٩٨٪), with the addition of ١٠٠ ml distilled water. It was used to detect the ability of bacteria to analyze gelatin (Collee ***et.al.***, ١٩٩٦).

٢-٢ *Methods:-*

٢-٢-١ *Subjects:-*

This study was including one hundred patients ٦٠ were females and ٤٠ were males. Fifty eight cases were clinically diagnosed as acute sinusitis and ٤٢ were diagnosed as chronic sinusitis patients who were attending the ENT department in Hilla Teaching Hospital during the period between October ٢٠٠٤ and July ٢٠٠٥. The age of the patient ranged from ١٠-٦٩ years. The primary clinical diagnosis of those patients was done by consultant ENT sergeant through clinical examination, sinus occipitomental view (CT-Scan) of sinuses or Nasoendoscopy.

Patients who were under antibiotic treatment or had stopped antibiotics therapy for less than one week were excluded. All patients were subjected to especially made case sheet which has been highlighted on patient's age, type of sample, antibiotic history, clinical feature, concomitant disease, clinical diagnosis as shown below:-

٢-٢-٢ *Collection of Specimens:-*

The proper specimens collected for bacteriological analysis are described below. Those specimens were collected under the help of advisory to avoid any possible contamination.

For acute sinusitis the specimens were collected by nasal swabs (middle meatus) especially for maxillary sinusitis excluded other sinusitis while antral wash (lavage) for chronic sinusitis. The nasal swabs were obtained as follows:

١- Sterile cotton swabs were moisted with two drops of sterile saline and carefully inserted into the patients nostril (the swab tip must be inserted up to ٢.٥ cm from the edge of the nares.

٢- The swabs were rotated ٥ times.

٣- Other swabs were inserted into the second nostril following the same way above (Fagnan, ١٩٩٨).

These swabs were immediately cultured and incubated for a further laboratory diagnosis.

In chronic sinusitis antral wash samples were obtained according to Sutter ***et.al.***(١٩٨٠) by using lavage instruments (Trocar and Canula) under local or general anesthesia. Sinus secretion was aspirated into sterile ٢٠ ml syringe. If aspiration yields nothing, then the canula was held in the sinus and one to two milliliter of sterile physiological saline was injected into the sinus through the canula and was aspirated again(this is called injection aspiration). Each specimen was immediately inoculated on the blood agar plates, chocolate agar plates and MacCoonkay's plates. All plates were incubated aerobically at ٣٧ C^{o} for ٢٤-٤٨ hrs.

٢-٢-٣ *Laboratory Diagnosis*

According to the diagnostic procedures recommended by Maccfaddin (٢٠٠٠); Baron and Finegold (١٩٩١); Prescott ***et.al***.(١٩٩٠), the isolation and identification of gram positive and gram negative bacteria in the nasal sinus of patients were performed as follows:-

٢-٢-٤ *Microscopy Examination and Colonial Morphology*

Microscopical examination included the examination of shape, gram stain reaction, arrangement of cell, capsule, flagella and spore formation which has been done for the different colonies, then the colonial morphology which included color and natural of pigments, translucency, edge, and elevation have been studied with respect to the ٢٤ hours bacterial growth. Plates which showed no growth were further incubated up to ٧٢ hours before discarding them as negative.

٢-٢-٥ *Physiological and Biochemical Tests*

٢-٢-٥-١ *Oxidase Test*

A filter paper circle was placed into a sterile plastic disposable petridish and moistured with several drops of the freshly prepared oxidase reagent, then a small portion of the colony to be tested was removed and rubbed on the filter paper changing in the color to blue or purple within ١٠ seconds indicated for a positive result (Baron and Fingold, ١٩٩١).

٢-٢-٥-٢ *Catalase Test:-*

By streaking the nutrient agar medium with the selected bacterial colonies and incubated at ٣٧ C^{o} for ٢٤ hrs then transfer the growth by the loop and put it on the surface of a clean slide and add a drop of (٣٠٪ $H_{٢}O_{٢}$), positive result when the gas bubbles appear (Barond Finegold, ١٩٩٦).

٢-٢-٥-٣ *Blood Hemolysis Test*

Blood agar medium was streaked with a pure culture of bacterial isolate to be tested and incubated at ٣٧ C° for ٢٤-٤٨ hrs. The appearance of a clear zone surrounding the colony is an indicator of β- hemolysis while the greenish zone is an indicator of α- hemolysis (Cowan, ١٩٨٥).

٢-٢-٥-٤ *Coagulase Test:-*

This enzyme was tested by two methods:-

a. Slide test for bund coagulase (clumping factor):-

A drop of human plasma was placed on a clean, dry glass slide, a drop of D.W. was placed next to the drop of plasma as a control. By a sterile loop an amount of the isolated colony was emulsified with each drop. When clumping the plasma, bacteria was observed and a smooth homogenous in the control; the result was recorded positively (Baron and Finegold, ١٩٩١).

b. Tube test for free coagulase:-

٠.٥ ml of Human plasma was placed in a glass tube and a visible portion of growth from isolated colonies was emulsified in the plasma by rubbing the material on the slide of the tube while holding the tube at an angle, then the suspension was incubated for ١-٤ hrs at ٣٧ C°; the presence of clot that cannot be resuspended by gentile; shaking was recorded as a positive result. The organism that fails to clot the plasma within ٢٤ hrs is considered as coagulase negative (Baron and Finegold, ١٩٩٦).

٢-٢-٥-٥ *Urease Production Test*

A slant of medium was inoculcated with the colony of tested organism and incubated at ٣٧ Cº and examined after ٤ hrs and ٢٤ hrs. Urea splitting organisms were identified by the change of the color from yellow to purple-pink (Cruikshank ***et.al.,*** ١٩٧٥).

٢-٢-٥- ٦ *Gelatin Hydrolysis:-*

Gelatin agar medium was inculcated with the colony of tested organism and incubated for ٣-٥ days at ٣٧ Cº, and then the plates were flooded in solution of Frazier reagent for ٥-١٠ min. Gelatin-liquefying organism was identified by the presence of clear zone around the colony of the organisms under test (Maccfadin, ٢٠٠٠).

٢-٢-٥-٧ *Citrate Utilization Test :-*

The surface of simmon's citrate slant medium was inculcated with colony of the tested bacteria and incubated at ٣٧ Cº for ١-٣ days. Conversion of the indicator's color from green to blue indicates that the organism was able to utilize citrate as a sole carbon source (Cruikshank ***et.al.,*** ١٩٧٥).

٢-٢-٥-٨ *Nitrate Reduction Test:-*

Nitrate broth was inculcated with the tested bacterial isolate and incubated at ٣٧ Cº for ٢٤hrs. Three drops of reagent A and ٣ drops **of** reagent B were added to the suspension of bacteria in broth positive test for reduction of nitrate to nitrite was indicated by the development of a red color within ٥ mins. (Baron and Finegold, ١٩٩١).

٢-٢-٥-٩ *Motility Test:-*

Tubes containing motility test medium were stabbed once at the center with an inoculcating needle and incubated at ٣٥ Cº for ٢٤-٤٨ hrs

motile bacteria speed out from the line of inculcating; but none motile bacteria grows only along the stab line, while the surrounding medium remains clear (Baron and Finegold, ١٩٩١).

٢-٢-٥-١٠ *Sugar Fermentation Test:-*

Tubes containing sugar broth were inculcated from ٢٤ hrs old bacterial culture and incubated at ٣٧ C° for ١-٣ days. The positive result was detected by a change in color of the broth from red to yellow, with or without the appearance of air bubbles in Durham tube (MacFacddin,٢٠٠٠).

٢-٢-٥-١١ *Kligler's Iron Agar Test for H_2S Production:-*

Only the colonies growing on MacConkey agar were touched by a straight wire and inoculated on the media by stabbing the butt of the tube and streaking the slant. Fermentation was detected by a change in the indicator phenol red to yellow. The PH changes in the butt and the slant of medium were recorded after ١٨- ٢٤ hrs of incubation of gas formation which is usually visualized as bubbles in the medium caused by the gas formed in the agar. Organisms can produce H_2S form black precipitate in the butt (Baron and Finegold, ١٩٩٦).

٢-٢-٥-١٢ *Indol Test*

Tubes containing a peptone water medium were inoculated with the colony of the tested bacteria and incubated at ٣٧ C° for ١٨ hrs, then several drops of kovac's reagent were added to the broth medium. After shaking, the appearance of the red ring on the surface was regarded as a positive result (Cruikshank ***et. al.***, ١٩٧٥).

٢-٢-٥-١٣ *Methyl Red Test:-*

The tubes of the (MR-VP broth) were inoculated with the selected bacterial colonies and incubated at ٣٧°C for ٢٤ hrs, then (٥ drops) of methyl red reagent were added to it. The appearance and observation of red colour means a positive result and a complete analysis of glucose (MacFaddin, ٢٠٠٠).

٢-٢-٥-١٤ *Voges Proskaur Test:-*

The tubes of (MR-VP broth) were seeded with the specific bacterial culture and were incubated at ٣٧°C for ٤٨ hrs., then we read the result by adding (٠.٦ ml of α- naphthol reagent) and (٠.٢ ml of ٤٠٪ NaOH solution); appearance of red colour after ١٥ min. means positive result due to partial analysis of glucose, which produce acetone or (Acetyl methyl-carbinol) (MacFaddin, ٢٠٠٠).

٢-٢-٥-١٥ *Mannitol Salt Agar Test:-*

The differentiation between ***Staphylococcus aureus*** and other Staphylococci e.g. ***Staphylococcus epidermidis*** was done by sub culturing selected colonies on mannitol salt agar for ٢٤ hrs at ٣٧ Cº. Colonies surrounded by a yellow halo indicating mannitol fermentation and isolates colony, is ***Staphylococcus aureus*** (MacFaddin, ٢٠٠٠).

٢-٢-٥-١٦ *Optochin Susceptibility Test:-*

A half of ٨٪ human blood agar plate was streaked with an inoculum form a pure isolates of the organism to be tested , then an optochin disc was placed in the center of the inoculum and incubated for ٢٤ hrs at ٣٧ Cº in a candle jar , then observation of zones of growth inhibition grater than ١٤ mm surrounding the disc was considered positive and it was presumptive indication of ***Streptococcus pneumoniae*** (Baron and Finegold, ١٩٩١).

٢-٢-٥-١٧ *Antimicrobial Sensitivity Test:-*

Two ml of brain heart infusion broth have been inoculated with an isolated colony of tested bacteria and incubated for ٢٤ hrs at٣٧ C°. After that, the turbidity of bacterial suspension has been adjusted according to McFarland standard tube (٠.٥). ٠.١ ml of bacterial suspension has been spread on the surface of Muller Hinton medium plate and left to dry. Antimicrobial disks have been placed properly and incubated for ٢٤ hrs at ٣٧ C° (Bauer ***et.al***., ١٩٦٦).

The inhibition zone have been measured by using a ruler in compared with inhibition zone determined by (NCCLs, ٢٠٠٠) and to decide the susceptible of bacteria to anti microbial agent whether being resist or sensitive.

٢-٢-٦ *Statistical Analysis*

Differences between means were compared by X^2 tests under confidence level of ٠.٩٥; the ***P*** value ≤ ٠.٠٥; the ***P*** value ≤ ٠.٠١ and ٠.٩٩ was considered a significant difference and ***P*** value > ٠.٠٥, ٠.٠١ was considered a non-significant difference.

Chapter Three
Results & Discussion

٣-١ *Age Related Disease:-*

Figure (٣-١) shows that the most affected age group in this study in both acute and chronic sinusitis was between ٢٠ to ٢٩ years old. These results agree with the results obtained by Sarmad (٢٠٠٥) and Baily **(**١٩٨١) who found that the affected age group ranged from ٢٠ to ٣٠ years old who were the most susceptible for sinusitis.

These results can be explained as this age group is more exposed to environmental factors such as allergens, climatic factors, air pollution and the extremely weather changes. Furthermore, this age group represents the active age group having the chance of person to person transmission. Statistical analysis by using Chi Square showed that there is a significant relationship between age and sinusitis ($p < ٠.٠٥$).

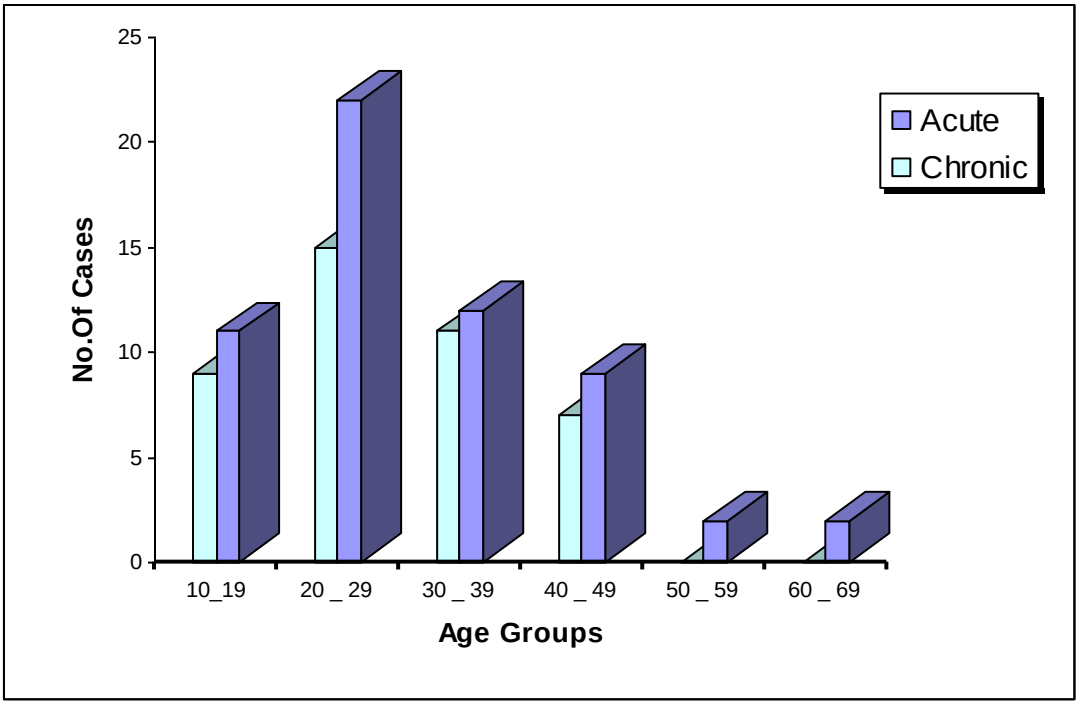

Figure ٣-١: Age Distribution of Acute and Chronic Sinusitis Disease.

٣-٢ *Clinical Features and Related Disease:-*

Like other diseases, the clinical presentation depends on the severity of the disease. The purulent discharge, nasal obstruction and periorbital pain are the major complaint in sinusitis as shown in the Table (٣-١).

In acute sinusitis the purulent discharge was observed in (٨٠٪), periorbital pain (٩٠٪) and nasal obstruction (٩٧٪). The results were in accordance with the results of a similar study done by Stalman ***et.al.*****(**٢٠٠١). Fever in acute sinusitis was the most frequent symptom. This result came to confirm the results being reported by (Brook, ٢٠٠٤) who states that fever is one of the most frequent features of patients suffering from acute sinusitis. In chronic sinusitis the purulent nasal discharge was observed in (٤٧٪), nasal obstruction (٩٠٪) and periorbital

pain observed in (٩٥٪). These results agree with the results obtained by Williams ***et.al.*** (١٩٩٣). The percentage of fever in acute sinusitis was found to be higher than that at chronic sinusitis. This may be due to induction of the immune system with chronic sinusitis and to the administration of antibiotics.

Table ٣-١ Clinical Features of Sinusitis Disease*:

Clinical features	Acute sinusitis	Chronic sinusitis
Purulent discharge	٨٠٪	٤٧٪
Periorbital pain	٩٠٪	٩٥٪
Nasal obstruction	٩٧٪	٩٠٪
Fever	٩٠٪	٣٥٪

* The clinical features were diagnosed by ENT expert.

٣-٣ *Clinical Conditions and Related to Disease:-*

According to the clinical conditions of sinusitis disease shown in Table (٣-٢), the most important predisposing conditions in chronic sinusitis were allergy (٩٠٪) and nasal polyp (٨٥٪) and Nasal septal division (٨٠٪). The results came to ensure the results of Wu ***et.al.***(٢٠٠٥), who stated that the anatomical abnormalities of nasal cavity can be the basic reason of sinusitis. In addition to that the edema of nasal mucosa and the change of nasal discharge caused by allergy triggering factor of chronic sinusitis. These abnormalities will interfere with ventilation and the free passage of air through the nasal chamber, and with secretion and movement of mucous blanket thus predispose to infections. The

frequency of diabetes mellitus and dental problem in chronic sinusitis was (٤٪) and (٢٪) respectively. These results were close to those results obtained by Sarmad (٢٠٠٥), who found that (٢.٣٪) of patients with sinusitis were suffering from diabetes mellitus versus (١٪) who have dental problems.

The percentage beginning with common cold in both acute and chronic sinusitis was high enough to be in agreement to those results obtained by Desrosiers ***et.al.*** (٢٠٠٢), who found that (٩١٪) of individuals with common cold will develop sinuses disease by secondary infection of bacteria, and this was also ensured by Buchman ***et.al.*** (١٩٩٤).

In acute sinusitis the percentages of nasal polyp, allergy and nasal septal division were ٢٠٪, ٢٣٪ and ١٢٪ respectively as shown in Table (٣-٢). Sarmad (٢٠٠٥) who found that allergy and nasal polyp were accounted for (١٨.٧٪) and (٧.٣٪) respectively in sinusitis cases while diabetes mellitus and dental problem was (٣٪), (١٪) respectively.

Table ٣-٢ Clinical Conditions associated with Sinusitis Disease:

Clinical conditions	Acute sinusitis	Chronic sinusitis
beginning with common cold	٩٧٪	٨٠٪
allergy	٢٣٪	٩٠٪
nasal polyp	٢٠٪	٨٥٪
Nasal septal division	١٢٪	٨٠٪
Diabetes mellitus	٣٪	٤٪
Dental problem	١٪	٢٪

٣-٤ *Sex Related Disease:-*

In this study, ١٠٠ specimens were investigated, ٥٨ specimens of them were from acute cases of sinusitis and ٤٢ specimens were chronic cases. Among the acute cases, ٥٠ specimens revealed positive results of bacterial culture versus ٨ specimens which revealed no growth for bacterial culture. Among the ٥٠ positive cases ٣٠ specimens (٦٠٪) were females versus ٢٠ specimens (٤٠٪) were males as shown in Figure (٣-٢). The result was in agreement with those results obtained by Stalman ***et.al.***(٢٠٠١) who found that (٦٦٪) of patients with acute sinusitis were females and (٣٤٪) were males. Moreover, a study done by Varonen ***et.al.***(٢٠٠٣) reported that (٧٠٪) of the patients with acute rhinosinusitis were females and (٣٠٪) were males.

Among the cases of chronic sinusitis ٣٥ bacterial cultures samples were positive and ٧ bacterial cultures were negative; ٢٥ (٧١٪) of the positive cultures were females while ١٠ (٢٩٪) were males as shown in Figure (٣-٢). The results were similar to the results obtained by Ron ***et.al.*** (٢٠٠٤) who found that (٦٨٪) of patients with chronic sinusitis were females and (٣٢٪) were males. From these results one can conclude that the incidence of sinusitis is common in females in comparison with that of males. The reasons for this are not quite clear. The available literature regarding sinusitis is quite rare and no explanation was found in that literature explains the variability of sinusitis between males and females. However the reasons for that are attributed to the common exposure of females (as house worker) to various allergens such as (house dust, flour, dust, foods and food flavors, heat, humidity and other allergens), or to a lesser degree pregnancy and lactating women can effect their immunity leading to an increase in infection. However physiology of pregnant

women like increase in Progesterone and Estrogen will lead to increase incidence of allergy. Statistical analysis by using Chi Square showed that there is a highly significant relationship between sex and sinusitis (***p*** < ٠.٠١).

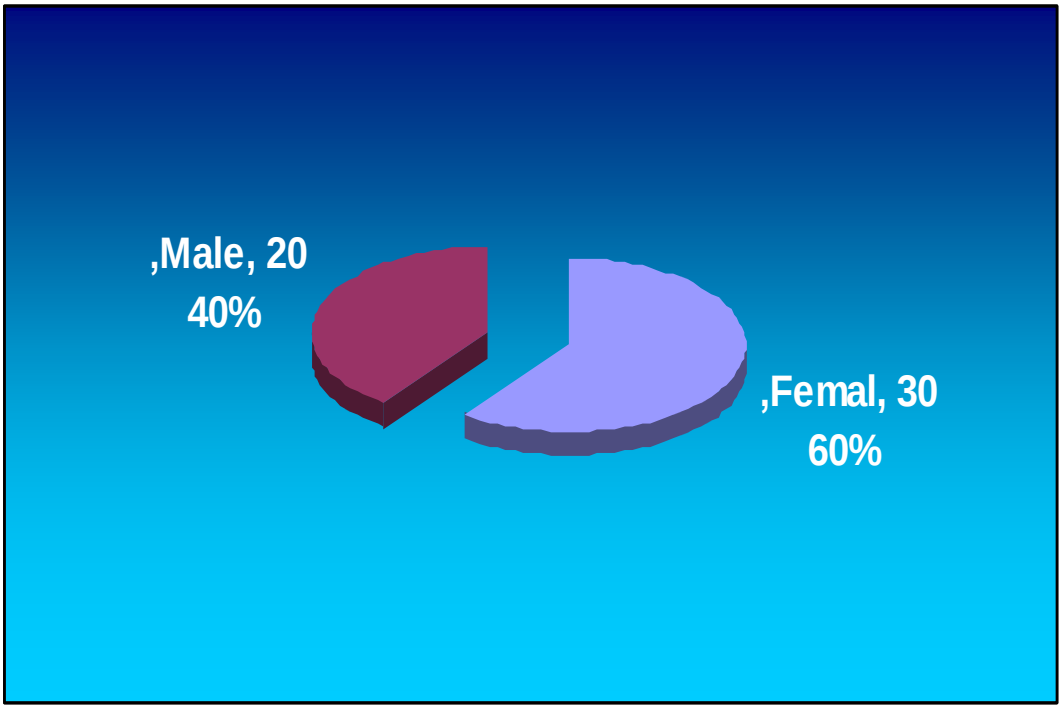

Figure ٣.٢: The Ratio of Male and Female in Positive Acute Sinusitis.

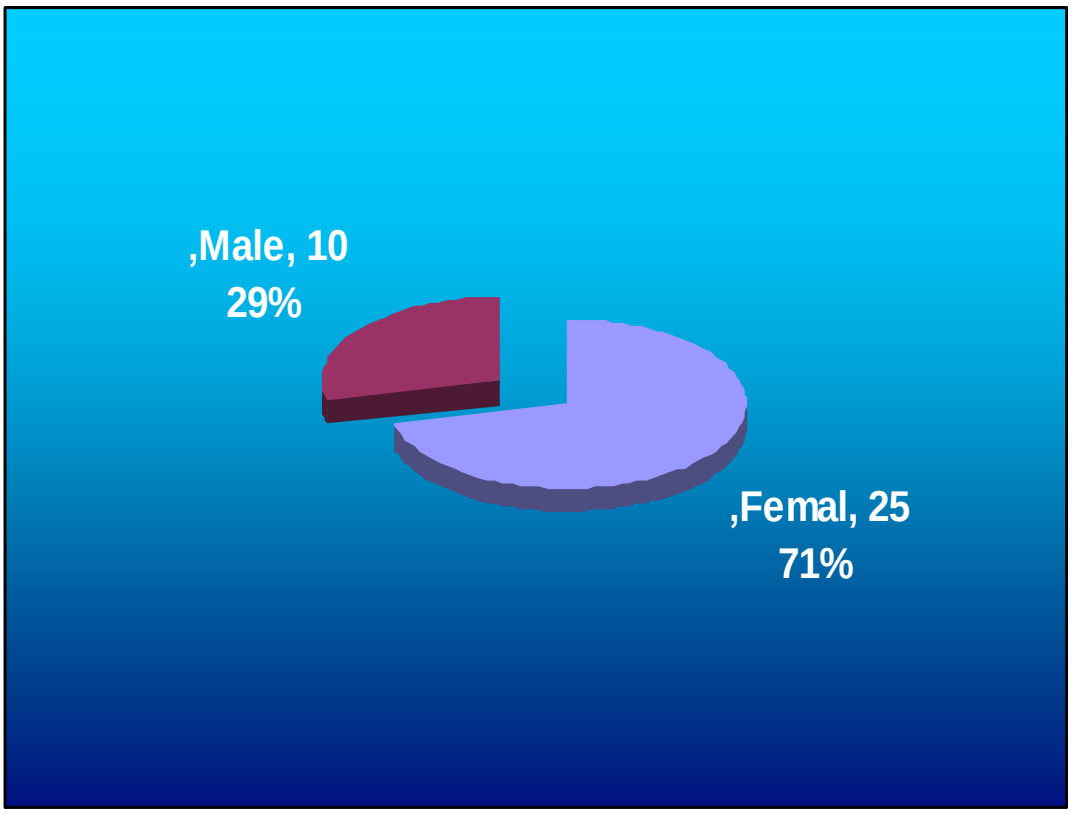

Figure ٣.٣: The Ratio of Male and Female in Positive Chronic Sinusitis.

٣-٥ *Isolation of Bacteria Associated with Sinusitis:-*

The study included ١٠٠ patients suffering from sinusitis who attend to Hilla Teaching Hospital-Department of Ear Nose and Throat (ENT). The results showed that (٨٥٪) of patients from both chronic and acute sinusitis revealed positive bacterial cultures as mentioned in Table (٣-٣).

As mentioned above, in chronic sinusitis the positive culture was in ٣٥ cases (٨٣.٣٪), while the negative culture was only in ٧ cases (١٦.٧٪). These results were in accordance with the results obtained by Hartog ***et.al.*** (١٩٩٥) who found that the positive cultures were as high as (٨١٪) versus the negative cultures which were (١٩٪). However the results of the study were not agreed with the results obtained by Aneke and Ezeanoluc

(٢٠٠٤) since they found that (٥٩٪) of the cases revealed negative cultures and (٤١٪) were positive for bacterial culture.

Table (٣-٣) shows that (٨٦.٢٪) of the patients in acute sinusitis have positive culture and (١٣.٨٪) of them had negative culture. These results were similar to the results obtained by Penttila ***et.al.*** **(**١٩٩٧). Some specimens of both chronic and acute sinusitis cases, being investigated, revealed negative bacterial culture. In chronic sinusitis, this could possibly be due to anaerobic microorganisms which colonizes the blocked sinus providing a suitable environment for them mainly ***Bacteroides spp.***, since they are described as the main causative agent of microorganisms in chronic sinusitis patients (Brook, ١٩٩٦). Unfortunately, the study focused mainly on aerobic and ⁄ or facultative anaerobes since anaerobes fastidious organisms are difficult to be isolated from infections sites and are often overlooked (Brook, ٢٠٠٢). The negative results of bacterial cultures can also be attributed to other microbial causes of sinusitis are not included in this study, such as viral or fungal infections.

Table (٣-٣) Number and Percentage of Sinusitis Culture Result

(Acute and Chronic):

Culture	Acute No. (%)	Chronic No.(%)
Positive	٥٠ (٨٦.٢)	٣٥(٨٣.٣)
Negative	٨ (١٣.٨)	٧(١٦.٧)
Total	٥٨ (١٠٠)	٤٢(١٠٠)

The study detected ٣٨ isolates of ***Staph. aureus,*** ١٤ solates from acute sinusitis and ٢٤ isolates from chronic sinusitis. These isolates were

subjected to sensitivity test against Oxacillin for detecting the Methicillin Resistant ***Staphylococcus aureus*** (MRSA) as these isolates are more resistant to antibiotics and more aggressive compared with Methicillin Sensitive ***Staphylococcus aureus*** (MSSA). Consequently the MRSA isolates ١١ isolates (٢٩ %) from both acute and chronic sinusitis versus ٢٧ (٧١٪) isolates are accounted for Methicillin sensitive ***Staphylococcus aureus*** (MSSA). There were ٩ (٣٧.٥٪) isolates from chronic sinusitis of MRSA and only ٢ isolates (١٤.٢٪) of MRSA from acute sinusitis.

These results can be compared with the results obtained by Manarey ***et.al.*** (٢٠٠٤) who found that only (٩.٢٢٪) of MRSA cause chronic rhinosinusitis where as Liu ***et.al.*** (٢٠٠١) found that (٩٦.٢٪) of MRSA were detected in chronic maxillary sinusitis.

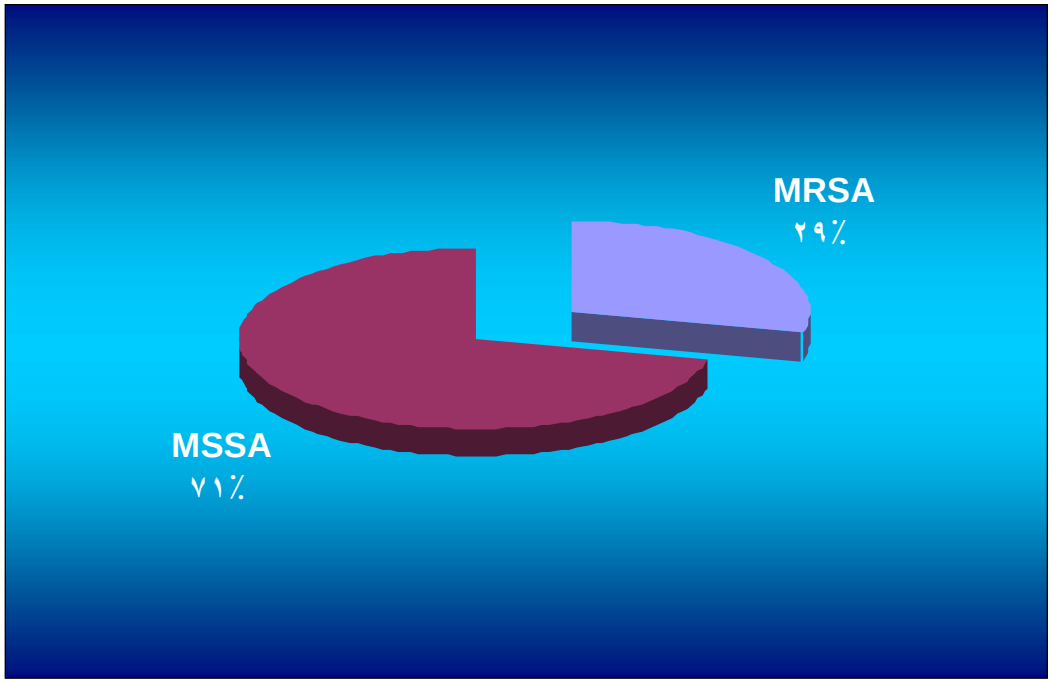

Figure ٣.٤: The Ratio of MRSA and MSSA in Sinusitis Disease.

٣-٦ *Types of Bacterial Isolates*

Table (٣-٤) shows that the most common organism being cultured from acute sinusitis patients was ***S. pnemonia*** (٢٥٪) followed by ***M. catarrahalis*** (١٨٪), ***S. aureus*** (١٦.٧٪), Coagulase negative Staphylococci

(١٤٪), Viridans streptococci (١٢٪), ***K. pneumonia*** (٨٪) and lastly (٦٪) isolates of ***S. pyogenes*** were also obtained.

The results of the present study agreed with the results obtained by Diaz and Bamberger (١٩٩٥); Brook and Frazier (٢٠٠٤) who showed that *S. pneumoniae* was the most common organism being isolated for sinusitis ٣٥٪ followed by *M. catarrahalis* (٢٨٪) and *S. aureus* (٢٥٪), Coagulase negative Staphylococci (٢٢٪), Viridans streptococci (١٩٪), *K. pneumoniae* (٨٪) with (٥٪) isolates of *S. pyogenes* furthermore Brook and Frazier (٢٠٠٥) stated that the most common organism causing acute sinusitis was *S. pneumoniae*. While other studies reported that *Haemophilus spp.* was the most predominant pathogen in acute sinusitis followed by *S. pneumoniae* (Kinman *et.al.*, ١٩٦٧; VanCauwenberg *et.al.*,١٩٧٦; Hannel *et.al*,١٩٨٨; Hannel *et.al.*,١٩٨٩ and Sarmad,٢٠٠٥). Pfaller *et.al.*(٢٠٠١) stated that the predominant type of bacteria being isolated from acute sinusitis was *M. catrrhalis* followed by *Haemophilus spp.* and *S. pneumoniae*.

Table (٣-٤) Number and Percentage of Acute Bacterial Isolates:

Bacteria	Number	Percentage %
S. pneumonia	٢١	٢٥
M. catarrahalis	١٥	١٨
S. aureus	١٤	١٦.٧
S. epidermidis	١٢	١٤
S. viridans	١٠	١٢
K. pneumonia	٧	٨
S. pyogenes	٥	٦
Total	٨٤	١٠٠

H. influenzae was reported to be the commonest isolate detected in sinusitis followed by ***S. pneumoniae, S.viridans,*** and ***K. pneumoniae*** **Mougthong *et.al.* (٢٠٠٥), while the study done by Brook (٢٠٠٥) found that the**

predominant cause of sinusitis was alpha hemolytic Streptococci followed by microaerophilie Streptococci and *S. aureus*. The variation in the causative agents of acute sinusitis is either due to the effect of antibiotics therapy on eliminating the sensitive bacteria allowing for the increasing predominance of other (Berg *et.al.,*١٩٨٨), or may be related to microorganisms geographical distribution and even may be related to patients conditions(Conrad *et.al.,* ٢٠٠٢).

Table (٣-٥) shows that the most common organisms isolated from chronic sinusitis patients were *S. aureus* (٤٢.٨٪), *S. pneumoniae* (٢٣.٤٪), *M. catarrhalis* (١٠.٧٪), *K. pneumoniae* (٩٪), Viridans streptococci (٥.٣٪), *E. coli* (٥.٣٪) and *Proteus mirabilis* (٣.٥٪). These results were in agreement with the results obtained by Sener *et.al.* (١٩٩٦) who pointed out that the most common organism in chronic sinusitis was *S. aureus* (٦٠٪), followed by *S. pneumoniae* (٤٠٪), *M. catarrhalis* (٣٠٪), *K. pneumoniae* (٢٥٪), Viridans streptococci (١٥٪), (٦٪) isolates of *E. coli* and (٤٪) of *Proteus mirabilis* . *S. aureus* was reported to be the dominant organisms habitant chronic sinusitis followed by *H. influenzae, E. coli* and *S. pneumoniae* (Namyslowski *et.al.,* ٢٠٠٤), while Sarmad (٢٠٠٥) found that the most predominant microorganisms isolated from chronic sinusitis was *H. influenzae, S. pneumoniae, M. catarrhalis, S. aureus,* Viridans streptococci and then some members of Enterobacteriaceae.

Consequently, *S. aureus* seemed to be the common dominant among bacteria resident in chronic sinusitis. This phenomenon may be explained by Bukharin *et.al.* (١٩٩٨) who stated that the biological properties of *Staphylococci* initiating the inflammation with feature of clinical course of maxillary sinusitis. These virulent characteristics determine the acute

course of the disease whereas the strain with persistent properties causes chronic course of maxillary sinusitis.

Table (٣-٥) Number and Percentage of Chronic Bacterial Isolates:

Type of Bacteria	Number	Percentage %
S. aureus	٢٤	٤٢.٨
S. pneumoniae	١٣	٢٣.٤
M. catarrhalis	٦	١٠.٧
K. pneumoniae	٥	٩
E. coli	٣	٥.٣
S. viridans	٣	٥.٣
Proteus mirabilis	٢	٣.٥
Total	٥٦	١٠٠

٣-٧ *Seasonal Variation :-*

The results shown in Table (٣-٦) indicate that the occurrence of sinusitis through the year showed an accumulation of cases during the cold season since the peak of incidences was observed in January.

Table (٣-٦) Number of Positive Obtained Cases Distributed on Months of the Year

years	Months	No. of Cases
٢٠٠٤	October	٤
	November	٦
	December	١٢
٢٠٠٥	January	١٧
	February	١٢
	March	١١
	April	١٢
	May	٧
	June	٢

	July	٢
	Total	٨٥

From these results one can conclude that sinusitis occurs as a secondary infection due to sinus ostia obstruction associated with the common cold or allergies and maxillary sinus is the most common (Diaz and Bamberger, ١٩٩٥). Most cases of common cold usually occur in temperate climate, so it appears in winter more than in summer (Macky and Bull,١٩٩٧). These results are also ensured by (Glezen, ١٩٩٨) who stated that the obstruction in nose due to infection with Rhinovirus or other virus that cause common cold will trap bacteria in the closed space resulting in suppurative sinusitis and otitis media which are the most common complication of URTI (upper respiratory tract infection). The results obtained by this study are in accordance with the results obtained by Stalman *et.al.* (٢٠٠١) who asserted that sinusitis cases are common in winter rather than in other seasons since (٧٠٪) of sinusitis cases were recorded in winter. Statistical analysis by using Chi Square showed that there is a significant relationship between months and number of cases (*p* < ٠.٠٥).

٣-٨ *Susceptibility of Bacterial Isolates to Antibiotics:-*

For more explanation the effect of different antibiotics on bacterial isolates being identified in this study will now be discussed:-

٣-٨-١ *Penicillins*

٣-٨-١-١ *Ampicillin*

Figure (٣-٥) shows that *M. catarrhalis, S. aureus* (both MRSA and MSSA) and *Proteus mirabilis* were fully resistant (١٠٠٪) to ampicillin. These results agree with the results obtained by (Kim *et.al.*, ٢٠٠٤; Rashmi *et.al.*,٢٠٠٥ and Melo-Cristino *et.al.*(٢٠٠٦) who observed that these bacteria were highly resistant to ampicillin. The resistance frequency

exhibited by *S. pneumoniae* and viridans group streptococci are accounted for (٧٣٪) and (٦٩٪) respectively while *S. pyogenes* isolates were sensitive. These results were more agreeable with the results obtained by Hsuch *et.al.* (٢٠٠٤) who found that the resistance rate of *S. pneumoniae* to ampicillin was (٧٠٪) while it was (٦٠٪) for viridians group streptococci whereas *S. pyogenes* was sensitive. Two isolates of *E. coli* (٦٦٪) and ٩ isolates of *K. pneumoniae* (٧٥٪) were resistant to ampicillin. These results were in accordance with the results obtained by Oteo *et.al.*(٢٠٠٥) who found that (٥٩.٩٪) of *E. coli* were resistant to ampicillin. Moreover Blandino *et.al.*(١٩٩٠) found that (٧٠٪) of *K. pneumoniae* were resistant to ampicillin. ٦٦٪ of *Staph. epidermidis* was found to be resistant to ampicillin. Hashemi *et.al.* (٢٠٠٥) reported that *S. epidermidis* was less resistant compared with *S. aureus* isolates.

٣-٨-١-٢ *Amoxicillin*

Figure (٣-٦) shows that (١٠٠٪) of *Proteus mirabilis*, (٥٠٪) of *E. coli* and (٧٠٪) of *K. pneumoniae* were resistant to amoxicillin. These results were in agreement with the results obtained by Quentin *et.al.* (٢٠٠٤) who stated that (٥٣.٤٪) of *E. coli* was resistant. Stock (٢٠٠٣) stated that *Proteus mirabilis* was naturally resistant to amoxicillin and Toy *et.al.* (١٩٩٣) who found that (٧٨.٦٪) of *K. pneumoniae* were resistant to this drug.

The susceptibility of *S. pneumoniae* and *S. pyogenes* are accounted for (١٠٠٪).These results were in accordance with the results obtained by Vanhoof *et.al.*(٢٠٠٥) who ensured that most of bacterial isolates remained fully susceptible to amoxicillin. Pichichero (١٩٩٥) found that *S. pyogenes* were highly susceptible to amoxicillin, while (٣٥٪) of Viridans streptococci were resistant to amoxicillin. These results were comparable with the results obtained by IKemoto *et.al.* (١٩٩٦) who found that (٤٠٪) of viridians group streptococci were resistant to amoxicillin. All isolates of MRSA (١٠٠٪) versus (٧٥٪) of MSSA were resistant to amoxicillin. These results agreed with the results obtained by Rajaduraipandi *et.al.*(٢٠٠٦)

who obtained that (١٠٠٪) of MRSA isolates were resistant to amoxicillin. Moreover Kingdom and Swain (٢٠٠٤) pointed out that (٧٨.٩٪) of MSSA were resistant to amoxicillin. The high resistance for beta-lactam drugs could be explained by the production of beta-lactamases by Staphylococci, gram positive bacteria, gonococci and others, moreover lack of penicillin binding protein (PBPs) or altered (PBPs) in Pneumococci. Sometimes failure of activation of autolytic enzyme in cell wall can result in inhibition without killing bacteria ex. Tolerance of some Staphylococci (Brooks *et.al.*, ٢٠٠٤)

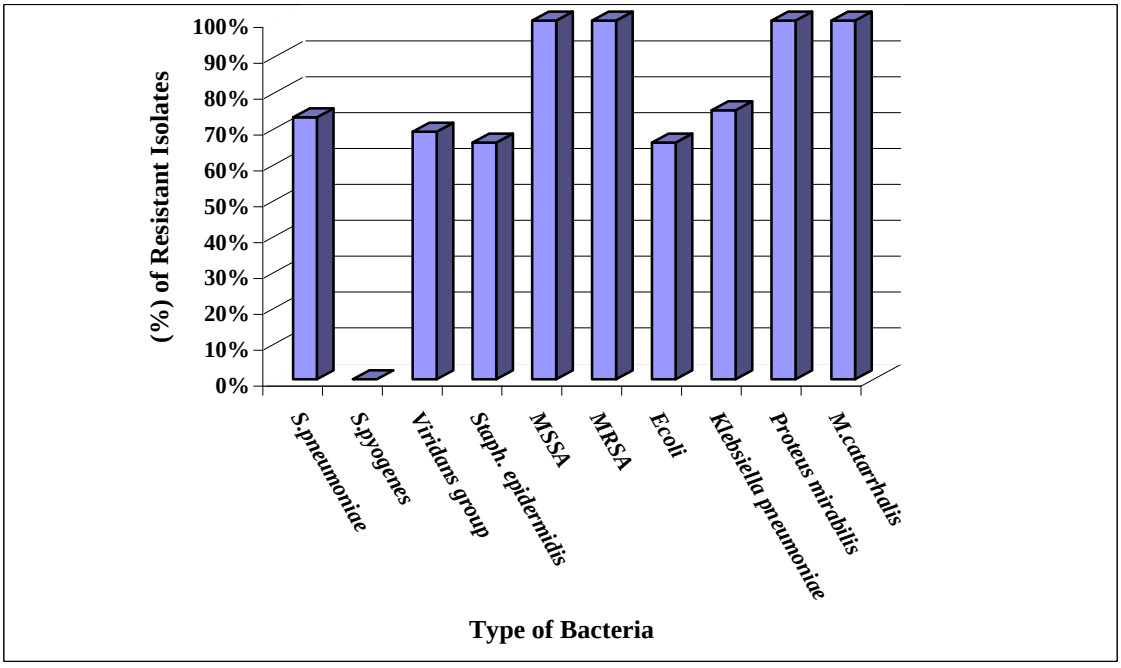

Figure ٣.٥:The Ratio of Resistant of Bacterial Isolates Against Ampicillin.

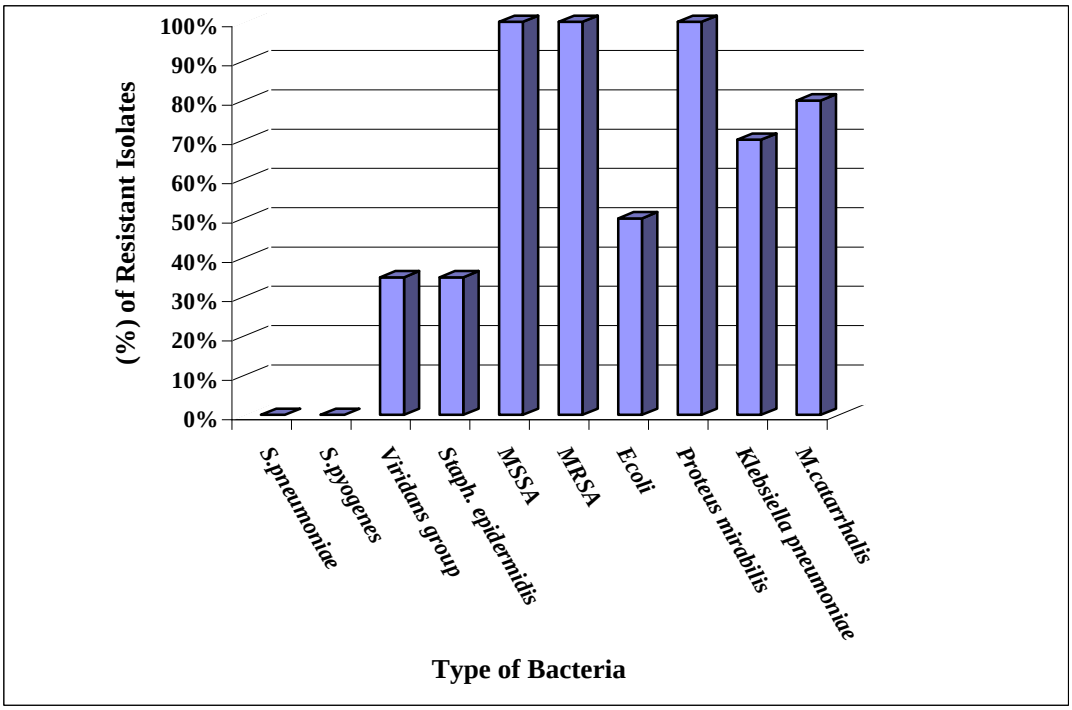

Figure ٣.٦: The Ratio of Resistant of Bacterial Isolates Against

Amoxicillin.

٣-٨-٢ *Aminoglycosides*

٣-٨-٢-١ *Gentamycin*

The results shown in Figure (٣-٧) indicate that only four types of bacterial isolates in this work were fully sensitive to gentamycin .These bacteria were ***S. pyogenes, K. pneumoniae, E. coli*** and ***Proteus mirabilis***. These findings correlated with other results being observed by other studies, since ***S. pyogenes*** exhibited sensitivity rate up to (١٠٠٪) Zhanel ***et.al.*** (٢٠٠٢) while the sensitivity rate of ***E. coli*** and ***Proteus mirabilis*** to gentamycin was (٩٥٪) and more than (٩٠٪) of ***K. pneumoniae*** were also sensitive to this drug (Shavidok, ١٩٨٧; Petrove ***et.al***., ٢٠٠٥ and Tonkic ***et.al.***, ٢٠٠٥). Moreover other types of bacterial isolates exhibited a percentage of resistance ranged from (٢٥٪) for ***Staph. epidermidis*** until they reached (٨٥٪) for ***M. catarrhalis***. (٧٦٪) for Viridans group, (٧٠٪)

for ***S. pneumoniae***, (٤٦.١٪) for MSSA and (٦٦٪) for MRSA. These results were in accordance with the results obtained by Hsuch ***et.al***. (٢٠٠٤) who ascertained that (٨٠٪) of ***S. pneumoniae*** were resistant to gentamycin. Rashmi ***et.al.***(٢٠٠٥) and Smith ***et.al.***(٢٠٠٢) showed that Viridans group Streptococci were highly resistant to gentamycin. In addition to that Adwan ***et.al.***(٢٠٠٥) mentioned that (٦٤.٣٪) of MRSA were resistant to gentamycin while Rohani ***et.al.(***٢٠٠٠) observed that (٤٠.٥٪) of MSSA were resistant to gentamycin. Deng ***et.al.*** (١٩٨١) revealed that (٨٠٪) of ***Staph. epidermidis*** were sensitive for this drug. 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 reason for resistance, especially in Streptococci. This mechanism of resistance was described as chromosomally mediated mechanism (Mims ***et.al.,*** ٢٠٠٤).

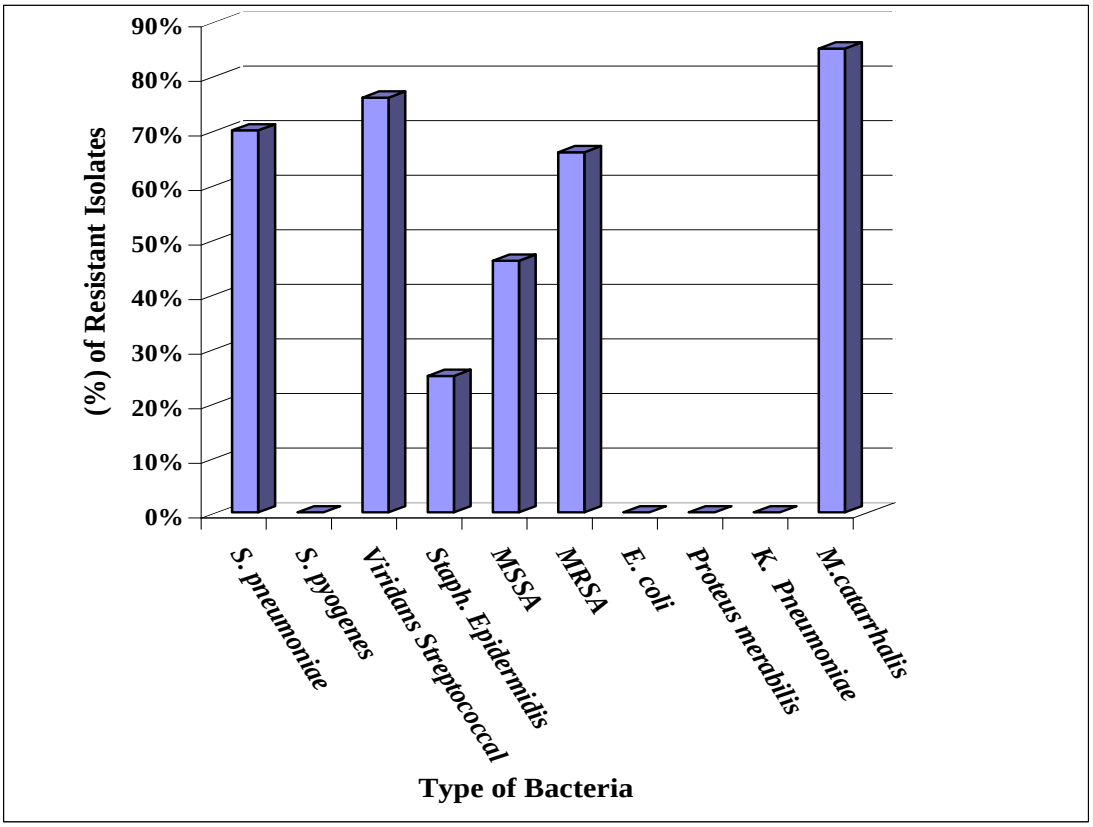

Figure ٣.٧: The Ratio of Resistant of Bacterial Isolates Against Gentamycin

٣-٨-٣ *Fluoroquinolon*

٣-٨-٣-١ *Ciprofloxacin*

Figure (٣-٨) shows that all types of bacterial isolates found in this test were fully susceptible for ciprofloxacin except (٥٨.٣٪) of MRSA, (٢٣٪) of MSSA and (١٢٪) of *M. catarrahalis* were resistant to ciprofloxacin. These results agreed with the results pointed out by other studies. Tonkic *et.al.*(٢٠٠٥) found that (٤.٤٪) of *E. coli* and (٤٪) of *K. pneumoniae* were resistant to ciprofloxacin; Brown and Mjrybak (٢٠٠٤) stated that all isolates of *S. pyogenes* were sensitive to ciprofloxacin and more than (٩٧٪) of *S. pneumoniae* were sensitive to ciprofluxacin. Inoue *et.al.*(٢٠٠٦) reported that viridians groups streptococci were highly sensitive to ciprofloxacin and Hsuch *et.al.*(٢٠٠٤) observed that (٩٠٪) of *M. catarrhalis* were sensitive to ciprofloxacin while Rohani *et.al.*(٢٠٠٠) stated that (٢٩.٢٪) of MSSA were resistant to ciprofluxacin. Moreover Adwan *et.al.*(٢٠٠٥) detected that (٣٢.١٪) of MRSA were resistant to ciprofluxacin.

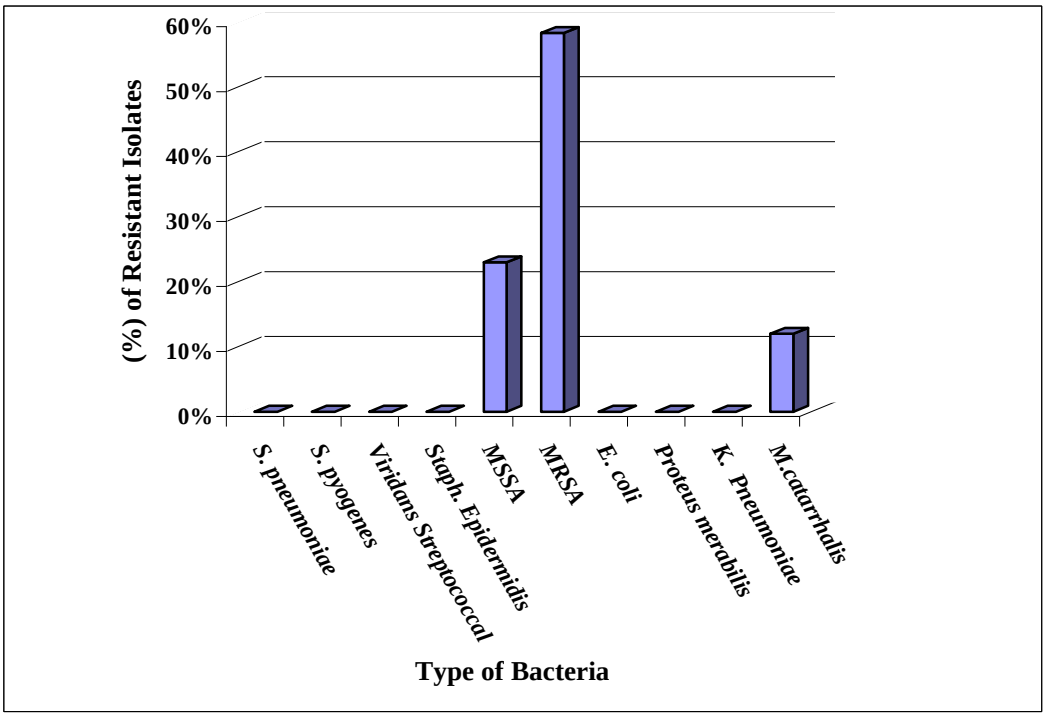

Figure ٣.٨: The Ratio of Resistant of Bacterial Isolates Against

Ciprofloxacin.

٣-٨-٤ *Cephalosporins*

٣-٨-٤-١ *Cefotaxim*

As shown in Figure (٣-٩) only MRSA, ***Proteus mirabilis*** and ***Klebsiella pnumoniae*** exhibited relative resistance represented by (٨٣٪), (٥٠٪) and (٢٠٪) respectively. While all other bacterial types included in this test were fully (١٠٠٪) sensitive for cefotaxim .These results came to confirm the results being reported by other studies, since Morrissey ***et.al***. (٢٠٠٥) found that all isolates of ***S. pneumoniae*** and ***M. catarrhalis*** were sensitive to cefotaxim. Mendes ***et.al.***(٢٠٠٤) stated that viridans group streptococci were highly susceptible to cefetaxim. Karlowsky ***et.al.,***(٢٠٠٤) reported that ***E. coli*** were sensitive to cefotaxim. Kim ***et.al.***(٢٠٠٤) found that a high percentage of ***P. merabilis*** was sensetive to cefotaxim. Balandino ***et.al.***(١٩٩٠) and Lee ***et.al***., (٢٠٠١) mentioned that (١٩٪), (٢٠٪) of ***K. pneumoniae*** were resistant to cefotaxim respectively. Deneg ***et.al.***(١٩٨١) found that all isolates of ***S. epidermidis*** and MSSA were sensitive to cefotaxim. ***S. pyogenes*** was found to be fully susceptible for cefotaxim (Brown and Mjrybak, ٢٠٠٤). Regarding the resistance of MRSA towards cefotaxim in this study, it was quietly in accordance with those results which have been recently reported by Asghar and Momenah (٢٠٠٦) who determined the resistance of MRSA against this antibiotic as high as (٨٥٪).

٣-٨-٤-٢ *Cephalothin*

The results show in Figure (٣-١٠) the percentage of resistance represented by (١٣.٣٠٪) for ***M. catarrahalis*** (٧٥٪) for ***K. pneumoniae*** **(**٦٦٪) for ***E. coli,*** (٥٠٪) for ***P. mirabilis*** and (٦٦%) for MRSA. All other bacterial isolates in this study were sensitive. These results were agreeable with those results obtained by Giglio ***et.al.*** (١٩٩٩) who

revealed **that the gram positive cocci was highly susceptible to Cephalothin. Stock (٢٠٠٣) observed that moderate percentage of *proteus mirabilis* was resistant to Cephalothin. Yeh and Chi (٢٠٠١) noticed that (٧٢٪) of *E. coli* were resistant to Cephalothin. Furthermore Inglis *et.al.* (١٩٩٤) stated that (٧٠٪) of *K. pneumoniae* were resistant to Cephalothin. Additionally, Morrissey *et.al.* (٢٠٠٥) reported that *M. catarrahalis* and *S. epidermidis* were highly sensitive to Cephalothin. In addition, Rohani *et.al.*(٢٠٠٠) found all strain of MSSA was susceptible to Cephalothin and Durmaz *et.al.*(١٩٩٧) obtained that (٦٠٪) of MRSA were resistant to Cephalothin. Antibiotics resistance is linked to prior exposure to antibacterial drugs because the first and second generation of cephalosporins are commonly prescribed for hospitalized patients (Liu *et.al.*, ١٩٩٩). Resistance to cephalosporins is due to special β-lactamases in gram negative bacteria and *Staphylococci* that hydrolyze and inactivate these drugs (Brooks *et.al.*, ٢٠٠٤).**

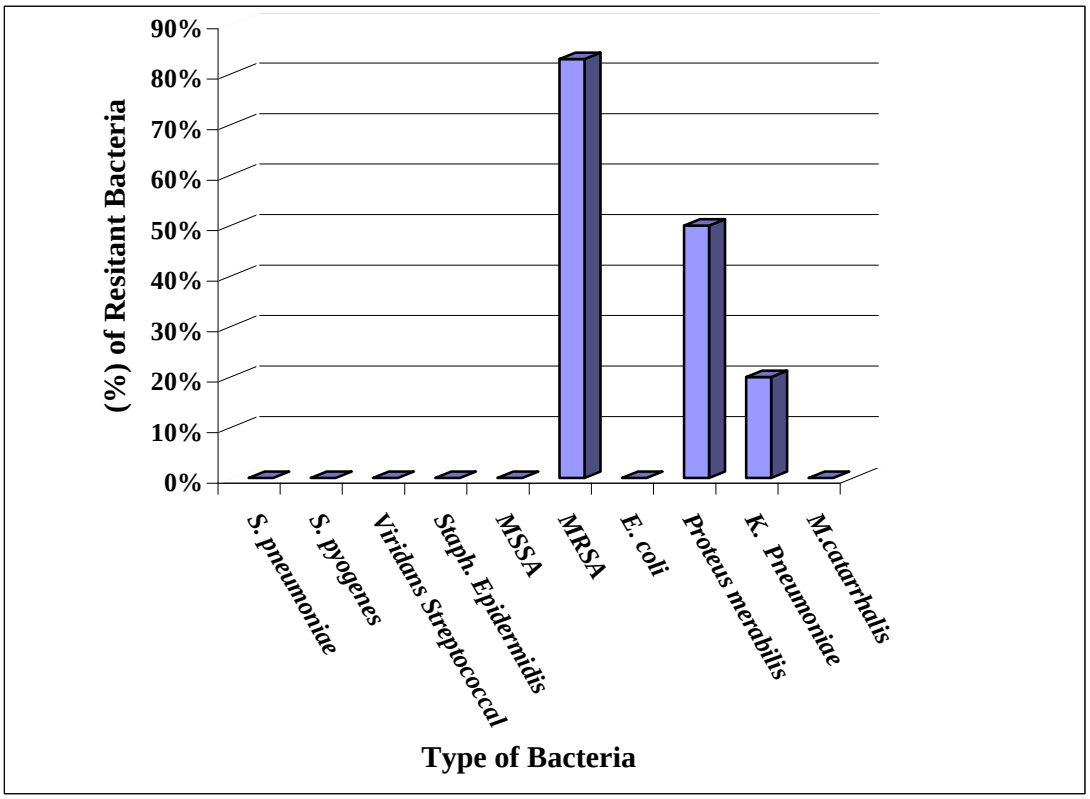

Figure ٣.٩: The Ratio of Resistant of Bacterial Isolates Against Cefotaxim. `

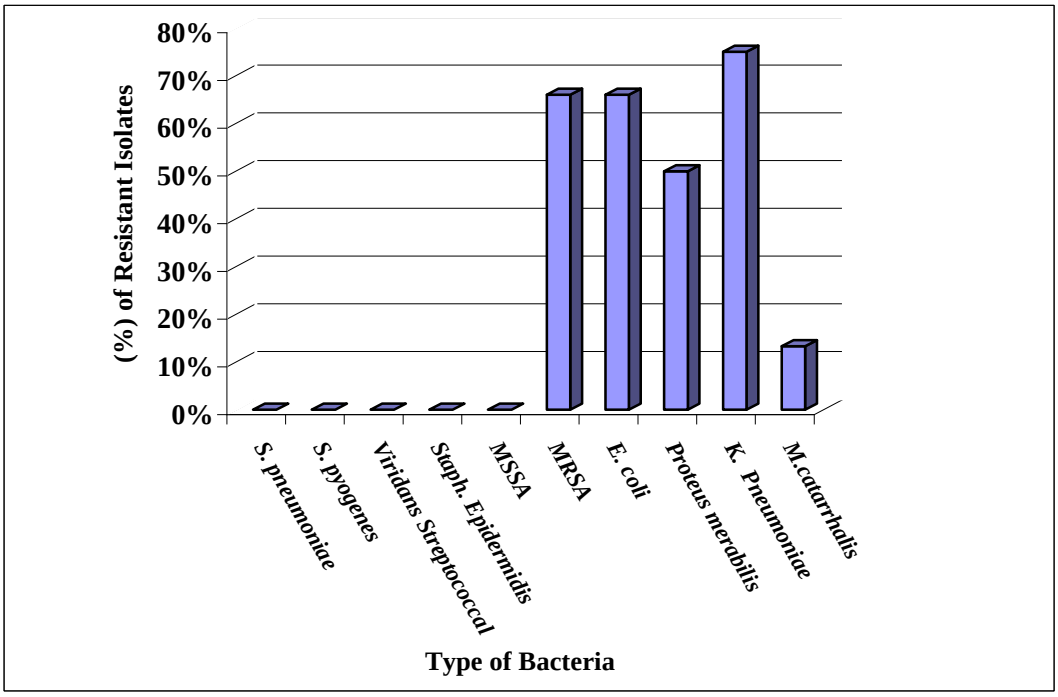

Figure ٣.١٠: The Ratio of Resistant of Bacterial Isolates Against Cephalothin.

٣-٨-٥ *Sulfonamide derivative*

٣-٨-٥-١ *Trimethoprim*

The results shown in Figure (٣-١١) indicate that ***S. pyogenes*** and ***Staph. epidermidis*** revealed no resistance, while the other types of investigated bacteria exhibited a percentage of resistance ranged from (١٣.٣٪) for ***K. pneumoniae*** up to (٩١٪) for MRSA. A moderate resistance was observed in MSSA and ***P. merabilis*** .These results were in accordance with Melo-Cristino ***et.al***. (٢٠٠٦) who found that (٢٧.١٪) of ***M. catarrhalis*** were resistant and Cardozo ***et.al.***(٢٠٠٦) who found that (٧.٣٪) of ***S. pneumoniae*** were resistant to trimethoprim. It was found that ***P. merabilis*** resisted Trimethoprim in a frequency of (٥٥٪) (Kim ***et.al.,*** ٢٠٠٤), while ***E. coli*** resisted Trimethoprim in a frequency of (٢١٪)

(Quentin ***et.al.,*** ٢٠٠٤). ***K. pneumoniae*** was observed to be resistant to Trimethoprim in a frequency at(١٣.٣٪) (Tonkic ***et.al***., ٢٠٠٥). Rohani ***et.al***.(٢٠٠٠) stated that (٤٧.٢٪) of MSSA were resistant while Pulimood ***et.al.***(١٩٩٦) found that (٩٧٪) of MRSA were resistant to trimethoprim. Moreover Rajaduraipandi ***et.al.***(٢٠٠٦) obtained (٦٣.٢٪) of MRSA isolates which were resistant towards Trimethoprim. The resistant to this antibiotic emerged to previous longer use of it leading to reduce cell permeability and pumping it from the resistant bacterial cell (Murray ***et.al.,*** ١٩٩٩).

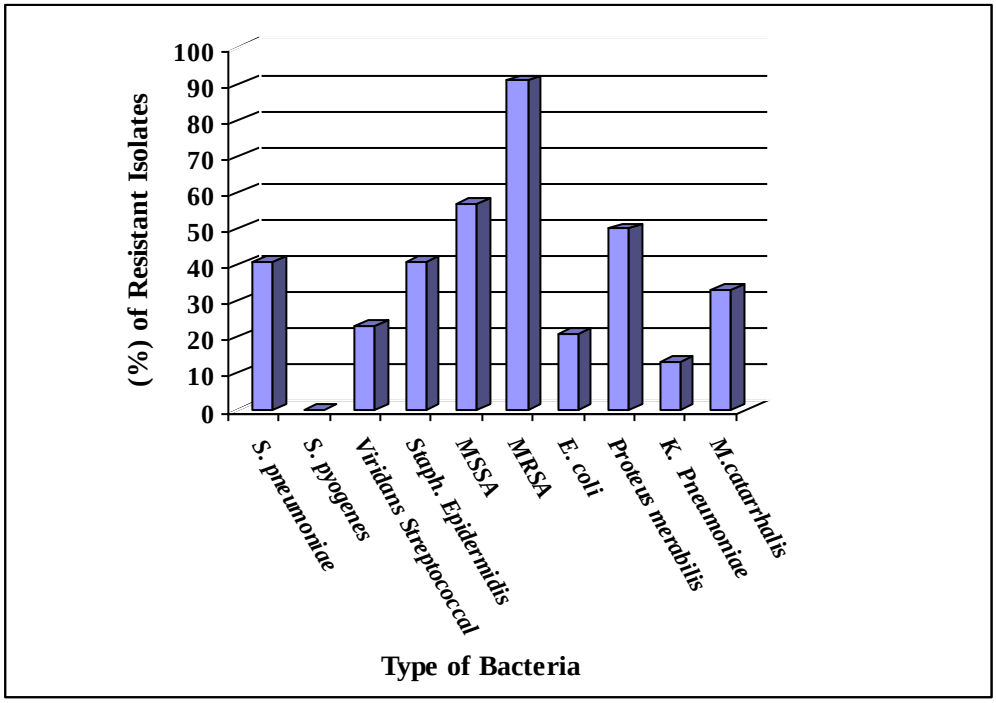

Figure ٣.١١: The Ratio of Resistant of Bacterial Isolates Against

Trimethoprim.

٣-٧-٦ *Vancomycin*

As shown in Figure (٣-١٢) all types of gram positive isolates were sensitive towards vancomycin, while all the negative isolates were resistant to it .These findings were closely correlated with the results being reported by Cardozo***, et.al.*** (٢٠٠٦); Ergin ***et.al.*** (٢٠٠٦) and Inoue

et.al. (٢٠٠٦) who pointed out that ***S. pneumoniae, S. pyogenes*** and Viridians group streptococci were highly susceptible to vancomycin .Moreover these results Matched the reports of Luh ***et.al.,*** (٢٠٠٠) and Hamze ***et.al.*** (٢٠٠٣) who showed that all isolates of MSSA and MRSA were susceptible to vancomycin .In addition Mendes ***et.al.,*** (٢٠٠٣) stated that ***S. epidermidis*** was highly susceptible to vancomycin.

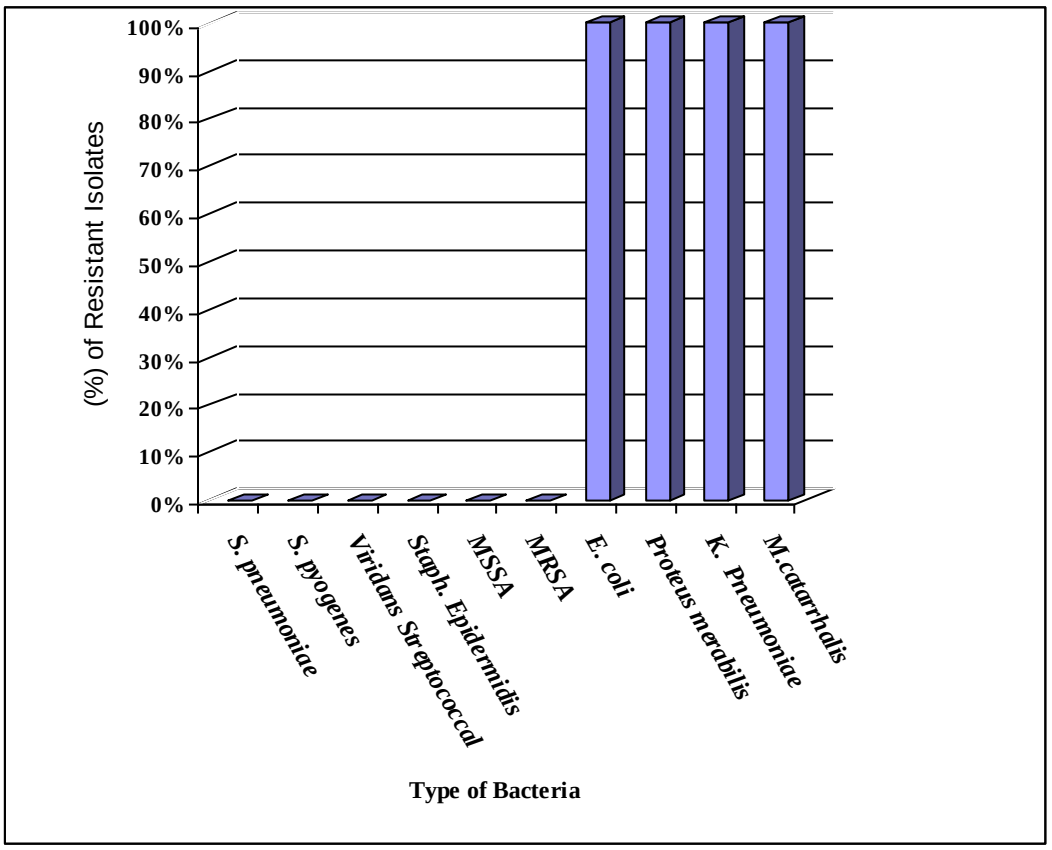

Figure ٣.١٢: The Ratio of Resistant of Bacterial Isolates Against

Vancomycin.

٣-٨-٧ *Tetracyclines*

٣-٨-٧-١ *Tetracyclin*

The results shown in Figure (٣-١٣) indicate that only two types of bacterial isolates represented by ***S. pyogenes*** and ***S. epidermidis*** were fully (١٠٠٪) sensitive for tetracycline. Other types of bacterial isolates showed different rates of resistance ranging from (٢٣٪) for Viridians group streptococci up to (١٠٠٪) for enteric group represented by (***Proteus mirabilis***, ***E. coli*** and ***K. Pneumoniae***). On the other hand, the resistance rate against tetracycline for ***S. pneumoniae*** was (٢٩٪), ***M. catarrhalis*** (٢٣.٨٪) and (٤٦.١٪) of MSSA isolates and (٩١.٦٪) of MRSA isolates. These results were in accordance with the results obtained by Stock (٢٠٠٣) who found that ***Proteus mirabilis*** was naturally resistant to all tested tetracycline. Ansari and Khatoon (١٩٩٩) reported that ***E. coli*** was resistant against tetracycline, while ***K. pneumoniae*** showed no response towards tetracycline (Toy ***et.al.,*** ١٩٩٣). Moreover Vanhoof ***et.al.*** (٢٠٠٥) mentioned that (٣٠.٥٪) of ***S. pneumoniae*** were resistant to tetracycline, while Inoue ***et.al.*** (٢٠٠٦) expressed ٣٠٪ of Viridians group streptococci were resistant to tetracycline and all isolates of ***S. pyogenes*** were sensitive . MSSA resisted to tetracycline in a frequency of (٤٧.٢٪) Inoue ***et.al.***(٢٠٠٦), while MRSA resisted to tetracycline in a frequency of more than (٩٠٪) (Rohani ***et.al.*** ,٢٠٠٠ and Asghr and Momenah ٢٠٠٦). The resistance of bacteria towards this antibiotic could be explained by efflux pump by an active transport protein pump (Jacopy, ١٩٩٣).

٣-٨-٨ *Macrolides*

٣-٨-٨-١ *Erythromycin*

As shown in Figure (٣-١٤), all types of bacterial isolates investigated by this study showed resistance to erythromycin in variable rates starting from (١٧٪) for ***S. pneumoniae*** and (٢٠٪) for ***S. pyogenes*** up to (١٠٠٪) for the members of Enterobacteriaceae represented by (***proteus mirabilis, E. coli*** and ***K. pneumoniae***). Moreover(٨٣.٣٪) of MRSA isolates were resistant to erythromycin, while a moderate resistance rate was observed in MSSA , ***S. epidermidis*** and Viridans group streptococci .Consequently these results can be compared with the results obtained by Melo-Cristino ***et.al.*** (٢٠٠٦) who showed that (١٨.٨٪) of ***S. pneumoniae*** and (١٨.٩٪) of ***S. pyogenes*** were resistant to erythromycin. Moreover Mendes ***et.al.*** (٢٠٠٣) stated that (٣٢.٦٪) of viridans group streptococci were resistant to erythromycin .Additionally, Rohani ***et.al.*** (٢٠٠٠) revealed that only (٤٥.٩٪) of MSSA isolates were resistant to erythromycin and the results obtained by Asghar and Momenah (٢٠٠٦) mentioned that (٨٢.١٪) of MRSA isolates were resistant to erythromycin. Toye ***et.al.*** (١٩٩٣), Zhang ***et.al.*** (٢٠٠٦) and Stock (٢٠٠٣) also obtained such results. Moreover**,** ***M. catarrhalis*** resisted to erythromycin in frequency of (١٣٪) (Inoue ***et.al.,*** ٢٠٠٦), while ***S. epidermidis*** resisted to this antibiotic in a frequency of (٣٥٪) (Pichiche, ١٩٩٥). The resistance of gram positive bacteria for these antibiotics resulted by the production of Erythromycin-resistant methylase resultant structural changes to rRNA prevent macrolide binding and allows synthesis of bacterial proteins to continue or by efflux (Pechere, ٢٠٠١), while the resistance of gram negative bacteria particularly (Enterobacteriaceae) can be ascribed to the production of esterase that hydrolyze macrolides (Chambers, ٢٠٠١).

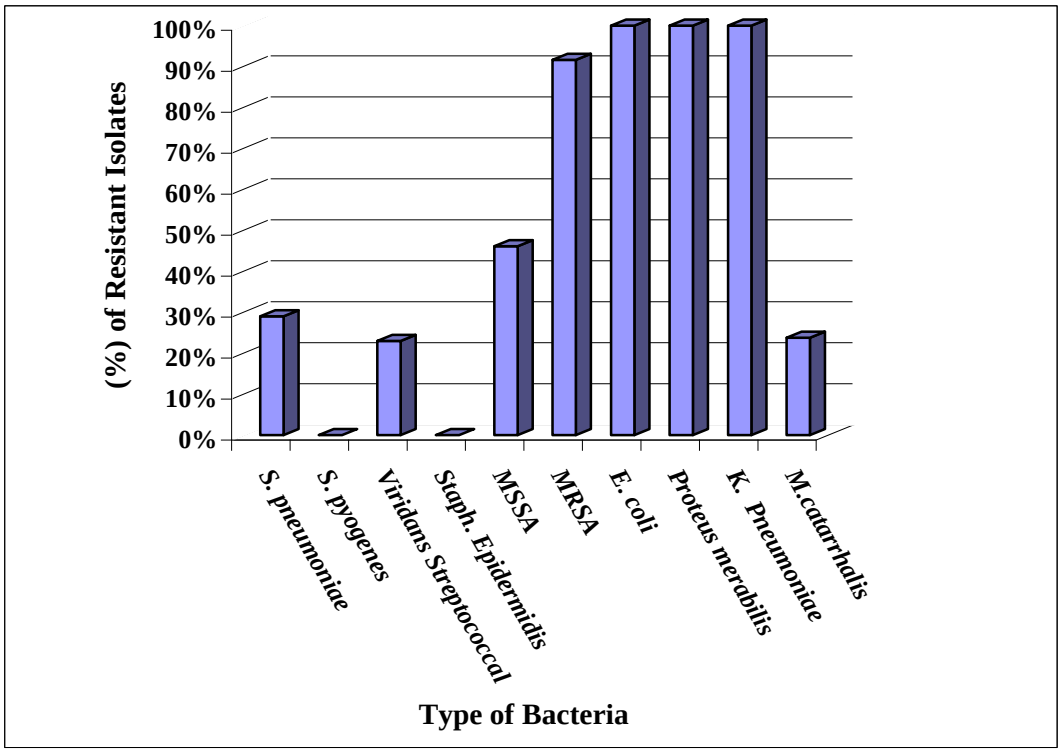

Figure ٣.١٣: The Ratio of Resistance of Bacterial Isolates Against Tetracycline.

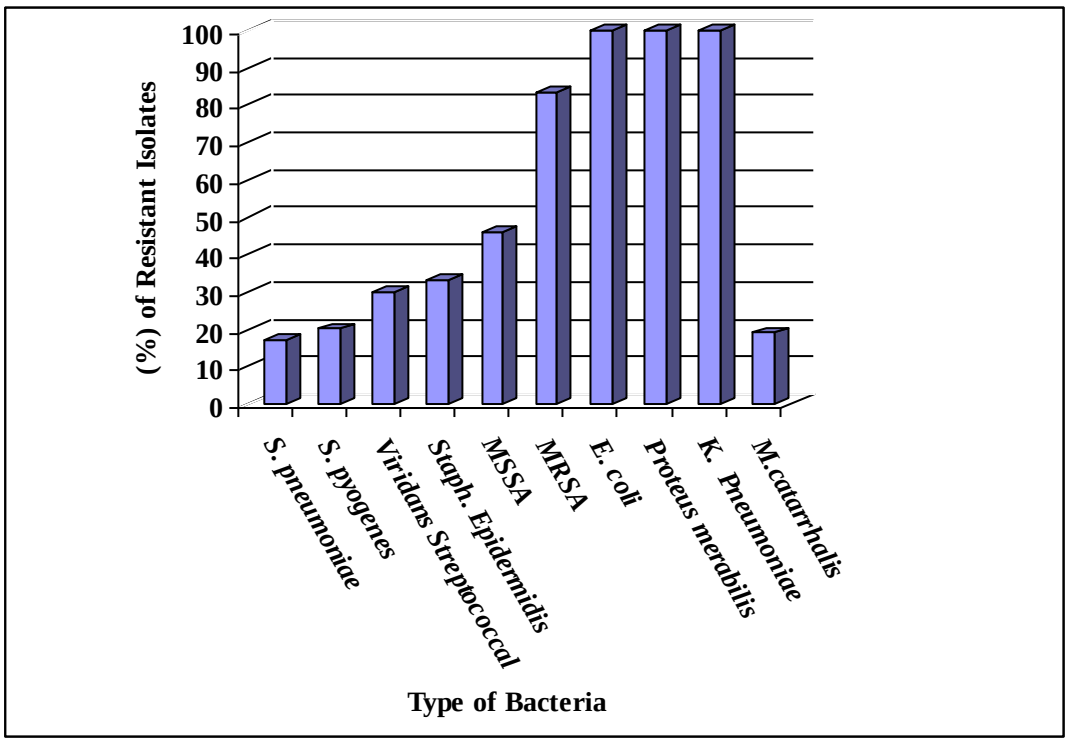

Figure ٣.١٤: The Ratio of Resistant of Bacterial Isolates Against Erythromycin

Conclusions & Recommendations

Conclusions:-

According to this study, the following could be concluded :

١- ٢٠-٢٩ years old persons are more suffering form acute and chronic sinusitis than other age groups.

٢- Females are more exposed for acute and chronic sinusitis.

٣- Nasal septal deviation and /or nasal polyps are predisposing for chronic sinusitis.

٤-Climatic allergens, food allergens, environmental allergens and common cold are mostly resulting in sinusitis.

٥-The proportionally increase in Methicillin Resistance ***Staphylococcus aureus*** (MRSA) is a serious risk for sever nosocomial infections.

٦-The most common type of bacterial isolates which isolated from acute sinusitis which were ***S. pneumoniae*** followed by ***M. catarrhalis***, ***S. aureus***, ***S. epidermidis***, Viridans streptococci, ***K. pneumoniae*** and ***S. pyogenes*** .

٧- The most common type of bacterial isolates which isolated from chronic sinusitis which were ***S. aureus*** followed by ***S. pneumoniae***, ***M. catarrhalis K. pneumoniae***, ***E. coli***, Viridans streptococci and ***Proteus mirabilis***

Recommendations:-

According to this study, the following could be recommended:

١-For proper diagnosis of the microbial etiology of sinusitis, culturing of sinus specimens is quietly necessary.

٢-Properly picking up the nasal swabs or maxillary sinus aspiration for laboratory diagnosis of the causative agents.

٣-Managment of the local predisposing causes such as polyp or nasal septal diviation which affect the normal sinus physiology.

٤-Ciprofluxacin and cefotaxim can be recommended as antimicrobial therapy for sinusitis.

٥-Prospective studies can be conducted to detect the role of microorganisms in sinusitis complications, the physical characters of sinus secretions and organisms causes sinusitis.

٦-Immunological studies could also be conducted to detect the level of serum antibody like IgA and IgG .

Appendices

Case Sheet Used in This Study

Pt. name	Sex:	Age	Address		date
Preliminary clinical diagnosis					
Associated history	A. common cold	b. Allergy	c-Polyp	D. Nasal septal deviation	E. Dental problem
F. Diabetes mellitus	J. Per orbital pain	H. Fever	I. Nasal obstruction	X ray finding and CT- Scan	
Type of sample	A. Nasal swab	B. Sinus antral wash			
Notes:-					

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سيفوتاكسيم و سيفالوثين و سيبروفلوكساسين و اريثروميسين و تيتراسيكلين و فانكوميسين و ترميثوبريم) للعزلات البكتيرية أن المضادين الحيويين سبروفلوكساسين و سيفوتاكسيم كانا الأكثر تأثيرا ضد العزلات البكتيرية المختبرة.

تم خلال هذا البحث جمع و دراسة ١٠٠ عينه (مسحات و غسيل الجيوب الانفية) من ١٠٠ مريض (٦٥ منهم إناث و ٣٥ ذكور) مصابين بالتهاب الجيوب الانفية (حسب التشخيص ألسريري الاولي من قبل اختصاصي الأنف والأذن والحنجرة) وممن أحيلوا إلى مستشفى الحلة التعليمي العام والعيادات ألتخصصية خلال فترة ١٠اشهر (من تشرين الأول ٢٠٠٤ إلى تموز ٢٠٠٥) . تراوحت أعمار المرضى الذين شملتهم الدراسة من ١٠-٦٠ سنه كان من بينهم ٥٨ حاله مرضية حادة و٤٢ حاله مزمنة لالتهاب الجيوب الانفية .

اظهرت النتائج أن أكثر فئة عمرية تعرضا لالتهاب الجيوب الانفية هي الفئة ألعمريه من ٢٠ إلى ٢٩ سنه وان النساء أكثر عرضه واستجابة للمرض مقارنه بالرجال.

اظهر ٥٠ مريضا (٨٦,٢%) ممن كانوا يعانون من الالتهاب الحاد نتائج موجبة اتجاه الزرع البكتيري مقابل نتائج سالبة ل ٨ مرضى (١٣,٨%) في حين اظهر ٣٥ مريضا (٨٣,٣%) من بين الحالات المزمنة نتائج موجبة للزرع البكتيري مقابل نتائج سالبة تمثلت ب ٧مرضى (١٦,٧%) .

الأنواع البكتيرية التي تم عزلها وتشخيصها خلال هذه الدراسة من الحالات ألمرضية إلحادة شملت أنواعا بكتيرية موجبة وسالبة لصبغة غرام جاءت في مقدمتها بكتريا ***Streptococcus pneumoniae*** حيث شكلت نسبة مئوية مقدارها ٢٥%، جاءت بعدها بكتريا ***Moraxella catarrahalis*** (١٨%) ، ثم ***Staphylococcus aureus*** (١٦،٧%) ، ثم Coagulase-negative Staphylococci(١٤%) ، ثم Viridians streptococci (١٢%) ، ثم ***Klepsiella pneumoniae*** (٨%) ، وأخيرا ***Streptococcus pyogenes*** (٦%).

أما في الحالات المرضية المزمنة فقد جاءت في مقدمتها بكتريا ***Staphylococcus aureus*** (٤٢،٨%) تلتها ***Streptococcus pneumoniae*** (٢٣،٤%) ، ثم ***Moraxella catarrhalis*** (١٠،٧%) ، ثم ***Klepsiella pneumoniae*** (٩%)، ثم ***E. coli*** (٥،٣%) ، ثم Viridians streptococci (٥،٣) ، وأخيرا ***Proteus mirabilis*** (٣،٥%) .

كان النوع المقاوم للمضاد الحيوي المشيلين من بين عزلات العنقوديات الذهبية Methicillin Resistance Staphylococcus aureus (MRSA) في كلا الحالتين ألحادة والمزمنة ، فقد مثل نسبة مئوية مقدارها ١٤% و ٣٧% على التوالي.

اظهرت نتائج اختبار فحص الحساسية للمضادات (امبيسيلين و اموكسيسيلين و جنتاميسين و

جامعة بابل
كلية الطب

دراسة بكتريولوجيه لالتهاب الجيوب الانفية في محافظة بابل

رسالة

مقدمة الى مجلس كلية الطب/جامعة بابل

كجزء من متطلبات نيل درجة الماجستير

في علم الأحياء المجهرية الطبية

من قبل

تيسير صفاء

ذو الحجة١٤٢٧ **٢٠٠٧ كانون الثاني**