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The staphylococci are among the most important bacteria which may cause disease in man. It is gram positive, non motile, non sporulating non capsulated spherical cells 0.8 to 1.0 um in diameter. It devides in two planes to form irregular grape like clusters (G.K. Staphylos means a bunch of Grapes) (Steewart, 1968).

Staphylocococci are responsible for over 80% of the suppurative diseases encountered in medical practice (Zinsser, 1976). As many as 10% of clean surgical wounds may become infected with staphylocococci (Briody, 1974).

It is well known that staphylocococci exhibit drug resistance more than other bacteria. The emergence of drug resistant strains often follows the wide spread theraputic use of antibiotics.

Penicillin was the first antibiotic to be used and its resistance appeared shortly after its introduction. The propertion of resistant strains encountered has continously increased (Munch-Peterson, et al., 1962).

Briody (1974) stated that penicillin was the drug of choice in treatment of staphylococcal infections because of excellent activity against Gram-positive cocci and remarkable freedom from dose related toxicity but more than 80%of hospital

infections and about 40% of community infections were caused by penicillin resistant organism.

Dyke, et.al.,(1966)stated that penicillin resistant strains of staphylococcus aureus (S.aureus), which became prevalent after 1945 were resistant to the antibacterial action of penicillin because they form penicillinase enzyme which attacks the B-lactam ring of the penicillin molecule.

The aim of the present work is to assess the sensitivity of the staphylococcus aureus isolated from wounds, abscesses, burns and nasal swabs to penicillin and other different antibiotics in order to (1) isolute the penicillin resistant strains and detection of penicillinase producing organisms by a rapid filter paper acidometric test using bromocresol purple as PH indicator and compare with penicillin disc diffusion method.

(2) Antibiogram pattern of the isolated staphylococcus aureus.

The results obtained might direct our choice to the proper line of therapy and affect our planning for future antibiolic policy.

REVIEW OF LITERATURE

CLASSIFICATION

Staphylococci were classified by Rosenbach (1884) according to pigment production into staphylococcus aureus forming a golden yellow pigment, and staphylococcus albus forming white pigment. The lemon coloured type : Staphylococcus citrus was later added by Passet (1885). The production of pigment by staphylococci formed the basis of much of the earlior classification, golden pigment being considered a property of pathogenic strains whilst the white strains were thought either to be less pathogenic or non pathogenic. Later work however, has shown pigment production to be an unreliable criterion for pathogenicity that has frequently led to confusion and mis understanding (Fairbrother, 1940, Williams Smith, 1959).

The previous method of classification is generally considered to be unsatisfactory, the main objection is that pigment production is not a constant characteristic but is dependen on a number of variable factors such as temperature of incubation, atmosphric condition and the nature of the medium, also there are considerable variations in the degree of pigmentation and a certain amount of objective difficulty is sometimes experienced in assessing the exact

colour of the pigment produced by some strains (Williams Smith, 1959).

Coagulase test constitutes an important criterion for classification and it is suggested that the genus staphylococcus should be primarily subdivided on this basis into coagulase positive and coagulase negative strains (Fairbrother, 1940), or staphylococcus aureus and staphylococcus epidermidis (Cowan and Steel, 1964).

Coagulase - Positive strains are pathogenic, usually ferment mannite, may produce aureus or albus pigment, form soluble haemolysins and should be designated staphylococcus pyogenes, coagulase - negative strains are non pathogenic usually non-mannitol fermenters, tend to form albus or citrus pigments, do not produce soluble haemolysins and should be designated staphylococcus saprophyticus (Fairbrother, 1940).

Baird-Parker (1963 - 1965) classified staphylococci into 6 subgroups. Staphylococcus aureus belonges to subgroup I, while the coagulase - negative strains belong to subgroups 2, 3, 4, 5, and 6. Table (1).

More recently Baird - Parker seperated staphylococcus aureus from staphylococcus epidermidis and classified the

latter in 4 subgroups (Topley and Wilson, 1975).

Strains of staphylococcus aureus can also be divided into a number of different types by a serological method which depends on their possession of different combinations of type specific surface antigens that are demonstrable in slide agglutination tests with absorbed anti sera (Cruick-shank, 1978).

Table (1) :

Classification according to Baird-Parker, 1963.	Original Classification
Subgroup I	S. aureus
II	S. albus, S.epidermidis, Saprophyticus, M-auran-tiacus, M. Violagabriella.
III	M. hyicus (S. Lactis)
IV	S.epidermidis
V	saprophyticus, M. Ceraly-ticus.
VI	S. albus, S. epidermidis, S. saprophyticus.

MORPHOLOGY AND STAINING PROPERTIES

Recently, Kloos and his colleague, (1985) characterized staphylococci as they are Gram-positive cocci(0.5 to 1.5 micrometer in diameter) that occur singly and in pairs, and irregular grape-like clusters. They are non motile, non sporeforming, and usually catalase positive and unencapsulated. Most species are facultative anacrobes. Their most obvious morphological characteristic is their marked tendency to occur as masses in grape like clusters. This happens as a consequence of the geometry of cell division. Cell division take place in successive perpenidcular planes, but there is incomplete seperation of the doughter cells and instead there is residual attachment along the division planes. The attachment point is usually eccentric to the division plane, resulting in irregular aggregates (Tzagaloff, 1977). Although clusters usually seen in pathogenic material and in growth on solid media, staphylococci may form short chains in liquid media (Cruckshank, 1975). Staphylococci stain readily and deeply with the usual basic dyes as the gram stain and are strongly gram-positive(Topley and Wilson's 1964).

Whether or not staphylococci form capsules has long been a matter of controversy. As early as 1931 Griffith described an encapsulated variants of staphylococcus aureus. It is now clear that mucoid variants of staphylococcus aureus that produce capsules which are readily visualized in early broth cultures by the india ink technic are relatively uncommon. Antisera to such encapsulated variants react with the homologous capsular antigen causing a quellung reaction (Davis, 1970).

Culture Characters

The staphylococci are facultative, anaerobes, but more a bundant growth is obtained under aerobic condition. Some strains also require an increased Co_2 tension. Growth occur over a wide temperature range 6.5 to 46 °C with an optimam of staphyloccus aureus of 30 - 37 °C, the optimum PH range is 7.0 to 7.5 for growth on chemically defined media, staphylococci require a number of amino acids and vitamins. Under anaerobic conditions uracil and a fermentable carbon source are also required (Zinsser, 1980)

Staphylococcus aureus grows readily on most routinly used labaratory media such as nutrient agar, trypticase soyagar, milk agar, blood agar and macConkey's agar.

On nutrient agar, colonies after aerobic incubation at 37 °C for 24 hours are 2 - 3 mm in diameter, have a mooth glistening surface, entire edage, firm consistancy and an opaque pigmented appearance. In most strains the pigmentation is golden, but in a few it is white. Colonies are smaller and pigmentation is absent on plates incubated anaerobically (Cruickshank , 1975).

On blood agar a zone of B-haemolysis sourrounds colonies of organisms that produce soluble haemolysins. Sheep blood is recommended for primary isolation from clinical materials. In the preparation of blood agar, human blood should not be used because of presence of non specific inhibitor or antibodies in blood that interfere with the development of staphylococcal colonies (Zinsser, 1976).

On milk agar, colonies as on nutrient agar but more intensely pigmented with clearer distinction between orange, yellow and cream buff strains. Zones of clearing around colonies due to digestion of heat coagulated casein by staphylococcal proteases (Cruickshank, 1975).

On macConkey's agar, colonies 0.1 to 0.5 mm,opaque, pigment often marked which may mask the pink of lactose fermentating strains (Stokes, 1980).

In broth uniform turbidity with some powdery deposits occur. Staphylococcus aureus also liquify gelatine stap from top after 5 days at 22 °C (Cruickshank, 1975).

Selective culture media, the majority of coagulase positive pathogenic staphylococci are able to grow in the

Presence of tellurite, reduction of tellurite occurs to give graysh black colonies on such medium. Most strains of staphylococcus aureus produce extracellalar deoxyribonuclease that may be detected by culture on D.N.A. containing medium. Also staphylococcus aureus produce alipase which results in a zone of opacity around colonies growing on egg agar. Lipase activity is most pronounced in strains of human origin grown under anaerobic condition (Freeman, 1979).

BIOCHEMICAL REACTIONS

The biochemical activities of staphylococci vary greatly from strain to strain, and there is no clear cut lines of demarcation between different groups of staphylococci or even between the staphylococci and micrococci. All staphylococci and many micrococci form acid from glucose aerobically, but the ability to do this anaerobically is now considered to be the most distincitive character of the staphylocicci (Topley and Wilson, 1975).

A wide range of sugars and other carbohydrates is utilized by staphylococci. Glucose, lactose, and mannitol are energy sources for staphylococci anaerobically. These sugars are also metabolized aerobically and so are other hexoses, pentoses and disaccharides (Braude et al., 1981). Under aerobic conditions, the major product of glucose dissimination is acetic acid with small amount of Co_2. Under anaerobic conditions, lactic acid is the principle product, and acetone is usually produced. The fermentation of mannitol by most strains of staphylococcus aureus is helpful in its differentation from staphylococcus epidermidis (Zinsser, 1980).

Staphylococcus aureus can grow in concentration of sodium chloride that are inhibitory to other microorganisms. Also catalase is produced by aerobically by grown cells. (Zinsser, 1980).

Energy is obtained via both respiratory and fermentative pathways . Intact pathways for glycolysis, the pentose phosphate and cetric acid cycles, are operative under appropiate growth conditions of both high and low-oxidation - reduction potential, this an obvious advantage to the organisms in its battle for survival in its natural habitate on mucosal surfaces and in competition with other bacterial species in the mixed microflora at the site of infection (Zinsser, 1976).

ANTIGENIC STRUCTURE

The phagocytic response of the host is a crucial factor in determining the initiation and out come of staphylococcal infections. In this process of host recognition and immunity, the cellular antigens of the staphylococcal cell especially the more superficial surface ones, are major determinants. The antigenic structure of staphylococcus aureus is very complex and more than 30 antigens were observed, but few have been well characterized (Zinsser, 1980).

Cellular antigens :

Species - specific polysaccharides A and antigens are two cell wall antigens which determine the immunological specificites of staphylococcus aureus and staphylococcus albus respectivly. Both were first isolated and identified as phosphorus - containing polysaccharides by (Julianelle and Wieghard, 1935). The first one extracted from pathogenic (aureus) strains, was designated the A polysaccharide. The second derived from non pathogenic (albus) strains, was termed B polysaccharide (Davis et al., 1970).

Polysaccharide A, a major antigenic determinant of all strains of staphylococcus aureus is the group specific polysaccharide A of the cell wall. The serological determinant of this polysaccharide is the N-acetyl glucosaminly ribitol unit of teichoic acid, specificity resides in the ∝ or B configuration of the glucosaminyl substituents. Polysaccharide A occurs with the mucopeptide in the cell wall in an insoluble state and requires lytic enzymes for release. Most adults have a cutaneous hypersensitivity reaction of the immediate type to polysaccharide A, and low levels of precipitating antibodies are found in their sera. The specific polysaccharide is not found in the non pathogenic staphylococcus albus, which contains instead glycerol teichoic acid with glucosyl residues rather than ribitol teichoic acid (Zinsser, 1976).

Protein A : A cell wall protein of staphylococcus aureus was described by (Verwey, 1940) and later designated protein A. Cells containing protein A are agglutinating by normal human serum. Protein A has the property of combining with the Fc protion of human immunoglobulin G and thus can be quantitated.

Protein A on the bacterial cell surface inhibits phagocytosis and is present in most coagulase positive strains of staphylococcus aureus. It is of some interest that the immunoglobulin G-protein A complex reacts with complement and and can thereby, deplete complement from serum. Such complement depleted sera can no longer potentiate antibody neutralization of herpes virues. By this mechanism, staphylococcal disease may bring about lowered host defenses against other microbial invaders (Freeman, 1979).

Capsular antigens :

Although staphylococci are rarely encapsulated,a few strains have been isolated that carry immunologically significant surface antigen. The capsular antigen found only in mucoid untypable strains of staphylococcus aureus that lack bound coagulase (clumbing factor)(Zinsser, 1976). like purified pneumococcal polysaccharide the capsular substance is a poor immunogen in rabbits, but does induce a protective immunity in mice. In solution it blocks, the agglutinating and opsonizing action of serum from animals immunized with intact cells, and on the organisms surface it exerts an antiphagocytic effect (Davis, 1970).

BACTERIOPHAGE - TYPES

Bacteriophages are viruses that attack bacteria, and in certain instances dissolve the parasitized bacterial cell. The action of bacteriophage is specific that is only certain phage or group of phages affects the given strain of bacteria. It has been found that specific bacterophages (Staphylophages) react with about 60% of coagulase positive strains. Coagulase-negative strains of staphylococci are not susceptible. Staphylococcus aureus can be classified into phage lytic groups as follows (Smith, 1976).

Lytic group	Phages in group
I	29, 52, 52 A, 79 and 80.
II	3A, 3B, 3C, 55 and 71.
III	6, 7, 42E, 47, 53, 54,75,77 and 83A.
IV	42 D
Not allotted	81 and 187.

Each phage group has some specificity in pathological lesion e.g. group I strains produce localized primary skin sepsis such as boil, carbuncles and styes. Group II strains

are often associated with spreading infection of the skin (Lacey, 1975). In other strains the production of enterotoxin is confirmed to phage group III and IV (Zinsser, 1976).

The appearance of new strains of staphylococcus aureus in hospital has been observed on a number of occasions in the last 30 years. Several of these strains were initailly recognized by their insusceptibility to lysis by phages of the current basic typing set. Their untypability was usually a result of prophage immunity. The frequency with which laysogenization occurs in staphylococcal strains that are endemic in hospitals may be attributed to the instability of the nasal flora in hospital patients (Topley and Wilson's, 1984).

Phage typing is not a static system. New strains that do not fit a particular pattern emerge frequently. Thus, the bacteriophages used in a standard typing set very over the years (Rose, 1983).

"ANTIBIOTIC SENSTRIVITY"

Staphylococci in general are sensitive to many antibiotics such as benzyl penicillin, cloxacillin, cephalosporins, tetracycline, chloramphenicol, erythromycin, fucidin, climdamycin, vancomycin, sterptomycin and gentamycin. Staphylococci are poorly susceptible to sulphonamides, except when sulphonamide is used in combination with trimethorprim (Duguid et al., 1978).

Staphylocci were among the first organisms recognized to hav the ability to resist antimicrobial therapy (Suchberget al., 1985), at the highest concentrations that can safely be achieved in the patient tissues, (Duguid et al., 1987).

Panicillin was the first antibiotic to be used, and penicillin resistance appeared shortly after its introduction depending upon the geographic area, 80 percent or more of strains occuring in the population are now penicillin resistant. Because of the prevalence of penicillin resistant strains in the general community is now as high as it is in the hospital, penicillin resistance must be presumed for all staphylococcal infections untile

sensitivity testing is performed. Resistance is due to the plasmid-mediated enzyme penicillinase (Lacey, 1975) which is a B-lactamase that splits the B lactam ring of the penicillin nucleus (Lacey , 1975).

In sever staphylococcal disease, unless the strain is sensitive to penicillin, penicillinase - resistant penicillins such as oxacellin, and methicillin are the drugs of choice. In patient allergic to penicillin, cephalosporin derivatives may be used. Combined therapy with an aminoglycoside such as gentamycin is often employed, but there is no clear evidence that such combinations are more effective than the use of the bacteriocidal penicillins or cephalosporins alone - Vancomycin is used in patients who are allergic to the penicillin and cephalosporins. Methicillin-resistant staphylococci are resistant to all anti-staphylococcal penicillins and frequently to multiple drugs, with varing susceptibilities to cephalosporins and aminoglycosides . Vancomycin is the treatment of choice for serious infections with methicillin - resistant staphylococci (Braude, 1986).

Tolerance is the term used to describe strains of staphylococci with a marked discrepancy between the concentration

of an antibiotic necessary to inhibit their growth and the concentration necessary to kill them. Mean inhibitory concentration for tolerant strains are similar to those for sensitive strains but the mean bactericidal concentration are (8 to 100) fold higher. These strains of staphylococci are usually tolerant to all antistaphylococcal penicillin and cephalosporins, the mechanism of tolerance seems to be a deficiency of the autolytic enzyme necessary to complete the destruction of the bacterial cell. (Braude, 1986) .

Superficial staphylococcal abscesses generally don't require antimicrobical therapy. Local application of moist heat, immobilization, incision and drainage usually suffice. In both serious and minor staphylococcal disease, infected foci such as foreign bodies or necrotic bone or tissue must be removed (Braude, 1986).

Resistance is caused by genetically controlled peculiarities of the metabolism or structure of the cell which enable it to escape the action of the drug. Among the biochemical changes which have been found in bacterial mutants selected for resistance are :

1- Increased destruction of the inhibitor :

2- Decreased permeability of the organism to the drug.

(3) Increased synthesis of an essential metabolite or drugs antagonist.

(4) Changes in the properties of enzymes, resulting in a different relative affinity of substrate and antagonist (Zinsser, 1976).

The aspect of genetic location is important, because many of the properties and products of S. aureus are genetically controlled by plasmids. plasmid regulation of antibiotic resistance, especially through the production of penicillinase is of profound clinical importance (Lacey, 1975). Penicillinase plasmids don't tend to carry other antibiotic resistance markers, with the exception of erythromycin. Hence, multiple drug resistance mediated by plasmids is unusual in S. aureus.There appear to be two molecular classes of staphylococcal resistance plasmids. The larger of those has a molecular weight of 20 X 10^6, may be isolated as a closed ring molecule, and its replication appears to depend on the cell division so that only one copy occurs in each cell. The other type is much smaller and has a molecular weight in the range of 3 x 10^6. The smaller type may carry resistance determinants for tetracycline or chloramphenicol and multibly independenty of cell division so that multiple copies are

produced in each cell (Braude, 1986).

Penicillin sensitive strains of S.aureus never mutate to become penicillinase producer, though sensitive strain can readily mutate into forms that are resistant to streptomycin, erythromycin, fucidine or novobiocin, and rarely into forms resistant to tetracycline or chloramphenicol. The multi resistant hospital staphylococci have propably arisen by a succession of mutations conferring resistance to an antibiotic may be transfered from a resistant to sensitive strain by phage transduction and such transferes may have contributed to the acquisition of resistance by some hospital strains (Cruickshank , 1978).

IMMUNITY

Most staphylococci appear to be killed once they are ingested by polymorphnuclear neutrophils, although a few may survive under experimental conditions. There is evidence that cell mediated immunity may play a role in host defence against staphylococcal infection (David. J. Doutz, 1976).

The granulocyte is responsible for the resistance against staphylococcal infection, once the organisms have penetrated the skin or mucous mambranes, mobile phagocytes migrate into the area in response to the stimulus of chemotactic factors (Zinsser, 1980). Once the organism has become attached to the cell membrane, the cell form pseudopodia around it (Wilkinson, 1977), the opposed membranes of the extended pseudopodia fuse and then invaginate, enclosing the microbe in an internalized vacuole, called a phagosome (Laurence and Robert, 1981). Through this process neutrophils undergo an increase in oxygen consumption, the production of hydrogen peroxide and an increase in glucose oxidation via the hexose monophosphate shunt, that result in what has been called the respiratory burst (Babior, 1978).

During the respiratory burst of neutrophils all the oxygen is transferred to superoxide and 80% of this is

converted to hydrogen peroxide (Root and Metcaff, 1977). Hydrogen peroxide by its self has bacteriocidal properties but in the presence of myeloperoxidase the potency of this system is increased at least five folds. (Denson and Uandell, 1979). Myeloperoxidase is released into the phagosome during granule phagosome fusion (Klebanoff, 1970), it combines with its substrate hydrogen peroxide to form an enzyme substrate complex that can oxidise a variety of compounds resulting information of toxic agents that can attack the microorganism (Klebanoff, 1980).

Phagocytosis of non encapsulated strains of staphylococci is promoted by either complement or antibody. For efficient phagocytosis of the more resistant encapsulated strains, however both antibody and complement are required. In most normal individuals, as well as in many with staphylococcal infections, complement is the primary source of opsonic factors. Sepecific IG G antibodies are present in the serum of most individuals as result of subclinical infection with staphylococci but the titer of these antibodies is relatively low (Zinsser, 1980).

Absence of antibody or abnormalities of the metabolism of complement leading to poor opsonization and occasional causes of increased susceptibility to staphylococcal infection (Topley and Wilson's, 1984).

The close association of organism with man through out his life, however is well estabilished, as in production of chronic, or latent infection, an important aspect of this host-parasite relationship is the development of a delayed hypersensitivity to staphylococcal antigens, exaggerated hypersensitivity may interfer with immunity. The sucess that has been countered by many clinicians in the use of autogenous vaccines for the treatment of the patients with recurrent boils has been attributed to a hyposensitization of the patients who has an excessive amount of delayed hypersensitivity to staphylococcal products (Wolffgang and Hilda , 1976).

VIRULENCE FACTORS IN STAPHYLOCCOCCI

Staphylococci can produce disease both through their ability to multiply and spread widely in tissues, and thr-ough their production of many extracellular substances (enzymes and toxins), that have an important role in disease production (Jawetz et al., 1982). These include :

A. Extracellular enzymes :

* Coagulase :

In the diagnostic laboratory pathogenic staphylococci are distinguished from non pathogenic strains by their production of extracellular coagulase that clot plasma (Zinsser, 1976). Coagulase is the most important soluble enzyme of S. aureus that converts the fibrinogen in citrated human or rabbit plasma into fibrin aided by an activator in the plasma called coagulase reacting factor (Cruickshank, 1978). Coagulase positive S. aureus is the conclusive identifing test for pathogenic strains. Although the actions of coagulase are not firmly established, some reserchers believe that coagulase contributes to the pathogenicity of staphylococci (1) by inhibiting the bacteriocidal activity of normal serum, and or (2) by inhibiting phagocytosis, which result from deposition of fibrin on the bacterial cell

surface (Milgrom, 1982).

Staphylocoagulase occurs in two forms soluble or free coagulase and bound coagulase or clumbing factor (Cruickshank, 1978).

* Free coagulase, is protein in nature and readily inactivated by proteolytic enzymes. It is antigenic and at least four antigenically distrinct types can be identified (Freeman, 1979).
* Bound coagulase is not released, but occurs on the cell surface of staphylococci, when these cells are mixed with plasma they undergo clumping by vitrue of fibrin precipitiation on the cell surface. (Freeman, 1979).

* Lipases :

Staphylococci produce several lipid hydrolyzing enzymes collectively referred to as "Lipases ". The lipases are active on a variety of substrates, including plasma and the fats and oils that accumulate on the surface areas of the body. The production of lipase apparently is essential in the invasion of healthy cutaneous and subcutaneous tissues. The decreased virulence of hospital staphylococci observed during the last 20 to 30 years parallels a decrease in

staphylococcal isolates that produce large amount of this enzyme. The decrease apparently is due to the presence of a prophage that blocks lipase production (Zinsser, 1980).

x Hyaluronidase :

Nearly all strains of S.aureus form hyaluronidase, but in varying amount. A clear relation between the amount of the enzyme formed and potentialy pathogenicity has not been established (Parker, 1983).

This enzyme hydrolyzes the hyaluronic acid present in the intracellular ground substance of connective tissue, thereby facilitating the spread of infaction, since inflammation antagonizes the spreading action of hyalurinidase, its importance in staphylococcal infections is limited to the very early stages of infection. (Abramson, 1972).

x Nuclease :

This enzyme is present in 90% of coagulase-positive strains of staphylococci, and is absent from most coagulase-negative strains. It is a compact globular protein consisting of a single polypeptide chain. The nuclease is a phosphodiesterase with both endo-and exonucleolytic properties, which can cleave either DNA or RNA to produce 3

phosphomononucleotides. Antisera produced in rabbits against the staphylococcal nuclease inhibit completely its enzymatic activity (Zinsser, 1980).

* Staphylokinase :

This fibrinolytic enzyme, which is produced by pathogenic staphylococci is similar in activity to streptokinase, which is produced by streptococci. The enzyme dissolves fibrin clots by mediating the conversion of plasmin. Production of this enzyme is a phage-mediated property resulting from lysogenic conversion. It is interesting that strains lose the ability to produce haemolysin when they acquire the capacity to produce staphylokinase. There is little evidence that this enzyme plays ascingificant role in pathogenicity (Milgrom, 1982).

* Phosphatase :

Phosphatase is an extracellular enzyme not related to pathogenicity. It is produced primarily by species found in phage group 1, particulary types 80 / 81. It is produced by coagulase-positive and coagulase negative strains (Robert, 1980)

B. Toxins Production :

Pathogenic staphylococci release a number of different exotoxins, their production in broth cultures is stimulated in an atmosphere of 30% carbon dioxide. The toxins most commonly elaborated include four immunologically distinct hemolysins, a non hemolytic leucocidin, enterotoxin (Davis, 1970), and epidermolytic toxin (Zinsser, 1980).

* Hemolysins :

The hemolysins of S.aureus are of interest and includes:

* Alpha hemolysin :

Pure alpha toxin has a sedimentation coefficient of 3.0 and a molecular weight of about 28,000. It consists of four different conformational forms, separable by electrophoresis. This toxin exhibits a wide range of biologic activities, including the hemolytic, lethal, and dermonecrotic effects observed following the injection of broth culture filtrates of certain strains of the organism (Zinsser,1976).

* Beta hemolysin :

Beta hemolysin is a protein having a molecular weight of 1 to 5 X 10^4, it is found in more than 90% of coagulase positive animals strains. Its enzymatic activity is related

to the sphingomyelin content of the erythrocytic membrane. Sheep erythrocytes are the most susceptible to the hemolysin (Robert, 1980). Beta hemolysin is a hot-cold hemolysin, i.e., hemolysis is increased if incubation at 37C° is followed by holding at lower temperatures, (Stored over night in refrigerator), (Frobisher and Fuersts, 1978), this mechanism is not fully understood (Robert, 1980).

* Gama hemolysin :

This hemolysin cause rapid lysis of the red blood corpuscles of the rabbit and sheep but not of the horse (Smith and Price, 1938). It is a protein non toxic for mice but large doses kill guinea pigs, it has some leucocidal action but is not dermonecrotic (Fackrell and Wiseman, 1976).

* Delta hemolysin :

Delta toxin is a small cytolytic polypeoptide produced and secreted by S.aureus and belongs to the family of surface active toxins that exhibit pronounced effects on a wide variety of cellular membrances. Although this class of proteins has been much studied by a wide variety of physical techinques, no consensus has been reached on their mode of action (Thomas, 1986).

* Leukocidin :

The panton-Valentin leukocidin (named after two of the orginal investigators) exhibit activity only against leukocytes. It is a protein composed of two units, F and S, which together have a molecular weight of 30,000 (Robert, 1980). It has no effect on erythrocyte and lymphocytes (Rose, 1983). Antibodies to leukocidin may play a role in resistance to recurrent staphylococcal infections (Jawetz et al., 1980).

* Enterotoxins :

Most entero toxigenic strains of staphylococci are coagulase positive, and belong to phage group III. These toxins are a major cause of food poisoning in humans. Disease caused by staphylococcal enterotoxins is characterized by sudden onset of nausea, vomitting, diarrhea and shock occuring within a few hours of ingesting contaminated food (Rose, 1983). The emetic effect of enterotoxin is probably the result of central nervous system stimulation after the toxin acts on neural receptors in the gut (Jawetz et al., 1980). Enterotoxins A and D are most frequently associated with staphylococcal food poisoning, and enterotoxin

B is the toxin most likely to be associated with hospital infection (Zinsser, 1980).

* Epidermolytic toxin (Exfoliative toxin) :

Exfoliative toxin is an extracellular product that cleaves the stratum granulousm layers of the epidermis and causes several clinical syndromes (Youmans, 1975). According to Parker and his colleagues (1955) Parker, (1959) most of the strains responsible belonged to phage-group II. Later reports, supported by laboratory tests for specific toxin, suggested that about one quarter of the strains did not belong to phage-group II and had a variety of other phage-typing patterns (Arbuthott, 1981). Infections with these organism in infants and young children are associated with generalized exfoliation (Ritter's syndrome). Localized bulous, impetigo, and generalized scarlatiniform eruption. The data suggest that exfoliative toxin is the cause of the skin changes in affected children (Briod, 1974).

STAPHYLOCOCCAL INFECTIONS

Staphylococci are normal inhibitants of the skin, mouth, throat and nose of man. They live in these areas without effect, but once passed the barrier of the skin and mucous, they can cause extensive disease (Smith,1976). The character of these infections is attributable, at least in part, to the properties of toxic products of the micro organism (Freeman, 1979). The pathogenesis of infection is associated with the formation of coagulase, alpha and gamma lysins. The tendency of the focus of infection to be walled off can be attributed to the action of coagulase, and the purulent character of the lesion, to the necrotic ability and killing of leucocytes by leucocidin and by the alpha and gamma toxins (Foster et al., 1963). The presence of protein A on the surface of the bacteria interfere with phagocytosis. Even after phagocytosis, coagulase positive staphylococci tend to persist in a viable form (Koening, 1972). The development of metastatic abscess occur by hemotogenous spread of the infection in the form of thromi and phagocytic cell containing viable micro organisms (Kloos, 1976).

Factors Predisposing to Infection :

Since the skin is an excellent barrier to bacterial infection, it is not surprising that invasion of the skin and subcutaneous tissues by staphyloccocci occurs most commonly when the continuity of the epithelium is disrupted (Elek, 1956). Invasive staphylococcal infections most often occur as complications of accidental and operative trauma and burns (Frobisher, 1978), skin areas affected by various cutaneous viral infections, and exfoliative dermatitis are particulary prone to become infected by staphylococci (Cluff et al., 1968, Colebrook, Duncen and Ross, 1948). Certain viral infection, for example, the sever tracheobronchitis produced by influenza viruses permits invasion of the lower resparitory tract by staphylococci and other bacteria (Louria et al., 1959).

Another group of individuals especially susceptible to serious staphylococcal infection consists of those with disorders of chemotoxis, phagocytosis, and intracellular bacterial killing by polymorphonulear leucocytes (Pabst et al., 1971). In Job's syndrome, in which recurrent, sever "Cold" abscesses and chronic eczema are associated with a very high serum IgE caused by S-aureus.

The leucocytes of these patients exhibit depressed leucotaxic response. (Hill et al., 1974). The "Lazy leucocytes syndrome " in which the polymorphonuclear leucocytes are in capable of responding to chemotaxis or phagocytosis, is also associated with recurrent Bacterial infections, especially those produced by staphylococcus aureus (Steermen et al., 1971, Miller and Schonaue, 1971).

Opsonins, including antibacterial antibodies and complement components, increase the efficiency of staphylococcal phagocytosis. Deficiencies in immunoglobulins or complement components (Primarily C_3) as a result of defective production, incomplete activity, hyper catabolism, or excessive protein loss increase the risk and severity of staphylococcal infections (Rose, 1983).

General systemic depressants of antibacterial resistance, such as dietary deficienies, metabolic disturbances, injections of bacterial endotoxins, and anaphylactic reactions have also been shown experimentally to in crease susceptibitity to staphylococcal disease (Frobishir and Fuersts, 1978).

The presence of foreign bodies in any site increases

susceptibility to staphylococcal infection. A large variety of foreign bodies, many iatrogenically introduced, hence been implicated, including sutures, plastic intravenous catheters. Vascular grafts or pacemakers, various types of prostheses used in cardiac, orthopedic , or ophthalmological surgury, and atirioventrcular plastic shunts employed to correct hydrocophulus (Elek, 1956 ; Bahnson, Spencer, and Bennett, 1957; Collins et al., 1968, Smits and Freeman, 1967).

Clinical Diseases :

Staphylococci produce two types of disease, invasive and toxigenic. The hallmark of invasive staphylococcal infection is abscess formation. Most often the abscesses are superficial (Furuncle), But in some cases the furuncle develop into barrowing lesions consisting of a number of interconnecting abscesses (Carbuncle) serious deep-seated disease usually is not seen in healthy people, But may occur in these debilitated by disease, malnutrition, extensive surgical procedure and immuno suppression (Braude, 1981).

I- Invasive Staphylococcal diseases :

1) Localized skin lesion :

Staphylococcal infection of the skin is the most common of all bacterial infection of the hair follicle. An extension into the subcutaneous tissue results in the formation of a focal suppurative lesion. The boil or furuncle. A carbuncle is similar to furuncle but has multiple foci and extends into the deeper layers of fibrous tissues.Carbuncle is limited to the neck and upper back where the skin is thick and elastic. (Zinsser, 1980).

Some individuals are subject to chronic furunculosis in which repeated attacks of boils are caused by the same phage type of S. aureus. There is little, if any, evidence of acquired immunity, indeed, delayed type hypersensitivity to staphylococcal products appears responsible for much of the information and necrosis that developed (Sherris,1984). In the development of the abscess tissue destruction at the site of inoculation is followed rapidly by hyperemia and vigorous accumulation of many polymorphonuclear leucocytes. The centre of the lesion soon becomes necrotic and a fibrin wall is formed at the site of intensive

hyperemia surrounding the lesion. The mature lesion consits of a fibrin wall surrounding by inflammed tissues and enclosing a central liquified core of pus containing staphylococci and leucocytes. As pus accumulates, it may drain towards the skin surface or into adjacent tissues, where it forms sinus tracts and secondary abscess. (Braude, 1986). Certain strains of S. aureus, especially of phage type 71, can cause bullous impetigo. A highly communicable superficial skin infection characterized by large blisters containing many staphylococci in the superficial layers of the skin (Sherris, 1984).

2) Systemic Infection :

Staphylococcus aureus may spread from a superficial lesion through the tissue planes, or along the lymphatics to the regional lymph Nodes, But it more often causes serious disease when it spreads by the blood stream (Topley and Wilson, 1984). Abscesses of the kidney result from deposition of staphylococci from the blood. They develop in the renal cortex, don't communicate with the renal collecting system (Topley, 1984). Osteomyelitic, septic arthritis, septic thrombophlebitis, and acute bacterial endocarditis are relatively common.

Liver, spleen, and pancreatic abscess are less common results of bacteremia. Hematogenous staphylococcal pneumonia is characterized by multiple metastatic foci in both lungs, and is incountered regulary in patients with endocarditis, focal infection of the organs, and in intravenous drug abusers (Braude, 1986).

Fulminating attacks of staphylococcal septecaemia may be associated with thrombocytopaenia, disseminated intravascular coagulation, glomerulonephritis and occasionally symmetrical peripheral gangrene (Rahal et al., 1968, Murray et al., 1977) Which may be attributable to activation of the alternative complement pathway (O'connor et al 1978).

Staphylococcus aureus is often present in considerable numbers in the urine in casses of staphylococcal septicaemia even in absence of renal abscess (Lee et al., 1978).

3) Staphylococcal Pneumonia :

Staphylococcus aureus may invade the lungs from the blood stream, givining rise to the formation of abscesses, more often it causes a primary pneumonia, this occurs mainly in the following classes of patient (1) young infants, especially when colonized by a particularly virulent

staphylococcal strains, (2) healthy young adults, secondary to influenzal infection, and (3) adults suffering from other serious diseases (Topley and Wilson, 1984). Staphylococcal pneumonia of infants tend to affect the premature or sickly, but epidemics may occur among groups of healthy new born infants in hospital, they were a feature of out breaks of sepsis due to staphylococci of the 52, 52A, 80 , 81 complex (Beavan and Burry 1956, Disney et al., 1956). The immediate mortality is high, and survivors may suffer from empyema, lung abscess or pneumocystocoele. In influenzal pneumonia appears to result from a massive invasion of the lung by strain of staphylococcus aureus carried by the victim and is a consequence of extensive damage to the mucosa of the lower respiratory tract by the virus. Post influenzal pneumonia is the main cause of death in healthy young people after influenza A infection (Slot, 1950, Oswald et al., 1958, Report, 1958).

4) Staphylococcal enterocolitis :

Staphylococcal enterocolitis is a serious disease characterized by diarrhea, dehydration, fever,nausea, abdominal pain, vomiting, and in some cases shock and

death. The disease is an infrequent but major hazard of broud-spectrum antibiotic therapy, often preceding major bowel surgery. The normal intestinal flora are inhibited by the antibiotics, but the growth of some staphylococci is unaffected because of their resistance. Under there circumstances, which favor the growth of staphylococci, pseudomembranous enterocolitis may develop (Briody,1974). Staphylococcal enterocolitis is to be distinguished from staphylococcal food poisoning, although it is propable that enterotoxins play a part in its pathogenesis (Freeman, 1979).

5)Urogenital Infections :

Cystitis and pyelonepharitis due to staphylococcus aureus occur rarely outside hospital, but may follow catheterization or operations on the bladder or prostate in hospital patient. They form only a very small proportion of hospital-acquired urinary tract infections but often have serious consequences, according to (Demuth, 1979), 15 per cent are complicated by bacteraemia, haematogenous spread to the kidney or perinephric tissues may occur in

pyaemic infections (Topley and Wilson, 1984).

6) Osteomyelitis and Pyoartheritis :

osteomyelitis is most frequently caused by staphylococcus aureus. It occur primarily in children under the age of 12 years and in most cases follows haematogenous spread from a primary focus usually a wound or furuncle. The organism localizes at the diaphysis of long bones. As the infection progresses, pus accumulates and emerges to the surface of the bone. When the infection occurs near a joint, staphylococcal pyoartheritis is a common complication. Pyoartheritis also may results directly from haemotogenous spread or by direct inoculation of staphylocci into the joint during intra articular injections. Staphylococcal joint infection destroys the articular cartilage and result in permenant joint deformity (Zinsser, 1976).

II - Toxinogenic Staphylococcal Diseases :

There are now three clinical syndromes in which the major manifestations are mediated by exotoxins instead of tissue invasion or bacteremia. These are staphylococcal food poisoning, the various forms of skin disease due to

epidermolytic toxin (Scaled skin syndrome), and the recently described toxic shock syndrome (Braude, 1986).

1) Food Poisoning :

Staphylococcal food poisoning results from production of staphylococcal enterotoxin in food before ingestion. It is an intoxication, not an infection (Sherris, 1984). Typically, staphylococcal food poisoning follows consumption of food such as custards, meats, pastries, or salad dressings that have been contaminated by enterotoxin producing organisms from a food handler. If the food is kept at a temperature that permits bacterial multiplication, enterotoxin production occurs. If the food are then heated, the organisms may be killed but the heat-stable enterotoxin persists, (Braude, 1981).

The illness is characterized by sudden onset, usually within 2 to 6 hours after consumption of toxin containing foods, and symptoms include nausea, vomiting and abdominal pain with diarrhea, fever does not occur. The mortality rate is very low and recovery usually is complete within 48 hours (Youmans, 1975).

2) Scalded Skin Syndrome:

It is characterized by stripping of the superficial layers of the skin from the underling tissues by the action of epidermolytic toxins. According to parker and his colleagues (1955, Parker, 1958), most of the strains responsible belonged to phage type II and were lysed only by phage 71.

In babies and young children, but very rarely in adults, the lesion may be more extensive and continous, affecting the skin of complete regions and sometimes of the whole body surface. This was described first by Ritter Von Rittershan (1878), hence the name Ritter's disease-and later by lyell (1956) as the "scalded skin syndrome" or toxic epidermal necrolysis (Lyell, 1969). The onset is adrupt, with generalized erythema closely resembling that of scarlet fever, within 1-2 days the skin become wrinkled and peels off on light stroking (Nikolsy sign). Then large flaccid bullae appear and extensive areas of the skin are exfoliated. There are signs of acute toxaemia, and death may occur unless the correct antimicribial treatment is given (Howells and Jones 1961), parker and Williams, 1961, lyell et al., 1969).

3) Toxic-Shock Syndrome :

Todd and his colleagues (1978) described under this name a characteristic syndrome of high fever, headache, confusion, conjunctival redding, subcutaneous oedema,vomiting and diarrhoea and profound hypotensive shock in older children and adolescents. In the more severe case, acute renal failure, disseminated intra vascular coagulation, peripheral gangrene, and even death sometimes occured.

Toxic shock syndrome is a serious, recently recognized disease, associated with staphylococcus aureus. It is most common in young women during of immediately after menstruation and has been associated with the use of highly absorbent intravaginal tampons (Sherris, 1984).

Staphylococci isolated from patients with toxic shock syndrome have been universally penicillin resistant and likely to belong to phage group I (Braude, 1986).

HOSPITAL INFECTION

Staphyloceccus aureus remains an important cause of hospital acquried infection, accounting for 15-20 percent of all nosocomial infections (Cressely et al., 1979). The problem of hospital staphylococcal infection begins with selection outside the hospital environment and ingreatly exagerated by conditions within the hospital (Briody, 1974). The source of infection is a patient or a number of the hospital personnel with a staphylococcal lesion. Patients with lesions draining pus externally are dangerous to others because of their ability to disseminate organisms by contamination of the environment. The rare documented cases of hospital personnel with mild staphylococcal lesions, such as furncles, paronychia or styes who are served as the source of epidemics. An infected surgeon is common source of infection among surgical patients and newborn infants (Zinsser, 1980).

Another source of infection is healthy carriers, they are a major source of infection in hospital, and many epidemics result from such cross infections. Studies have shown that nasal carriers have a greater chance of post

operative sepsis than non carrier. Within the hospital population the strains that appear in infection carry a large number of antibiotics - resistant plasmids that are lost when the patient leaves the hospital (Robert, 1980).

In the hospital environment, many factors contributing to hospital - acquired infection, the wide use of antibiotics which are generally bacteriestatic, not bacteriocid, staphylococci well endowed for survival and consequently antibiotics - resistant strains have developed, complicated surgical techniques with a greater exposure of tissues at operation for a longer period of time than ever before, valuable drugs, such as the corticosteroids, depress the patient's resistance to infection (Smith,1976). The wide spread unsatisfactory aseptic techniques lead to staphylococcal infection with many phagetype, in aspetic techniques, infections are usually associated with a single phage type, although these infections may be traced to a single nasal carrier, an out break is generally associated with a single phage type that is widely distributed among the staff and the patients. Staphylococci of phage type 80/81, for example have been responsible for many

hospital epidemics (Briody, 1974). Despite the development and use of numerous antimicrobial agents, staphylococcal infections have remained a major problem in hospitals (Nahmias and Shulman, 1972), and dangerous cause of morbidity and mortality (Musher, 1977 and peacock et al.,1980).

Because of the frequency and seriousness of hospital acquired staphylococcal disease, control efforts have been concentrated. These consist of (1) good hygienic care and proper disposal of material contaminated with pus, (2) isolation of patients with staphylococcal diseases particulary these with open wounds or pneumonia, (3) barring contact of known carriers, with highly susceptible individuals, new born infants or compromised patients, (4) avoiding indiscriminate use of antibiotics, and (5) strick adherence to propor operating room procedures (Miligram,1982).

PENICILLINASE PRODUCTION BY STAPHYLOCOCCUS AUREUS

The first claims that an enzyme produced by bacteria able of destroying penicillin was put in (1940) by Abrahim and Chain. This fact drew the attention of workers on penicillin resistance to search for this penicillinase in both sensitive and in vitro resistant strains of staphylococci, but early workers failed to demonstrate the presence of this penicillinase (Abraham and Chain, 1940). Later on, other workers searched for penicillinase in strains developing resistance to penicillin in vivo and they were able to demonstrate it in those staphyylococcal strains developing resistance to penicillin in vivo which proved to be penicillinase producers (Spink, 1945) while these developing resistance in vitro do not produce penicillinase (Spink and Ferris, 1945). But later on it was demonstrated that those strains developing resistance in vitro are also capable of producing penicillinase (Gould, 1955 a). In 1965, the enzyme commission perpetuated the partial view of B-lactamases when it described the enzyme as "Penicillin amido B-lactam hydrolase " these enzymes

hydrolysis the cyclic amide bond of susceptiable B-Lactam ring of antibiotics to give antibiotically inactive products. In case of penicillin, the products of hydrolysis are penicilloates which are stable and easily detectable (sykes, 1982).

Occurance and origin

The enzyme is widely distributed amongest bacterial species and there is no accurate description of penicillinase of non bacterial origin (Citri and Pollock, 1966). Staphylococcal penicillinase is generally regarded as a true extracellular enzyme according to the definition of pollock (1962), although a high proportion of the total enzyme activity is intracellular. The production of penicillinase by S. aureues is under the control of a plasmid associated gene (Novick, 1963).

Coles and Gross (1969) using a S. aureus strain which excretes up to 40% of its total penicillinase as extracellular enzyme and retains 60% of the exopenicillinase in a cell bound state, have shown that the cell bound enzyme may be released by incubating the cells in 0.15M Soduim cirate at 37 C° for short peroids. The bound

enzyme could be librated within organic anion phosphate, arsenate of the polyanion heparine, RNA or dextran suplhate (Abramson, 1972).

Variants of staphylo-exopenicillinase

Three variants of staphylococcal exopenicillinase can be distinguished on chemical, enzymelogical and immunological grounds. These types are A,B and C. Enzyme type A has a higher specific activity than type B but has similar specific activity with type C. All three enzymes have the same amino acid analysis and they also have small but significant differences in kinetics of action when hydrolysing benzyl penicillin, methicillin, cloxacillin and cephalosporin (Richmond, 1965). Staphylococcal strain that have established endemically in hospitals are members of phage-groups I and III, which penicillinase is always of immunological type A or C (Dyke, Parker and Richmond, 1970).

Genetics of Penicillinase

It has been found that penicillinase-producing strains of S-aureus each harber an extrachromosomal element of plasmid, which apparetly carries all the genetic

information necessary for penicillinase synthesis, these plasmids comprise linkage groups containing several markers and in that undergo such genetic events as mutation, segregation, and recombination. A certain amount of variability has been encountered among the penicillinase plasmids harbored by different staphylococcal strains, it has been found that (1) there are at least three molecular variants of the enzyme itself, (2) most but not all, of the penicillinase plasmids carry a genetic determinant resistance to mercuric ions, (3) plasmids carried by very small number of the strains bear a determinant of resistance to erthyromycin, (4) the plasmids determine the fraction of penicillinase excreted into the medium during growth and this also varies from strain to strain (Novick and Richmond,1965).

Characteristics of penicillinase and factors affecting its synthesis

It was suggested that penicillinase was really modified peptidase concerned primarly with cell wall metalolism but presently oriented toward B - lactam ring of penicillin and cephalosporins as a result of natural selection (Abramson, 1972). The molecular weight was calculated

to be 29, 600.analysis of the amino acid content of the pure enzyme revealed that the molecule contains no cysteine and has lysine as the N-terminal amino acid. The PH. activity curve of the pure enzyme was determined over the range PH 4.0 - 9.0 (Richmond, 1963).

It is apparent that potential exopenicillinase does not accumulate within the cells grown in the presence of dextran sulphate. Failure of exopenicillinase to find an unoccupied site in the cell wall may lead to inhibition, possibly by a mechanism of the feed back type, of synthesis of total penicillinase. Maximum inhibition is reached at the same dextran sulphate concentrations that cause complete cessation of the formation of surface bound penicillinase. (Cloes and Gross, 1967).

Cloes and Gross (1965a) proved that glucose, amino-acid, magnesuim and phosphates were required for induction of penicillinase. Penicillinase synthesis in a wildtype inducible strains of S.aurus increased with uptake of iron and with increasing acidic PH of the media. The enzyme was inactivated by trypsin and inhibited by iodine and potasuim iodide (Abramson, 1972).

Stable L-form colonies were found by Rosdahl and Vejlsgaard (1970) to be unable to produce penicillinase after 70 to 100 subcultures on methicillin centaining substrate. When the resistant coccal forms were transformed into L-forms during treatment with penicillin in vivo, they became sensitive to this antibiotic after reversion to coccal forms. The loss of penicillinase activity may be due to the loss of penicillinase plasmid (Abramson, 1972).

Assay of penicillinase

After the discovery of penicillinase, one of the B-lactamase group of enzymes in 1940 and the demonstration of its role in penicillin resistance of S.aureus (Kirby, 1944) many methods began to be developed for the detection and quantitation of this enzyme. In 1962, Wollf and Hamburger puplished an evaluation of seven methods of determinating staphylococcal penicillinase which were then current. Some of the principles being employed at that time have been adopted in more recent methods but none of the procedures remins popular today,(Lucas, 1979). In recent years, however, the emergence of penicillinase producing strains of Haemophilus influenzae and Neisseria gonorrhoeae has given a new impetus to interest in these

enzymes. A large number of new, simpler, or more rapid methods have been described (Lucas, 1979). One of these methods which is simple is a filter paper acidometric test, using bromocresol purple as PH indicator for detecting penicillinase producing S. aureus gave complete agreement with the chromogenic cephalosporin and iodometric method (SNG, Yeo and Rajan, 1981).

In this study, we will discuss a filter paper acidometric test using bromocresol purple as PH indicator for detecting penicillinase producing S.aureus.

MATERIALS AND METHODS

MATERIAL & METHODS

MATERIAL

(1) Staphylococcal strains isolated from different pyogenic infection (infected wounds, abscesses, burns 75 cases) and 50 nasal swabs of apparently healthy individuals.

(2) Culture media :

- Nutrient agar.
- Blood agar.

(3) Antibiotic discs used penicillin, tetracyctine, chloramphencol, rafampicin, cefalotine and gentamycin.

(4) Reagents used in the preparation the acido metric filter paper test.

- 5% crystalline penicillin solution.
- 2% bromocresol purple indicator.
- 0.05 molar phosphate buffer, PH 8.0

METHODS

1- Collection of specimens :

Pus samples were taken by sterile cotton swabs from infected surgical wounds, abscesses and infected burns from patients attending the out patient clinic in Zifta Hospital, also nasal swabs were taken from apparently healthy individuals(nasal carriers)

II- Isolation and identification of staphylococcal isolates:

Swabs were cultured on 5% sheep Blood agar plates as well as nutrient agar plate. The inculated plantes were examined after 24 hours incubation at 37 C°, for the presence of colonies with typical morphology of staphylococci, and surrounded by clear zone of haemolysis on sheep's blood agar plates.

Identification of isolated staphylococcal strains was based on :

1- Microscopical examination of gram stained films to demonstrate the gram positive cocci in clusters.

2- Biochemical Reactions : Variable, mannite fermentation is of special importance because is displayed by the pathogenic members of staphylococci of human origin.

3- Coagulase Production test :

It was carried out both by the slide and the tube method :

a- Slide coagulase test was done according to the method by (Sydney et al., 1978), which detects the coagulase bound to the organism, a homogenous suspension of the test organism was made in drop of sterile (0.85% Nach) on a slide then mixed with a drop of undiluted citrated plasma. Coagulase positive staphylococci clump with 15 sec, because coagulase precipitates the fibrin in the plasma on the cell surface. A control was included where no plasma was added to exclude auto agglutination of staphylococci.

b- Tube coagulase test was done according to the method described by (Sydney et al., 1978), which detect the free coagulase.

0.1 ml of over night broth culture was added to 1.0 ml of freshly prepared $\frac{1}{10}$ dilution of human plasma in wassermann tubes, the tubes were incubated at 37C° and read after 1, 2, and 4 hours, if no clot has appeared in this time, it was left over night at room temperature and again

examined. A control tube containing diluted plasma was included, the clotting of the plasma indicated a coagulase positive strains.

III- Antibiotic sensitivity of the coagulase positive staphylococcal strains.

Sensitivity of isolated strains of staphylococcus aureus was done using a set of antibiotics by filter paper disc diffusion method on sensitivity agar medium (Cruickshank , 1975). This is an agar diffusion method in which bacteria isolated in pure culture were used to prepar a broth suspension by which the surface of an agar plate was flooded, the excess was pipetted off and the surface of the medium allowed to dry. The antibiotic discs were then placed on the surface of the plate. The result was read after 24 hours incubation at 37 °C.

The antibiotics used were :

Penicillin G	10 ug / disc
Chloramphencol	25 ug / disc
Tetracycline	30 ug / disc
Cefalotine	30 ug / disc
Gentamycin	10 ug / disc
Rifampicin	30 ug / disc

The organism under test was considered sensitive if the inhibition zone measured was 12 mm or more.

IV - Penicillinase Production :

The ability of isolated strains of staphylococcus aureus resistant to penicillin to produce the enzyme penicillinase was measured using an acido metric method described by (Sng et al., 1981).

Reagents :

5% crystalline penicillin solution

0. 2% bromo cresol purple indicator

0.05% molar phosphate buffer, PH. 8.0

(37.5 mg $K H_2 Po_4$ and 842 mg $Na_2 Hpo_4 2Ho_2$ in 100 ml distalled water).

5 gm of crystalline penicilline and 200 bromocresol purple were dissolved in 100 ml of 0.02% molar phosphate buffer, forming penicillin solution which was kept - 20C° if stored for 3 months but when the solution was in use it was kept at 4C°

The test :

A peice of whatman No 1 filter paper was placed in petri dish, the penicillin solution was then dropped on

the paper to saturate it, the filter paper appeared violet in colour. With bacteriological loop a few number of colonies was spread over an area of 5 mm in diameter on the filter paper, several strains may be tested on the same paper seperated from each other by more than 1 Cm. Then observed for the appearance of yellow zones around the colonies producing penicillinase due to the formation of penicilloic acid as a result of opening of the B.lactam ring of penicillin under the effect of penicillinase.

The yellow colour was best seen by looking through the botton of the petri dish against indirect light.