Introduction
&
Aim Of Work

INTRODUCTION

The genus Staphylococcus consists of cluster- forming Gram positive cocci . It includes two important species Staph. aureus and Staph. epidermidis .

Staph. aureus is much more important as regards its pathogenicity. It is the cause of a wide range of pyogenic infections . Also it is found as commensal parasite in about 20-30% of healthy people " carriers " (Duguid, et al . 1978) .

Staph. epidermidis is harmless commensal that grows on the whole surface of the skin, in the nostrils and mouth of all persons, but it occasionally acts as opportunistic pathogen (Duguid, et al . 1978).

Rapid and reliable differentiation of Staph. aureus from other strains of staphylococci is important in clinical bacteriological laboratory (Essers & Radebold, 1980).

As approximately 97% of staphylococci isolated from pathogenic processes elaborate coagulases, enzymes that cause the coagulation of plasma, the slide & tube test are usefull for diagnostic purposes (Joklik, et al 1984).

Weckman & Catlin (1957), & Di Salvo (1958) recommended determination of DNase as valuable criterion of the pathogenicity of the strain .

Most strains of Staph. aureus ferment mannitol so, mannitol fermentation , among other tests, distinguishes Staph. aureus from Staph. epidermidis (Bailey & Scott, 1974).

Essers & Radebold, (1980) introduced a new method, latex agglutination test, for identification of Staph. aureus.

AIM OF THE WORK.

As the biochemical activities of staphylococci are not easy to determine , even when care is taken to use standard techniques , the aim of this work is to evaluate the efficacy of the latex agglutination test as a rapid and easy method in detection of Staph. aureus strains.

Review Of Literature

HISTORICAL INTRODUCTION

Cocci were first observed in diseased tissues and in pus obtained from human abscesses . These organisms were called " micrococci " by Von Recklinghausen (1871), " Microsporum septicum " by Klebs (1872), and " monads " by Hueter (1872) . But Billroth (1874) classified them on the basis of their cell arrangements into " monococcus " " diplococcus ", " streptococcus ", and " staphylococcus ".

In (1880) a Scottish surgeon, Sir Alexander Ogston observed that a cluster-forming coccus was the cause of certain pyogenic abscesses in man. He named this pyogenic organism Staphylococcus and he proposed this name for the cluster-forming cocci to distinguish them form Billroth's chain-forming streptococci. At the same time, Louis Pasteur (1880) reached similar conclusion in France. Rosenbach (1884) made a thorough study of the staphylococci and adapted the generic name Staphylococcus proposed by Ogston.

The name staphylococci is very descriptive and appropriate name and is derived from the Greek noun Staphylo

NATURAL HABITAT

Staphylococci are normally present on the body surface of many species of mammals and birds , in the air , dust , milk , food , and sewage (Parker, 1984).

Staph. aureus and Staph. epidermidis are members of the endogenous microbial flora of man (Youmans, et al. 1975). Staph. aureus is isolated from about 35-50% of the healthy adults (Williams & Harber, 1946). These pathogenic strains are present in the nose, skin, and large intestine, but the nose is the most important site of the carrier state (Briody & Gillis, 1974). The colonization of Staph. aureus in the anterior nares provides a convenient source for colonization of the skin (Turk & Porter , 1978).

Skin is frequently inhabited by both Staph. aureus and Staph. epidermidis and they are commonly found in the following areas : umbilicus,axillae,perineum, face, hands, and hair (Youmans, et al. 1975).

In the gastrointestinal tract they may also be found but they do not commonly produce gastroitestinal disease

resistant strains may develop, multiply, and produce disease (Youmans, et al. 1975).

CARRIERS :

** Asymptomatic staphylococcal carriers : can be divided into :

a- Persistent carrier , those who harbor a specific type of Staph. aureus for prolonged periods .

b- Occasional carriers , those who sporadically harbor pathogenic staphylococci .

c- Intermittent or transient carriers , those who harbor one staphylococcal type for a certain period and then harbor a different type.

d- Non carriers , those who never, or only rarely, carry virulent Staph. aureus strains.

A number of factors may be involved in the maintenance of the carrier state, but the immune state of the host is the most important factor .

** Symptomatic carriers : are those who are suffering from overt staphylococcal diseases (Youmans, et al. 1975).

CLASSIFICATIONS

Staphylococci is the only genus of medical importance in the family Micrococcaceae .

Rosenbach (1884) divided the staphylococci according to pigment production into 2 species : Staph. pyogenes aureus, and Staph. pyogenes albus. Passet (1885) added another species , Staph. pyogenes citreus .

In this classification golden yellow pigment (aureus) being considered a property of pathogenic strain whilst the white strains (albus) were thought either to be less pathogenic or non (Fairbrother, 1940 ; Elek & Levy, 1950 ; Williams Smith, 1959).

Since most strains freshly isolated from staphylococcal diseases produced a golden yellow pigment , the organism was named Staph. aureus to distinguish these strains from the non-pathogenic strains that usually produce white or lemon yellow (citreus) colonies .
As pigment production is a variable trait of staphylococci its correlation with pathogenicity is unreliable (Joklik, et al . 1984). Moreover , albus variants may be obtained from aureus strains and still give the other reactions of the parent strains (Fairbrother, 1940) .

The above method of classification is generally considered to be unsatisfactory . The main objection is that, pigment production is not a constant characteristic but is dependant on a number of variable factors as temprature of incubation, atmospheric conditions and the nature of the medium . Also, there are considrable variation in the degree of pigmentation and a certain amount of difficulty is sometimes experienced in assessing the exact colour of the pigment produced by some strains (Williams Smith, 1959).

Other methods of classifications have been tried using biochemical, serological, heamolytic and pathogenic activities as a basis . These methods have been proved to be of limited values for general application (Fairbrother, 1940).

It was found that pathogenic staphylococci are distinguished from non-pathogenic strains by their production of extracellular coagulases that clot plasma (Joklik,et al . 1984).As coagulase production constitutes an important criterion for classification, Cowan & Steel(1964), classified staphylococci into coagulase +ve or Staph. aureus and coagulase -ve or Staph. epidermidis .

Baird-Parker (1963- 1965) classified staphylococci into six subgroups, the first subgroup I contained Staph. aureus while coagulase -ve staphylococci were classified

subgroups 2 & 5 originally described by Baird-Parker as these organisms were separated on the basis of the phosphatase production which was shown to be produced by members of both subgroups if a more sensitive test is used to detect this enzyme.

Baird-Parker (1972) separated Staph. aureus from Staph. epidermidis and classified the later in 4 groups. The main criteria separating these two species are shown in Table (1).

Strains of Staph. aureus can also be divided into number of different types by a serological method which depends on type-specific surface antigens that are demonstrable in slide agglutination tests with absorbed antisera . There are three main serotypes I - III and some minor types. But serotyping has been much less extensively used in epidemiological studies than phage typing becauce of technical difficulties (Duguid,et al . 1978).

Another type of classification depends on host-adapted varieties. Hájek & Marsálek (1971) recognized 6 biotypes A-F of which two (E & F) were subsequently transferred

Table (1) : Differntial Characteristics of Species Staphylococcus. Cited from Baird-Parker (1972).

Characteristic	aureus	epidermidis
Coagulases	+	-
Mannitol		
Acid aerobically	+	d
Acid anaerobically	+	-
Alpha toxin	+	-
Heat-resistant endo-nucleases	+	-
Biotin for growth	-	+
Cell wall		
Ribitol	+	-
Glycerol	-	+
Protein A	+	-

+ = most (90% or more) strains positive.

- = most (90% or more) strains negative.

d = some strains positive , some negative.

Nielsen (1970) observed that the DNA from Staph. aureus was not hybridized with the DNA from Staph. epidermidis . This observation differentiates these two species.

Table (2) : Biotypes of Staph. epidermidis.

Cited from Baird-Parker (1972).

Characteristic	Biotype			
	1	2	3	4
Acetoin	+	-	+	+
Phosphatase	+	+	-	-
Acid aerobically from:				
Lactose	+	d	-	d
Maltose	+	-	d	d
Mannitol	-	-	-	+

+ = most (90% or more) strains positive.

- = most (90% or more) strains negative.

d = some strains positive , some negative.

Table (3): Subdivision of Staph. aureus into biotypes. Data cited from Hájek & Marsálek, (1974).

	Biotypes					
	A	B	C	D	E	F
Origin of biotype	Human	Pigs, Poultry	Cattle, Sheep	Hares	Dogs	Pigeons
Fibrinolysin	+	-	-	-	-	-
Pigment	+	+	+	V	-	-
Coagulation of:						
Human plasma	+	+	+	+	V	+
Bovine plasma	-	-	+	-	+	+
α-Haemolysin	+	V	V	-	-	V
β-Haemolysin	V	V	+	+	+	+
Reduction of tellurite	+	+	+	+	W	-
Clumping factor	+	+	+	+	V	-
Growth on crystal violet agar	-	V	V	-	+	+
Typed by adapted phages	H	H	H,B	H	C	-

+= more than 80% of strains positive , -= more than 80% of strains negative
V= some strains positive, some negative, W=weak reaction
H= basic international human phage set B=bovine phage set C=canine phages

ULTRASTRUCTURE & CELL COMPOSITION

The architecture of a staphylococcus varies with conditions of culture and appears to be similar to other Gram +ve organisms . Log phase cells of a standard strain , as seen in thin section, reveals nucleoids , mesosomes , and a trilaminar cytoplasmic membrane that is separated from the cell wall by a periplasmic region ,

The thickness of the wall of young cells normally varies between 18 - 25 nm. (Joklik, et al 1984).

The cell wall of Staph. aureus consists of three major components : peptidoglycan , the ribitol teichoic acid and protein A (Baird-Parker, 1972).

*** Peptidoglycan

It comprises 40 - 60 % of the weight of cell wall, and is quite characteristic of this genus .
It consists of glycan composed of regularly alternating N-acetylglucosamine and N-acetylmuramic acid residues joined together through β- 1,4 glycosidic linkages .
In staphylococci , all of the N- acetylmuramic

*** Teichoic acid

Attached to , or in close proximity to , the peptidoglycan is the teichoic acid which, depending on the species, contains either ribitol in case of Staph. aureus or glycerol in Staph. epidermidis linked to a sugar or an amino sugar (Baird-Parker, 1974).

Teichoic acid is an essential component of the phage receptor of Staph. aureus. It also plays an important role in the maintenance of normal physiologic functions. By regulating the cationic environment of the bacterial cell, it controls the activity of autolytic enzymes that function in growth of the cell wall and separation of the daughter cells (Joklik, et al. 1984).

Mutants completely lacking teichoic acid can exist, showing that it is not essential for viability, but they are phage-resistant, grow more slowly than ordinary type organisms, and produce large bizarre nonseparating cells with abnormal cross-wall structure (Joklik, et al. 1984) Removal of teichoic acid renders staphylococci susceptible to lysozyme (Parker, 1984).

*** Protein A

The major protein component of the cell wall of Staph. aureus is protein A, and about 1/3 of which is released into the medium during cell growth (Joklik, et al. 1984).

Protein A, was first described by Verwey, (1940) and rediscovered by Jensen,(1958) ,who showed that it was responsible for a precipitation line in agar gel between crude acid extracts of Staph. aureus and normal human serum. Forsgren & Sjöquist, in (1966) isolated it from the cell wall of Staph. aureus .

Protein A ,which is found in virtually all strains of Staph. aureus is the group-specific antigen, and it appears to be loosely bound to the cell wall and is probably attached to the peptidoglycan and can be demonestrated at the cell surface by the electron microscopy (Grov & Rude, 1967 ; Nickerson ,et al. 1970). Oeding (1965) showed that protein A was absent from all Staph. epidermidis, but Forsgren ,(1970) indicated that occasional strains formed it .

Depending on the method of isolation , its molecular weight ranges between 13,000 & 42,000 (Morse, 1981). Purified protein A contains a preponderance of basic amino acids , and its removal from the bacterial cell increases the -ve charge of the cell surface (Joklik, et al. 1984).

The amount of protein A formed by human

cytosis more than strains that contained lesser or none of this protein .

Small amounts of other proteins and carbohydrates are also present in cell walls of staphylococci ; these correspond to the various type-specific precipitinogens & agglutinogens which have been shown to be usefull in the serological typing of this organism (Oeding, 1965).

Some strains of Staph. aureus may possess a capsule or slime layer that may be responsible for increased virulence (Wiley & Maverakis, 1968).

MORPHOLOGY & STAINING PROPERTIES

The staphylococci are rounded or somewhat oval cells with an average diameter of 0.8 - 1.0 μm. The size varies from strain to strain and depends on the age of culture and the medium on which it is grown . All staphylococci are non-motile, non-flagellated, and non-sporing (Parker, 1984). Some strains of *Staph. aureus* may possess a capsule or slime layer (Wiley & Maverakis, 1968). Encapsulation is more apparent in vivo than in vitro cultivation (Morse, 1978) .

Cells are characteristically grouped in aggregates resembling clusters of grapes (Morse, 1981). This is because, cell division takes place in successive perpendicular plans with incomplete separation of the daughter cells and the attached point is usually eccentric (Tzagaloff & Novick, 1977). Moreover, daughter cells may shift from their original site of attachment (Morse, 1981).

In broth cultures, short chains and diplococcal forms are common, but irregular clusters are characteristically found in solid cultures smears (Joklik, et al. 1984).

Staphylococci stain with most aniline dyes and are uniformly Gram +ve in young cultures (Parker, 1984). In

GROWTH AND CULTURAL CHARACTERS.

Staphylococci grow readily on most bacteriologic media under aerobic or microaerophilic conditions (Jawetz, et al. 1986). Although more abundant growth is obtained under aerobic conditions, some strains require an increased CO_2 tension (Joklik, et al. 1984).

For aerobic growth a medium containing as many as 12 amino acids is required, together with adenine and thiamine (Fildes, et al. 1936 ; Mah, et al. 1967). In addition to these requirements, Richardson, (1936) found that uracil and a fermentable carbon source are essential for anaerobic growth .

Growth occurs over a wide temperature range, 6.5 to 46 °C, with an optimum of 30 to 37 °C (Joklik, et al. 1984). They can also grow at pH ranged between 4.2 and 9.3 while the optimum is 7.0 - 7.5 (Genigeorgis,et al.1971). The minimum value of water activity in which growth has been observed is 0.86 (Troller, 1972).

** NUTRIENT AGAR :

They form smooth, circular, opaque colonies, 1-2 mm

inous, adherent to the medium and difficult to emulsify . Heavily capsulated strains may form mucoid colonies. Colonies of most strains of Staph. aureus are golden yellow but may be pale yellow or even completly white. Those of coagulase -ve staphylococci are usually white and may be pale yellow (Parker, 1984).

Pigmentation and pigment-enhancing media.

Hammond & White, (1970) declared that colony pigmentation resulted from the presence of carotenoid pigments in the cell membranes of this organism and white colonies were produced by strains that were low in carotenoid pigments. Jawetz, et al .(1980) found that no pigmentation was produced anaerobically or in broth.

Pigment production may be best observed by growth on agar plates at 37°C for 24 h. , followed by incubation at room temp. for an additional 24 to 48 h. (Joklik, et al. 1984) . It is also optimal by the use of a special pigment-enhancing medium such as 33% full fresh milk agar (Christie & Keogh, 1940). According to Willis & his colleagues (1966) milk agar gave irregular results and 10% cream agar was preferable.

By observing the effects of lipids on pigmentation

fatty acids are metabolised by individual strains must then depend on the production of appropriate dehydrogenating enzymes. The white strains of Staph. aureus which are preferably regarded as non-pigmented, are presumably deficient in enzymes that are essential for carotenoid biosynthesis (Willis, et al. 1966). Glycerol monoacetate agar 1% also favours pigmentation and two distinct types of pigmentation orange and lemon yellow occur among Staph. aureus strains grown on this medium (Willis & Turner,1962)

Many workers (Turner & Willis,1962 ; Willis & Turner, 1963 ; Willis,et al. 1964) found that there was a relation between pigment production on glycerol monoacetate agar and antibiotic sensitivity and mercury sensitivity . They found that the yellow pigmented organisms were generally multiple antibiotic resistant and mercury resistant . Orange and buff strains were more usually sensitive to antibiotics.

** Blood agar :

Staph. aureus is usually surrounded by a zone of clear haemolysis . Strains of Staph. aureus may produce one or

midis strains are also haemolytic (Morse, 1981). The haemolytic property may be lost in stock cultures or after a number of transfers (Bailey & Scott, 1974) . In the preparation of blood agar , human blood should not be used because of the presence of non-specific inhibitors or antibodies, so sheep blood agar is recommended for primary isolation from clinical materials (Joklik, et al. 1984).

** Milk agar :

Colonies are similar to those on agar but more intensely pigmented , with clear distinction between orange, yellow, and cream-buff strains . Zones of clearance around colonies are due to digestion of heat coagulated casein by staphylococcal proteases (Cruickshank, et al. 1975) .

** Mac Conkey's agar :

Tiny pinkish colonies appear (Cruickshank, et al. 1975)

** In broth :

Uniform turbidity with some powdery deposit is observed (Cruickshank, et al. 1975).

Results in filiform growth with liquefaction from top (Cruickshank, et al. 1975).

Barber & Kuper (1951) recommended phenolphthalein phosphate agar as an indicator medium allowing easy provisional identification of Staph. aureus colonies. All strains form phosphatase so liberate phenolphthalein which reacts with the ammonia placed in the lid of the Petri dish to give the colonies a bright pink colour .

Staphylococci tolerate higher concentration of sodium chloride (7.5- 10 %) than many other kinds of bacteria (Cruickshank, et al. 1975).

Salt containing media as mannitol salt agar is recommended as a selective medium for its isolation . Colonies of salt-tolerant Staph. aureus appears on this medium surrounded by a readily visible yellow halo, which indicates mannitol fermentation , after 24 to 48 h. incubation (Bailey & Scott, 1974).

Finegold & Sweeney, (1961) found that polymyxin agar was another selective medium for Staph. aureus by inhibition of Staph. albus , Gram -ve bacilli and other bacteria .

Dwarf or G forms of Staph. aureus can be isolated from most strains by growth under conditions in which the normal forms are inhibited, including growth in the presence of lithium chloride, barium chloride, gentian violet, acridines, and varios antibiotics (Hale,1947 ; Browning & Adamson, 1950 ; Wise & Spink, 1954). Of particular importance in medical microbiology are the small colony-variant (G forms) that may appear on plates inoculated with materials from infected tissue. These form very minute colonies which may be confused with colonies of streptococci or haemophilic bacteria (Youmans, et al. 1975). Most dwarf forms show a tendency to reversion on continued subcultures (Parker, et al. 1984).

Osmotically unstable L forms, deficient in cell wall material, can be induced by agents that affect cell wall synthesis or structure for example penicillin or lysostaphin . It can be propagated in vitro in hypertonic medium (Morse, 1981).

Both L & G forms are resistant to many drugs, especially those affecting the cell wall, and it has been postulated that recrudescent Staph. aureus disease is sometimes due to reversion of persisting dormant L or G forms

bulk of the population in cultural characteristics, (colony type, pigment, haemolysis) in enzyme equipment, in drug resistance, and in pathoginicity .

METABOLISM

The metabolism of staphylococci is respiratory and fermentative (Baird-Parker, 1972).

The staphylococci possess enzymes that allow normal respiratory metabolism . The most important of these are cytochrome a, b and o, which contain firmly bound prosthetic group capable of donating or accepting electrons as they undergo oxidation or reduction . They are bound to the cell membrane of the staphylococcus and form the membrane-bound electron transport system (Morse, 1981).

A wide range of sugars and other carbohydrates is utilized by staphylococci . Under aerobic condition, the major product is acetic acid , lactic acid is principal product , and acetion also is usually produced (Joklik, et al. 1984).

Catalase which is produced aerobically by grown cells is important for splitting hydrogen peroxide and preventing accumulation of this high toxic compound. Catalase activity helps in distinguishing staphylococci from streptococci , which are catalase deficient (Morse, 1981 ;

BIOCHEMICAL & BIOLOGICAL ACTIVITIES

The biochemical activities of staphylococci are not easy to determine, even when care is taken to use standard techniques, yet these are the characters upon which we have to rely in routine practice to distinguish between staphylococci and micrococci (Parker, 1984).

A wide range of sugars and other carbohydrates are utilized by staphylococci, particularly in presence of air, with the production of acid but gas is not detectable by standard procedures (Baird-Parker, 1974).

All staphylococci and many micrococci form acid from glucose aerobically, but the ability to do this anaerobically is now considered to be the most distinctive character of the staphylococci (Parker, 1984). Cowan & Steel, (1964) found that the results of tests for the fermentation of glucose depended very much on the exact composition of the medium, and the standard technique should be used (Report 1965). However, the test may give a -ve or equivocal results with some staphylococci that form very little acid from glucose either aerobically or anaerobically (Parker, 1984).

Other sugars as maltose, lactose, sucrose and

from human sources ferment mannitol. Also some strains of coagulase -ve staphylococci do so (Cruickshank, et al. 1975). The only staphylococcus which forms acid from mannitol anaerobically is the Staph. aureus, but a standard medium must be used to detect this character (Evans & Pate, 1980). Hugh & Ellis, (1968) showed that Staph. epidermidis did not usually utilize mannitol as a fermentable substance for anaerobic growth.

Few staphylococci acidify raffinose, starch, dulcitol and salicin (Parker, 1984). Staphylococci acidify glycerol aerobically but micrococci do this rarely (Schleifer & Kloos, 1975).

Many staphylococci and all Staph. aureus, are both methyl red +ve and Voges-Proskauer +ve , indole is not produced, oxidase -ve and with rare exceptions catalase +ve . Most staphylococci produce ammonia from arginine, hydrolyse urea, and reduce nitrate to nitrite. Some of them produce a trace of hydrogen sulphide (Deisseroth & Dounce, 1970 ; Parker, 1984) .

Phosphatase production is useful in the classification of staphylococci . In Staph. aureus it is

Lipolysis is common in staphylococci . The ability to hydrolyse tributyrine is a character of all strains of Staph. aureus and of many other staphylococci (Parker, 1984). The production of opacity in egg yolk is due to a lipase , only produced by coagulase +ve staphylococci (Gillespie & Alder, 1952) .

Nearly all strains of Staph. aureus and many other staphylococci, have proteolytic effect on gelatin, coagulated serum (Cruickshank, et al. 1975), and digest casein rapidly in the presence of serum (Fisk & Mordvin, 1943).

The most important differential characteristic of staphylococci is the demonstration of coagulase production as there is a high correlation between its production and pathogenicity (Youmans, et al. 1975).

Most strains of Staph. aureus form coagulase which reacts in the tube coagulase test and bound coagulase which is detected by slide method. A small minority of strains form only one type (Cruickshank , et al. 1975).

Reading the tube coagulase test depends on the degree of clotting in the coagulase plasma : (Turner & Schwartz, 1958)

* -ve = No evidence of fibrin formation.
* 1 +ve = Small unorganized clots.
* 2 +ve = Small organized clot.
* 3 +ve = Large organized clot.

Coagulase production has been accepted as the primary criterion for differentiating pathogenic from commensal strains (Forsgren, 1970).

There is a close correlation , in staphylococci ,between coagulase production and DNase activity. So Weckman & Catlin, (1957) & Di Salvo, (1958) recommended determination of DNase as another valuable criterion of the pathogenicity of the strains.

The above two tests (coagulase, DNase) , rely on the ability of Staph. aureus to produce the respective enzymes (Zarzour & Belle, 1978).

RESISTANCE OF STAPHYLOCOCCI

1- TO PHYSICAL AGENTS :

a- Heat :

The staphylococci are among the most resistant non-sporing organisms . Staphylococci show no particular resistance to heat and are usually killed by a temperature of 60 °C in ½ an hour .(Parker, 1984).

Three conditions may alter the heat resistance of staphylococci :

1- Age of cells heated : Walker & Harmon,(1966) found that logarithmic phase cells of bacteria were less resistant than cells during the stationary phase .

2- Composition of heat menstruum : Substrates in which the staphylococci are present have a variable & generally unpredictable effect on heat resistance (Baird-Parker, 1972). For example, the heat resistance of Staph. aureus does not appear to change significantly in the presence of meat protein. (Gross & Vinton, 1947 ; Thomas,et al. 1966). Kadan, et al. (1963) found that the addition of sucrose to milk might result in a large increase in heat resistance but, the addition of other carbohydrates might not increase heat resistance. Amin, et al.(1966) ; Calhoun & Frazier,(1966) found that the presence of glucose or

hydrogen peroxide reduced the heat resistance of Staph. aureus, while Scott & Strong, (1964) found that sodium alginate increased it . Staphylococci suspended in fat are extremely heat resistant, resistance is greater in dry than in moist fat, (Yesair, et al, 1946).

3- Recovery conditions : Growth conditions are extremely important for the full recovery of the heat damaged cells. Allwood & Russell,(1966) showed that the optimum temperature and pH for the recovery of heat damaged cells of Staph. aureus were 32°C and 6.0 .

As heating cause leakage of cytoplasmic constituents, decrease in metabolic activities, degradation of ribosomal RNA , and partial denaturation of cell protein (Iandolo, 1965 ; Stiles & Witter, 1965 ; Iandolo & Ordal, 1966 ; Bluhm & Ordal, 1968) . For repair, an energy source such as glucose is required, together with a mixture of amino acids and phosphate (Iandolo & Ordal, 1966).

b- Drying :

In broth or agar tubes sealed with paraffin and kept in the ice-chest , cultures may remain alive for months. Staphylococci dried on threads retain their vitality for 3-6 months , and from dried pus they have been cultivated after 2-3 months (Parker, 1984) .

were generally resistant to freezing and dried slowly.

d- Radiation :

Staphylococci are moderatly resistant to gamma radiation and X-rays (Baird-Parker, 1972). Resistance depends on a variety of factors, such as the physiological state and age of the organism, the composition of the radiation menstruum , the presence of sensitizers or protectors, and the atmosphere and temperature of the radiation (Hugo, 1971).

At -79 °C, Staph. aureus cells are more sensitive to UV than at ambient temperatures . The wave length of UV is also important, and there is a peak of activity against staphylococci at between 250 & 280 nm (Baird-Parker 1972).

e- Hydrostatic pressure :

Baird-Parker (1972) found that high hydrostatic pressure would inhibit the growth of staphylococci and might result in the destruction of some cells

2- RESISTANCE TO DISINFECTANTS :

Staphylococci are very sensitive to some aniline dyes as crystal violet. Fatty acids inhibit the growth of staphylococci and the highly unsaturated acids have a more powerful action on coagulase +ve than on coagulase -ve strains (Parker, 1984).

3- RESISTANCE TO ENZYMES THAT ATTACK THE CELL WALL :

Staphylococci are uniformly resistant to lysozyme, but some micrococci are sensitive to it. On the other hand, staphylococci are generally sensitive to lysostaphin, but most micrococci are resistant to it (Schindler & Schuhardt, 1964 ; Lachica, et al. 1971). According to Schleifer & Kloos (1975) if an organism is sensitive to lysostaphin and resistant to lysozyme it is probably a staphylococcus.

4- RESISTANCE TO ANTIMICROBIAL AGENTS :

Strains of Staph. aureus isolated before 1942 were sensitive to a wide range of antibiotics, but a few of them were resistant to benzylpenicillin by virtue of the

from patients and carriers outside hospitals and most of those present in hospitals are resistant to benzylpenicillin. Many of the hospital strains are also resistant to several other anti-staphylococcal antibiotics (multi-resistant strains) and these strains are often responsible for hospital cross infection and may be highly virulant (Duguid, et al. 1978).

Resistance to penicillin is due to the ability of some Staph. aureus strains especially those in phage grops 1 and 111 (Briody & Gillis, 1974) to produce penicillinase enzyme which is a β- lactamase that splits the β- lactam ring of the penicillin nucleus (Lacey, 1975) .

The enzyme penicillinase is generally coded for on a plasmid and only rarely on the chromosome (Dyke & Richmond, 1967; Peyra et al. 1969). Although four different types of penicillinase plasmids have been recognised, no naturally occuring strain carries more than one type . Some penicillinase plasmids also carry markers conferring resistance to erythromycin and certain metal ions, but with the exception of plasmids displaying resistance to both penicillin and erythromycin, there is no tendency to build up multiple antibiotic- resistance plasmids . In Staph. aureus, the plasmids carrying

neomycin and kanamycin are independent entities (Joklik, et al. 1984).

Duguid, et al. (1978) stated that penicillin sensitive strains of Staph. aureus never mutate to become penicillinase-producing, though sensitive strains could readily mutate into forms that were resistant to streptomycin, erythromycin, fucidin or novobiocin and rarely into forms resistant to tetracyclines or cloramphenicol.

The multi-resistant staphylococci have probably arisen by a succession of mutation conferring resistance to these different drugs in strains that were at first only penicillin resistant. Plasmids that bear genes conferring resistance to an antibiotic may be transferred from a resistant to a sensitive strain by phage transduction (Duguid, et al. 1978).

Some strains of Staph. aureus carry genetic determinants for bacteriocin production. These genes are extrachromosomal and in many ways analogous to the colicinogenic factors of the enteric bacteria.
The production of the bacteriocin, staphylococcin, is

phage product . Its spectrum is wide and includes β-haemolytic streptococci, pneumococci, other staphylococci, corynebacteria and several bacillus species . Gram negative bacteria and the producer strains are resistant to its action (Joklik, et al. 1984).

Determination of susceptibility of pathogenic strains to antibiotics are important in both eradication and treatment (Klastersky, et al. 1971).

ANTIGENIC STRUCTURE

The antigenic structure of Staph. aureus is very complex, and more than 30 antigens were observed. The biologic and chemical properties of only a few number have been well characterized (Joklik, et al. 1984).

Staphylococci contain both polysaccharides and proteins antigens that permit a grouping of strains to a limited extent (Jawetz, et al. 1980).

Julianelle & Wieghard (1934 , 1935) extracted a polysaccharide from pathogenic Staph. aureus (type A) . A similar, but chemically and immunologically different substance (type B) was obtaind from non pathogenic staphylococci . They considered that virulence was associated with the presence of a specific polysaccharide.

Cellular antigens of Staph. aureus :

** Polysaccharide A :

A major antigenic determinant of all strains of Staph. aureus is the group-specific polysaccharide A of the cell wall. The serologic determinant of this polysaccharide is the N-acetyl-glucosaminyl ribitol unit of teichoic acid (Joklik, et al . 1984).

Although in the cell wall, polysaccharide A is associated with the peptidoglycan in an insoluble state and

requires lytic enzymes for release (Joklik, et al. 1984), there is evidence that some teichoic acid appears at the cell surface (James & Brewer, 1968) , and that antibody to it may be detected by agglutination (Sanderson, et al. 1961).

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 (Joklik, et al. 1984).

This group-specific polysaccharide A is not found in Staph. epidermidis, which contains instead glycerol teichoic acid with glycosyl residues rather than ribitol teichoic acid . In Staph. epidermidis the group-specific antigen is referred to as polysaccharide B (Joklik, et al. 1984).

** Protein A :

This group-specific antigen is the major protein component of the wall of Staph. aureus and is found in varying amounts in over 90% of Staph. aureus strains (Joklik, et al. 1984).

Protein A , is of special interest because it is precipitated by all normal human sera. This reaction is not secondary to antibodies formed as a result of constant contact with Staph. aureus as was believed (Joklik, et al. 1984).

of human IgG_1 , IgG_2 , IgG_4 , but not with IgG_3 (Kronvall & Williams, 1969 ; Skvaril, 1976 ; Duhamel, et al. 1979). It binds also to some human IgM and IgA_2 (Morse,1981).

The reactivity between protein A and IgG can be detected in Fc fragments and heavy chain preparations but not in $F(ab')_2$, Fab , F'c fragments, pepsin component 11 or 111 , as well as it is absent in light chain preparations (Kronvall, et al. 1970).

Protein A not only combines with human Ig, but it also reacts directly with the Fc portion of IgG of many mammalian species (Forsgren & Sjöquist, 1966 ; 1967 ; Forsgren, 1968 ; Kronvall, et al. 1970).

Protein A is constituted of 4 highly homologous Fc-binding regions, each consisting of approximately 60 amino acid residues (Joklik, et al. 1984).

Morse, (1981) found that the interaction with the Fc portion of IgG resulted in a variety of biologic effects including :

* Activation of complement by both the classical and alternative pathways .
* Hypersensitivity reactions as local wheal and flare reaction, the Arthus phenomenon, local and systemic

through competition with the Fc receptors of phagocytes for the Fc portion of opsonic antibody (Peterson, et al. 1977).

* In vitro induction of proliferation of both human T & B lymphocytes (Sakane & Green, 1978).
* Moreover, protein A in the cell wall may mask important antigenic sites or creat steric hindrance of specific opsonin due to its non-specific reaction with IgG (Dossett, et al. 1969).

Protein A is also a true antigen and reacts with the Fab portion of specific antibody (Morse, 1981).

** Capsular antigens :

Few strains have polysaccharide antigenic capsules (Parker, 1984). The observation of Price & Kneeland, (1954; 1956) suggested that many non-capsulated strains had similar material but in smaller amount.

The capsular antigen is found only in mucoid-untypable strains of Staph. aureus that lack detectable bound coagulase (clumping factor) (Joklik, et al. 1984). Capsulated strains give a negative clumping reaction, persumably because the clumping factor is covered by extracellular polysaccharides (Parker, 1984).

At least 4 antigenically distinct type-specific capsular antigens had been reported (Wiley & Maverakis,1968).

** Clumping factor (bound coagulase) :

Uncapsulated strains of Staph. aureus clumps when suspended in fibrinogen-containing solutions. There are apparently specific receptors on the bacterial surface for fibrinogen and the clumping is due to cross-linkage of the cells by fibrinogen . The receptors have not been characterized (Morse, 1981).

** Colony-compacting factor :

It is a surface component, thought to be protein A but Yoshida, et al. (1977) considered that, compacting factor was not clumping factor, protein A or teichoic acid, but a distinct polysaccharide.

Extracellular antigens of Staph. aureus :

Most of the extracellular substances wether enzymes or toxins produced by Staph. aureus are likewise antigenic (Jawetz,et al. 1980) .

DETERMINANTS OF PATHOGENICITY

The ability of Staph. aureus to survive in the animal host, may be attributed to their ability to produce extracellular enzymes ,toxins , and cellular components (Youmans, et al. 1975).

1- Surface Antigens :

Surface components that possess antiphagocytic activity are of obvious advantage to Staph. aureus in its initial establishment in the host (Joklik, et al.1984).

2- Extracellular Enzymes :

A- COAGULASES :

Staphylocoagulases occur in two forms :
Soluble or free coagulase which is demonstrated by tube method , and bound coagulase (clumping factor) that can be detected by slide test (Duguid, et al. 1978).

Soluble or Free Coagulase :

Pathogenic Staph. aureus is distinguished from non-pathogenic by its production of extracellular coagulase that clots citrated or oxalated plasma (Joklik, et al. 1984). This enzyme-like protein is produced during the logarithmic phase of growth (Cruickshank, 1937 ; Briody & Gillis, 1974).

Coagulase, does not clot fibrinogen directly but first reacts with a plasma constituent, coagulase-reacting factor (CRF), which is most probably prothrombin, to form a coagulase-CRF complex (thrombin-like substance) which acts on fibrinogen to form fibrin. Formation of the coaglulase-CRF complex is not dependant on calcium ions and is relatively insensitive to heparin , thus differing from thrombin (Briody & Gillis, 1974 ; Morse, 1981).

The coagulase-thrombin product not only causes fibrinogen clotting , but also possesses proteolytic and esterolytic activity similar to that of thrombin. Some of the fibrinopeptides released possess pharmacologic activity comparable to that of bradykinin on smooth muscle. This activity , together with defibrination , could perhaps, in the absence of anticoagulant, contribute to the overall manifestations of staphylococcal disease (Joklik, et al. 1984).

Also, coagulase may contribute to pathogenicity by inactivation of a bactericidal substance in normal serum or by protecting the cocci with a fibrin barrier against phagocytosis (Duguid ,et al. 1978).

Coagulase is antigenic . At least 7 antigenically distinct extracellular coagulases have been recognized, but the clotting of plasma by each coagulase proceeds according to the above outlined mechanism (Briody & Gillis, 1974).

Zen-Yoji ,et al. (1961) noted a correlation between coagulase antigenic type and phage group , and suggested that coagulase typing could be used on strains of non-phage-typable staphylococci . Abramson, (1972) noticed that a single strain produced a single antigenic type of coagulase .

Maximal coagulase production occurs at a pH between 7.3 and 7.9 , although growth is not optimal (Abramson, 1972). Pariza & Iandolo,(1969) found that active growth was not necessary for its production but active protein synthesis, stimulated by ribosomal regeneration was necessary for its production .

Bound coagulase (clumping factor) :

Duthie, (1954) introduced the term bound coagulase for a factor responsible for the clumping associated with the slide coagulase test. He indicated that the clumping factor was antigenically distinct from free coagulase .

Bound coagulase is not present in culture filtrates of Staph. aureus but is attached to the cell wall and can be libarated from it by autolysis. It converts fibrinogen directly to fibrin without (CRF) (Briody & Gillis, 1974).

B- HYALURONIDASE :
****

Nearly all strains of Staph. aureus produce it, but

in varying amounts .A clear relation between the amount of the enzyme formed and pathogenicity has not been established (Parker, 1984).

This enzyme hydrolyzes the hyaluronic acid present in the intracellular ground substance of connective tissue, thereby facilitating spread of infection. The importance of this enzyme is limited to the very early stages of infection (Joklik, et al. 1984).

C - STAPHYLOKINASE :

It is one of the proteolytic enzymes of Staph. aureus with fibrinolytic activity . The dissolution of clots by this enzyme is mediated by its activation of plasma plasminogen to the fibrinolytic enzyme plasmin (Jöklik, et al. 1984).

D- NUCLEASE :

The elaboration of a heat-resistant nuclease, appears to be uniquely associated with Staph. aureus strains (Chesbro & Auborn ,1967).

The ability of a microorganism to produce nuclease per se , unless the nuclease is shown to be heat resistant, is not diagnostic of Staph. aureus (Stickler & Freestone, 1971).

It is present in the cell at or near the cell surface

It is a compact globular protein consisting of a single polypeptide chain . It can cleave either DNA or RNA (Joklik, et al. 1984).

E- STAPHYLOCOCCAL PHOSPHATASES :
****

Both acid and alkaline phosphatases are produced by almost all strains of Staph. aureus (Malveaux and San Clemente, 1967 ; Tirunarayanan , 1968).

Barber & Kuper, (1951) reported a close correlation between coagulase and phosphatase production , and used phenolphthalin phosphate agar for its detection.

These enzymes are not specific for Staph. aureus and a number of other staphylococci , micrococci , and other organisms produce them (Baird-Parker, 1963).

F- LIPASES :
****

Staphylococci produce several lipid-hydrolyzing enzymes collectively referred to as " lipases " . The lipases are active on a variety of substrates including plasma and fats and oils that accumulate on the surface areas of the body . The production of lipase is apparently essential in the invation of healthy cutaneous and subcutaneous tissues (Joklik, et al. 1984).

G- PENICILLINASE ENZYME

groups 1 and 111 to produce penicillinase enzyme which opens the β - lactam ring of the penicillin molecule thus inactivates it . Penicillinase is a genetically constitutive enzyme whose phenotypic expression is enhanced by the presence of penicillin substrate (Briody & Gillis,1974).

Penicillinase enzyme is generally coded for on a plasmid and rarely on the chromosome (Dyke & Richmond, 1967 ; Peyru, et al. 1969). And can be transferred to organisms which lack it by phage transduction (Briody & Gillis, 1974).

At least 4 immunological types of staphylococcal penicillinase are known (Richmond, 1965 ; Rosdahl, 1973). These types differ in their specific activity and in the extent to which they appear extracellularly (Parker, 1984).

3- Toxins :

Staph. aureus produces a complex series of filtrable toxins many of them have been studied and shown to be of importance in the pathogenesis of staphylococcal infection (Williams Smith,1959).

Four major groups of staphylococcal toxins have been defined on the basis of their biologic activity : Cytolytic toxins (haemolysins & leucocidin), enterotoxins, epidermolytic toxin, and pyrogenic exotoxins (Joklik, et al. 1984).

CYTOLYTIC TOXINS :

The haemolysins and leucocidin elaborated by Staph. aureus are the best defined members of this group. They are extracellular proteins , induce the formation of neutralizing antibodies and are antigenically distinct from the other staphylococcal toxins (Joklik, et al.1984).

1- The haemolytic toxins :

Four distinct haemolysins are produced by Staph. aureus and many strains manufacture more than one type (Wiseman, 1975). All cause β (clear) haemolysis . They have different lytic spectra with respect to the susceptibility of various erythrocyte species and are also cytotoxic for cells other than erythrocytes . The haemolysins are antigenic proteins and their activities are neutralized by specific antisera (Morse, 1981).

a- Alpha-Haemolysin :

Is the most commonly encountered toxin in clinical isolates of Staph. aureus (Morse, 1981).

It is a heat-labile protein with a molecular weight of 44,000 , it can be converted to a toxoid by formalin treatment (Briody & Gillis, 1974).

This toxin exhibits a wide range of biologic activities including the haemolytic, lethal, leucocidal,and dermonecrotic effects . Alpha-toxin disrupts lysozymes and

is cytotoxic for a variety of tissue culture cells . Human macrophages and platelets are damaged but monocytes are resistant . There is injury to the circulatory system, muscle tissue, and renal cortex tissue (Joklik, et al. 1984).

The toxin lysis rabbit erythrocytes rapidly at 37 °C ; sheep cells are less sensitive to its action but horse and human cells are almost completely resistant (Joklik, et al. 1984 ; Parker, 1984). Reaction with erythrocytes involves two sequential steps ;

1- An initial interaction between toxin and cells that result in the prelytic release of K^+.

2- Actual lysis of the cell and release of haemoglobin . The precise mechanism by which alpha-toxin damages the cell membrane has not been establashed (Joklik, et al.1984).

Formation of alpha-toxin in broth is better when it is incubated in 20% CO_2 or if 0.8% nutrient agar is incubated in 20% CO_2 for 24 hours (Briody & Gillis,1974).

b- Beta-Haemolysin (Staphylococcal sphingomyelinase) :

This toxin is found in many strains of animal origin but is produced by less than 20% of human strains (Morse, 1981).

The most striking activity of this toxin is its ability to produce a hot-cold lysis, i.e. an enhanced haem-

olytic activity if incubation at 37 °C is followed by a peroid at 4 °C or at room temperature .

The toxin is an enzyme with substrate specificity for sphingomyelin and lysophosphatides. Sphingomyelin degradation is the membrane lesion that leads to haemolysis when the cells are chilled :

$$\text{Sphingomyelin} + H_2O \xrightarrow[Mg^{2+}]{\beta\text{-toxin}} \text{N-acylsphingosine} + \text{phosphorylcholine.}$$

Erythrocytes from different animal species exhibit impressive differences in their sensitivity to β-toxin . A correlation exists between toxin sensitivity and content of sphingomyelin , most of which is located in the outer leaflet of the lipid bilayer of the erythrocyte membrane and thus accessible to exogenous toxin (Mudd, 1970).

This toxin acts on sheep and ox but not on rabbit or human red corpuscles . Staphylococci that produce β -lysin give rise to zones of darkening around the bacterial growth on sheep blood agar plates . β-lysin is formed in absence of CO_2, though its production is enhanced by this gas

It is antigenically distinct from alpha-lysin and is not dermonecrotic (Parker, 1984).

c- Gamma-Haemolysin :

It is a protein that is antigenically distinct from

other haemolysins, It acts strongly on sheep , rabbit , and human erythrocytes . Also, it has some leucocidal action but it is not dermonecrotic (Parker, 1984).

Gamma toxin consists of two components which act synergistically, both being necessary for haemolysis and toxicity , but detailed information on the chemistry and biologic effects of it is lacking (Joklik, et al. 1984).

Gamma-toxin is inhibited by sulfonated polymers including agar, and hence its activity is not readily seen on blood agar plates (Morse, 1981).

d- Delta-Haemolysin :

This toxin is a relatively thermostable surface active toxin . It is an antigenic protein , consists of low molecular weight subunits and has a broad range of lytic and cytotoxic activity, probably due to a nonspecific detergent like action on membranes (Morse, 1981).

Erythrocytes, macrophages, lymphocytes , neutrophils and platelets are damaged by it . It has also a similar effect on spheroplasts and protoplasts of other bacteria. Its activity is inhibited by phospholipids in the serum (Morse, 1981; Joklik, et al. 1984).

Hoffmann & Streitfeild,(1965) found that this toxin had antibiotic activity on certain Gram +ve organisms ,

but this activity now is in dispute .

2- Leucocidin :
****

In addition to the leucocytotoxic effects of some of the haemolysins, Staph. aureus produces a distinct non-haemolytic leucocidal substance, Panton - Valentine (PV) leucocidin (Morse, 1981).

It attacks polymorphonuclear leucocytes and macroph-ages but no other cell type is attacked. The unique response of leucocytes to it , is an altered permeability to cations and the other changes which occur are secondary to this initial event , resulting in degranulation of leucocytes (Joklik, et al. 1984).

The toxin is composed of two proteins which are electrophoretically separable , the F (fast) and S (slow) components. They act synergistically on the cell membrane of the leucocyte to induce its cytolysis. Each component is inactive alone. Both of them are highly antigenic and have been toxoided (Joklik, et al. 1984).

Leucocidin alone is not responsible for pathogenicity, but it enhances staphylococcal invasiveness by resisting phagocytosis (Joklik,et al. 1984).

11- ENTEROTOXINS :

It has been estimated that at least 50% of Staph. aureus strains produce enterotoxins (Bergdoll,1972) that give rise to staphylococcal food poisoning , when the toxin is ingested in the food (Parker, 1984).

Enterotoxins are trypsin-resistant proteins that resist boiling and have molecular weights of 28000-35000 , Their mode of action is uncertain (Parker, 1984). But, it appears that , the emetic receptor site of staphylococcal enterotoxin is the abdominal viscera , from which the sensory stimulus reaches the vomiting center via the vagus and sympathetic nerves . Enterotoxin induced diarrhea has been attributed to inhibition of water absorption from the lumen of the intestine and to increased transmucosal fluid flux into the lumen (Joklik, et al. 1984).

Enterotoxins are a potent mitogen for lymphocytes . They are pyrogenic and enhance Gram -ve lethality .

Six types (A, B, C_1, C_2, D, E), of which five are noncross-reacting , have been identified . Enterotoxins A and D are frequently associated with food poisoning ; enterotoxin B is the toxin usually associated with hospital infection. A new staphylococcal enterotoxin , enterotoxin F , has recently been isolated from toxic shock syndrome(Joklik, et al. 1984). Bergdoll, et al. (1974 ; 1976) found that many unidentified enterotoxins were pro-

duced by a large number of enterotoxigenic Staph. aureus.

Production of enterotoxin is confined primarily to phage groups lll and lV . Potent toxoids to the enterotoxins may be produced by treatment with acidic or neutral formaldehyde solution (Joklik, et al. 1984).

A plasmid coding for enterotoxin production has been found , but recent evidence suggests that the genetic determinants may be chromosomal in nature (Shafer & Iandolo, 1978).

lll- EXFOLIATIVE TOXIN (EXFOLIATIN, EPIDERMOLYTIC TOXIN):

Coagulase positive staphylococci of phage group ll have the capacity to produce a soluble protein which is associated with a spectrum of dermatologic diseases that includes generalized exfoliation , localized bullous impetigo , and generalized scarlatiniform eruption . On the basis of clinical similarities and common causative agent the term "Staphylococcal Scalded Skin Syndrome" has been proposed to describe these clinical manifestations (Briody & Gillis, 1974).

The action of the toxin is to cause separation of epidermal cells along the line of cleavage just below the stratum granulosum , but the cells are apparently not killed (Parker, 1984).

referred to as epidermolytic toxins A and B , have been isolated (Kondo, et al. 1975). Both have molecular weights of about 25,500 but they differ in other physical properties .Toxin A is sometimes called the stable toxin because it is resistant to boiling . Toxin B is referred to as labile toxin as it is inactivated at 60 °F . Toxin A is chromosomally determined , but toxin B may be extrachromosomally mediated as it can be eliminated by DNA intercalating agents (Warren , et al. 1974).

1V - PYROGENIC EXOTOXIN :

They are proteins of low molecular weight that are pyrogenic in rabbits and mice . They enhance susceptibility to endotoxic shock. Also they are potent non-specific mitogens for lymphocytes, and are immunosuppressive . They are antigenically distinct from all other staphylococcal extracellular products (Parker, 1984).

Three antigenic types of pyrogenic exotoxin have been described by Schlievert, et al. (1981). Types A and B are formed by most strains of Staph. aureus but type C appears to be more limited in distribution . Pyrogenic exotoxins have not been identified in Staph. epidermidis (Parker 1984). Staphylococcal toxic shock syndrome is attributed to the tpye C pyrogenic exotoxin (Schlievert, et al. 1981; Parker, 1984).

STAPHYLOCOCCAL INFECTIONS

Staph. aureus, which is part of man's normal flora, will produce local skin infections at some time in the life of most individuals . Problems in host defenses predispose to serious local infections, and bacteremia with or without metastatic abscess formation (Shulman & Nahmias, 1972).

** PREDISPOSING FACTORS :

1- Skin :

The skin is an excellent barrier to bacterial infection, any disruption of its continuity predisposes to staphylococcal invasion (Elek, 1956). Abrasions, wounds, surgical incisions, burns, skin areas affected by various cutaneous viral infections and exfoliative dermatitis are particularly prone to become infected by staphylococci (Colebrook, et al. 1948 ; Cluff et al. 1968).

Local staphylococcal infection, may result from bypassing skin barrier by drug addictors (Hussey & Katz, 1950) and rarely from jetgun immunization (Kassanoff,et al. 1971).

2- Prior viral infections :

Certain viral infections may predispose to staphylococcal infections . Sever tracheobronchitis caused by influenza viruses permits invasion of the lower respiratory tract by staphylococci (Louria, et al. 1959). This increased susceptibility is due to the ability of this organism to survive and even multiply in the viscid pulmonary secretion (May & Roberts, 1969).

3- Deficiencies in humoral immunity :

Patients with defects in serum opsonins or in complement components may be unable to phagocytize staphylococci adequetly, so are prone to infection (Miller & Nilsson, 1970).

4- Leucocyte defects :

Pateints with disorders of chemotaxis, phagocytosis and intracellular bacterial killing by polymorphonuclear leucocytes are susceptible to serious staphylococcal infection (Shulman & Nahmias, 1972).

High susceptibility is also found in individual with various forms of granulocytopenia , either congenital or

5- Presence of foreign bodies :

Their presence in any site increase susceptibility to staphylococcal infections . A large variety of them have been implicated including sutures,plastic intravenous catheters,vascular grafts or pace makers,various types of prosthesis,and atrioventricular plastic shunts employed to correct hydrocephalus (Elek, 1956 ; Bahnson, et al. 1957 ; Phillips & Tyre, 1958 ; Smith & Freeman, 1967 ; Bentley & Lepper, 1968 ; Collins et al. 1968 ; Rohan & Miller, 1969 ; Eykyn, et al. 1970).

6- Abuse of antibiotics :

Patients who are given antibiotics which are not effective against the staphylococci fall in the susceptible group (Louria & Kaminski, 1962).

7- Hospitalized debiletated patients :

Those who have serious underlying disease or who have undergone extensive surgery are susceptible to infection with staphylococci (Joklik, et al. 1984).

8- Miscellaneous illnesses with less-well-understood defects in host resistance :

As diabetes mellitus, alcoholism, mucoviscidosis, coro-

** CLINICAL MANIFESTATIONS :

Two types of diseases are produced by Staph, aureus, invasive and toxinogenic type.

INVASIVE LESIONS OF STAPH. AUREUS :

No single component or product of Staph. aureus has been shown to be the primary determinant of either the initiation or the progression of invasive lesions.

Therefore, many factors may be involved (Morse, 1981).

According to the portal of entry, pathogenesis, and prognosis Shulman & Nahmia, (1972) divided the invasive type of Staph. aureus diseases into 2 major groups ;

1- Infections at local sites with or without spread :

The basic lesion in this group of diseases is the abscess formation with a central core of dead leucocytes and bacteria.

a- Skin :

Staph. aureus infection of the skin is the most common of all bacterial infections (Shulman & Nahmias, 1972). It can enter through a break in the skin or through hair follicle or ducts of sweat or sebaceous glands (Nolan & Beaty, 1976).

Depending on the location and

infection occurs in newborn infants (Briody & Gillis, 1974).

Recurrence of staphylococcal skin infection in the same individual is a common event (Johnson, et al. 1960). Such patients are usually persistent nasal carriers of Staph. aureus . Perineal carriage is important for recurrent lesions below the waist (Tullach,et al. 1960).

b- Eye :

It is another common site of Staph. aureus infection with the conjunctiva being most frequently involved. The sebaceous glands of the eyelids may be infected. Infection may also follows occular surgery (Shulman & Nahmias, 1972).

c- Paranasal and pharyngeal infection :

Staphylococci can involve any of the paranasopharyngeal areas such as parotids, sinuses, peritonsillar and retropharyngeal regions (Shulman & Nahmias, 1972).

d- Primary staphylococcal pneumonia :

When staphylococci are present in the upper respiratory tract, they may be aspirated into the lung, producing a primary staphylococcal pneumonia (Shulman & Nahmias, 1972). Infants under the age of 6

it frequently follows viral infections as influenza or measles (Petersdorf, et al. 1959 ; Miller & Jay, 1962).

Patients with underlying illness as leukemia, cancer, or chronic lung disease, and other hospitalized patients may develop a staphylococcal superinfection (Shulman & Nahmias, 1972).

This type of pneumonia is patchy and is characterized by the presence of abscesses, empyema and often terminates fatally (Briody & Gillis, 1974).

e- Urogenital infections :

Staphylococci infrequently produce local infections in the urethera and may involve the bladder with or without an associated ascending pyelonephritis (Many, et al. 1967).

Staphylococcal abscesses of Bartholin's glands are frequent (Gati & Toth, 1967). Also Staph. aureus can cause prostatitis (Magid & Khafaga, 1965).

f- Breast infection :

Staphylococcal mastitis may occur in newborns and in women, particularly in the puerperium (Smith,1958 ; Goodman & Benson, 1970).

g- Staphylococcal pseudomembranous enterocolitis :

This infection occurs primarily in hospitalized patients

whose normal bowel flora has been suppressed by the oral adminestration of wide spectrum antibiotics that selectively permit overgrowth of drug-resistant entero-toxin producing strains of staphylococci (Joklik, et al. 1984).

This acute colitis is characterized by diarrhea, dehydration, fever, abdominal pain, vomiting and in some cases shock and death (Briody & Gillis, 1974).

2- Metastatic or systemic staphylococcal infections :

Trauma and debilitating diseases predispose to staphylococcal bacteremia resulting in profound toxaemia and multiple abscesses formation (Joklik, et al. 1984).

Sources of bacteremia are varied and sometimes obscure (Cluff, et al. 1968). However, the skin, the respiratory tree, or the gastrointestinal tract is the usual primary site of infection (Jensen, et al. 1969).

The most common sites in which the staphylococci are lodged include : lungs , bones , kidneys , brain , heart, spleen , and skin tissues (Cluff, et al. 1968).

TOXINOGENIC LESIONS OF STAPH. AUREUS :

1- Staphylococcal food poisoning :

strains . The food is usually contaminated by food handlers who have the organisms on their hands (Joklik, et al. 1984).

Contamination of food frequently occurs after cooking if it is held at a temprature which permits staphylococcal growth and multiplication. Proper refrigeration i.e. at temprature below 7 °C will prevent growth and toxigenesis (Youmans, et al. 1975).

A variety of foods have been implicated in epidemics of Staph. aureus food poisoning but ham products , cold meats , salads , cream filled desserts and custards are commonly involved (Braude, et al. 1981).

All types of enterotoxins have been implicated in outbreak of food poisoning , although types A ,B and D are most common (Braude, et al. 1981).

Symptoms which appear abruptly 2-6 hours after ingestion of the food consist of sever cramping , abdominal pain , nausea ,vomiting , and diarrhea . Sweating and headache are seen but fever is not a common feature. Recovery is usually rapid, occurs within 6-8 hours (Joklik, et al. 1984).

2- Staphylococcal Scalded Skin Syndrome :
****

Staph. aureus is associated with a spectrum of derm-

diffuse exfoliative disease with systemic toxaemia. These conditions are caused by an epidermolytic toxin that is produced only by Staph. aureus belonging usually but not exclusively to phage group 11 especially type 71 (Jefferson, 1967 ; Lyell et al. 1969 ; Melish & Glasgow, 1970 ; Melish, 1981).

There are three basic syndroms that together are termed the Staphylococcal Scalded Skin Syndrome (SSSS) (Melish & Glasgow, 1970). These syndromes are :

1- Bullous impetigo.

2- Diffuse exfoliative disease.

3- Staphylococcal scarlatiniform eruption.

The (SSSS) primarily affects neonates and children under 4 years of age (Joklik, et al. 1984).

3- Toxic Shock Syndrome (TSS) :
****

Toxin producing strains of Staph. aureus have been implicated in this multisystem disease that primarily affects young women. Onset of illness usually occurs during menses (Joklik, et al. 1984).

IMMUNITY

Humans are highly resistant to infection by the staphylococcus . Billions of the organisms must be introduced in order to elicit an observable response . How much of this resistance is of a natural type and how much is acquired, in response to repeated natural exposure to organisms that constitute part of the normal flora, have not been defined (Joklik, et al, 1984).

The interaction between staphylococci and phagocytic cells plays an important role in the critical early stages of infection . Chemotactic activity is generated by a number of different mechanisms . Early in the infection , staphylococcal proteases generate their own chemotactically active fragments of complement components. Later, specific antibody may generate chemotactic activity through the classic complement pathway . By virtue of their ability to activate complement , all major cell components of Staph. aureus contribute to the generation of chemotactic factors (Joklik, et al. 1984).

Phagocytosis of uncapsulated strains of Staph. aureus is promoted by either complement or antibody , but capsulated strains require both of them . Once staphylococci

degraded within the phagocytic vacuoles (Joklik, et al 1984).

Antibodies to a variety of components and products of Staph. aureus are found in normal humans , but there is no correlation between the presence of these antibodies and the occurrence and the progression of disease (Morse, 1981).

In experimental animals , cell-mediated immunity may restrict the spread of organisms from the inoculation site but local reactions are often more severe . Thus, the characteristic morphology and confinement of the staphylococcal abscess may be related to the effects of cell-mediated immunity (Morse, 1981).

Material & Methods

MATERIALS

1- CASES EXAMINED :

68 patients with different pyogenic lesions were examined for staphylococcal infections .

11- MEDIA :

Media used in this study were prepared according to the manufactory instructions .

a- ORDINARY MEDIA :

1- Nutrient Agar (lab m),

2- Standard Meat Extract Broth (Cruickshank, et al. 1975).

3- Sheep Blood Agar (Cruickshank, et al. 1975).

b- SELECTIVE MEDIUM :

1- Mannitol Salt Agar (bio Mérieux).

c- MEDIA FOR STAPHYLOCOCCAL ACTIVITIES :

1- DNase Agar (Oxoid).

2- Milk Agar (Christie & Keogh, 1940).

3- Mannitol test medium (Cruickshank, et al. 1975).

111- CHEMICALS AND REAGENTS :

1- Human Plasma : Fresh plasma obtained from human

2- Peptone Water (Cruickshank, et al. 1975).

3- Normal Hcl .

4- Glycine Saline Buffer pH. 8

5- Phosphate Saline Buffer pH. 7.4

6- Latex Reagent (Essers & Radebold, 1980).

Latex suspension (Dow) was diluted 1 : 8 with glycine saline buffer (pH 8) . Equal volumes of the diluted latex suspension and human plasma (Ethylene-diamine-tetra-acetate treated , diluted 1: 1,000 with glycine-saline buffer) were incubated for 30 min at 56 °C in a shaking bath . The coated particles were washed two times with saline and suspended in phosphate-buffered saline(pH 7.4) contaning 0.02 % sodium azide and 0.05 % human plasma .

1V- OTHER MATERIALS :

1- Dry clean glass slides .

2- Sterile cotton swabs (bio Mérieux).

3- Test tubes .

4- Petri dishs .

METHODS.

1- Identification of the causative bacterial agents :

From 68 pyogenic lesions , sterile cotton swabs were taken and plated directly without delay on each of the following media :

1- Nutrient agar ,

2- Sheep blood agar .

3- Mannitol salt agar .

Also , direct films stained by Gram stain were prepared.

The plates were incubated for 24 h. at 37 °C and the suspected Staph. colony was examined .

1- On nurtient agar :

Colony was smooth , glistening , opaque , about 2-3 mm in diameter , butyrous in consistency . Pigmentation was not clearly apparent and varied between white and golden yellow .

2- On sheep blood agar :

Colony was surrounded by a zone of clear heamolysis .

3- On mannitol salt agar :

Colony was surrounded by a yellow zone due to

prepared .

The colony proved to be staphylococcus was subcultured on agar slope , incubated for 24 h. at 37 °C and the growth was kept in the ice-chest for further study .

2- Biochemical and biological tests for Staph. aureus :

a- PIGMENT PRODUCTION :
****

By growing the isolated culture on milk agar at 37 °C for 24 h. , the pigment produced was easily detected .

b- MANNITOL FERMENTATION TEST :
****

Inoculating the mannitol tubes with the test culture and after 24 h. incubation at 37 °C the tubes were examined,

Change of the colour from yellow to pink indicates acid production i.e. positive test .

c- COAGULASE PRODUCTION :
****

1- Slide Coagulase Test :- (Williams & Harper, 1946).

On the surface of a clean dry slide, a drop of saline (0.85 % NaCl) solution or water was placed , in which one

Coarse clumping becoming visible to the naked eye within 5 - 10 sec is a positive result . A slower reaction is a negative result .

2- Tube Coagulase Test : (Gillespie, 1943).

Citrated human plasma obtained freshly at the time of the test was diluted 1 : 10 with saline (0.85 % NaCl). 1 ml of the diluted plasma was placed in small tube which was inoculated with 0.1 ml of an 18- 24 h. broth culture of each sample to be tested .

The tubes were incubated at 37 °C and examined after 1 , 3 , 6 , hours .

Appearance of clot indicated positive result . If no clot appeared , tube was left up to 24 h. at room temprature and examined at intervals to detect the appearance of any clot .

Control tubes of known coagulase positive (coagulase +ve) and coagulase negative (coagulase -ve) cultures and a tube of uninoculated plasma were done .

The conversion of the plasma into a soft or stiff gel , indicates a positive result .

d- DNase ACTIVITY : (Blair, et al. 1967).
****

The organisms to be tested were either streaked or

spotted on a small area of the DNA plate . The plates then incubated at 37 °C for 18 - 24 h.

Flooding of the plates with Normal Hcl would precipitate the DNA and turn the plate cloudy .

The appearance of a zone of clearance (absence of turbidity) around the colony indicates DNase production and a positive result .

e- LATEX AGGLUTINATION TEST ! (Essers & Radebold,1980).

****

One to two colonies were emulsified homogeneously on a clean dry glass slide with one drop saline (0,85 % Nacl) and one drop of the latex reagent . the slide then was rocked backward and forward for about one minute and the result was observed .

Appearance of visible agglutination indicates positive result .

Fig (1) : Haemolytic activity of different isolates of Staph. aureus on sheep blood agar plates.

Fig (2) : Mannitol fermentation by Staph. aureus on mannitol salt agar.

Fig (3) : DNase agar medium revealing two types of Staph. colonies

A +ve = Colony surrounded by a zone of clearance .

B