Staphylococcal Infections In The Nineties

Thesis
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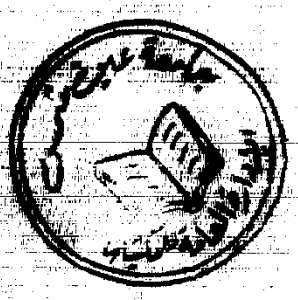

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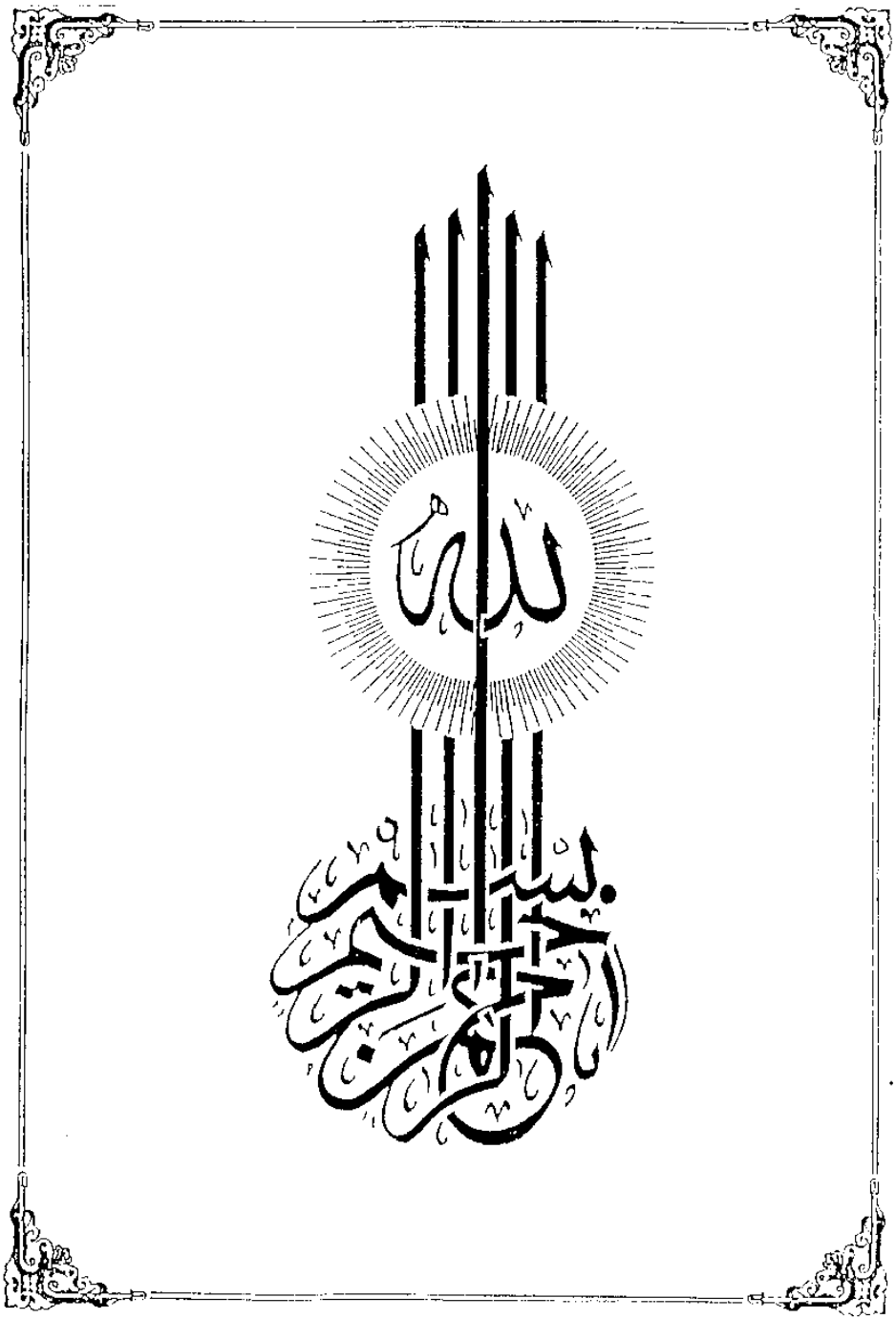

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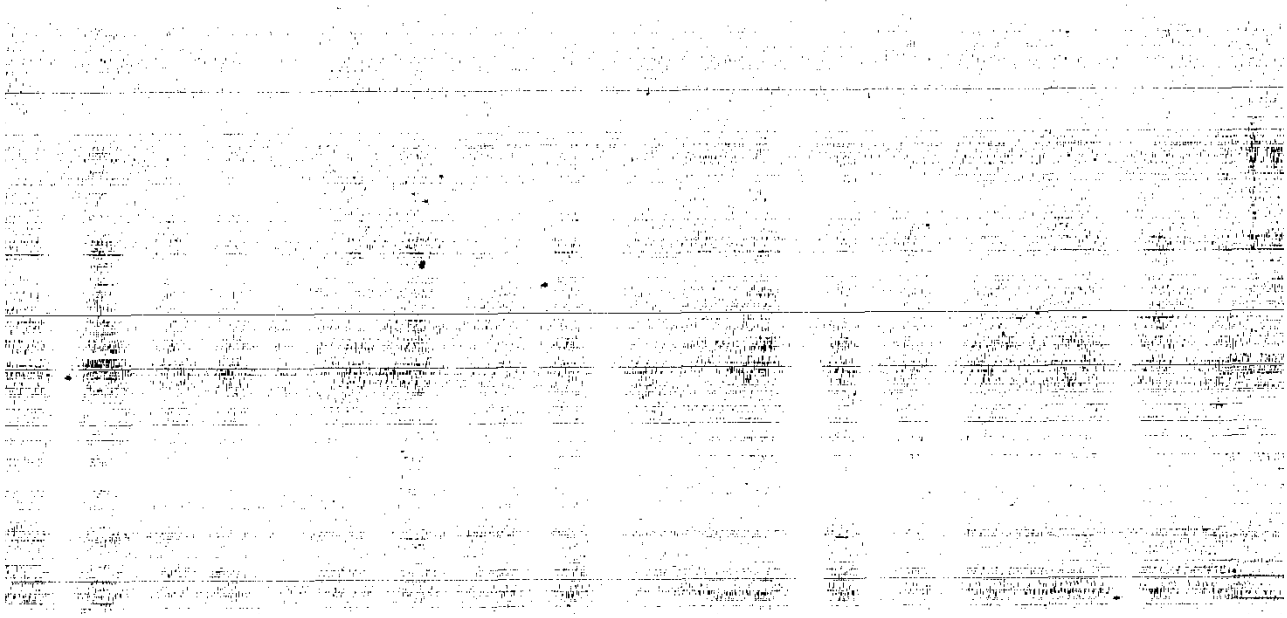


LIST OF ABBREVIATIONS

MRS	methicillin-resistant staphylococci
MRSA	methicillin-resistant *Staphylococcus aureus*
CNS	coagulase-negative staphylococci
CSF	cerebrospinal fluid
MIC	minimum inhibitory concentration
MBC	minimum bactericidal concentration
PMNL	polymorphonuclear leucocyte
TSS	toxic shock syndrome
iv	intravenous
im	intramuscular
UTI	urinary tract infection
Tn	Transposon
IS	insertion sequence
R	resistance
PBPs	penicillin binding protein
TMP-SMX	trimethoprim-sulphamethoxazole
H-acq	hospital-acquired
Comm. acq	community-acquired
Isol.	isolate
Inf.	infection
Interv.	intervention

INTRODUCTION
AND
AIM OF THE WORK

INTRODUCTION AND AIM OF THE WORK

Staphylococci may not be the most notorious of the microbial enemies of man, but they are certainly the most familiar of the bacterial pathogens and were the first to be recognised in clinical material. Once the germ theory of disease had received general credence in the latter end of the 19th century, it was inevitable that staphylococci should be quickly discovered in pus. In the event, it was the Scottish surgeon, Alexander Ogston, in a paper read to the 9th Congress of the German Chirurgical Society in 1880, who provided the first systematic description of the role of staphylococci in infection.

Indeed, no organism is as versatile and resilient as *Staph. aureus*. Not only is it a formidable pathogen with a battery of virulence factors, but it is the most elusive of opponents, requiring all the resources of the antimicrobial armamentarium to hold it in check. The other 'pyogenic cocci' have been more or less controlled by antibiotics, but *Staph. aureus*, despite being inherently susceptible to most antibacterial agents, has repeatedly demonstrated its ability to recover from a succession of 'knock-out blows' (Greenwood, 1986).

The range of activities of *Staph. aureus*, the most important species, stretches from passive commensalism, to severe, life-threatening sepsis and the elaboration of extremely potent toxins. The sudden appearance a few years

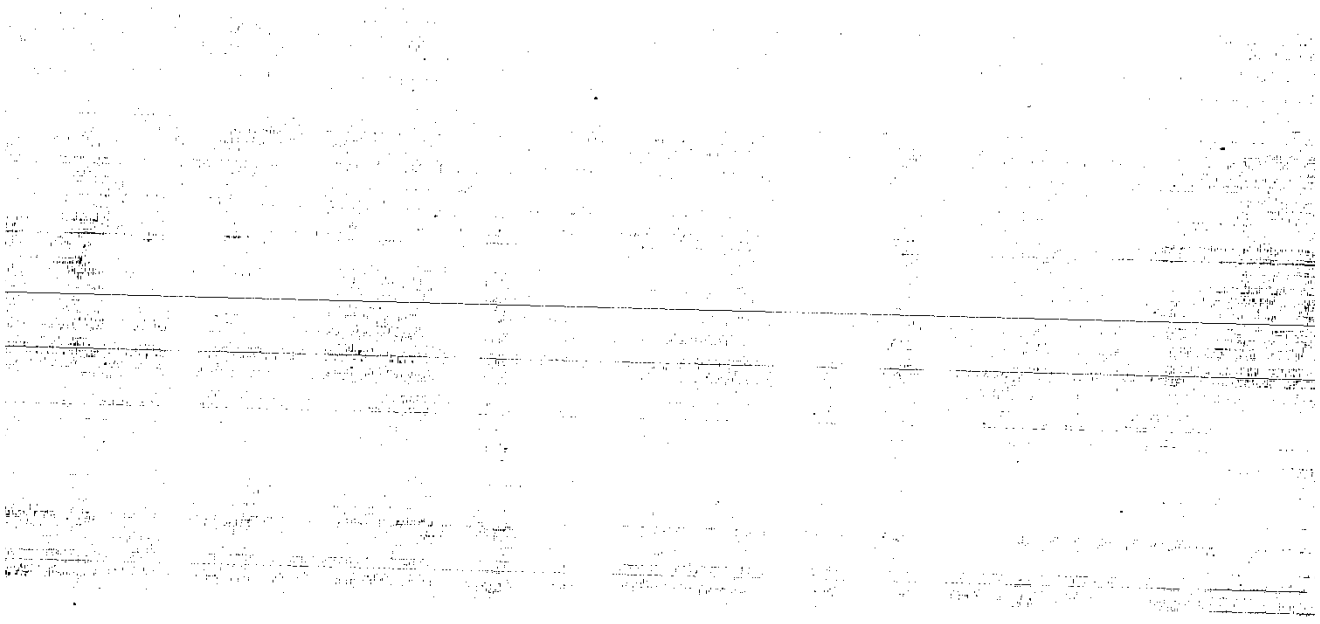
methicillin and other antistaphylococcal drugs. However, *Staph. aureus* was able again to overcome their activity by the selection of methicillin-resistant strains (MRSA) (Haley et al., 1982). These are usually resistant to several other antibiotics (Kayser, Berger-Bachi and Beck, 1986). At present, vancomycin and teicoplanin are the most active against MRSA; so far resistance to them seems rare or absent (Williams and Grunebery, 1984). However, knowing the past, we have to ask whether this situation will hold in the future.

The mechanisms underlying the expression of resistance of *Staph. aureus* to penicillin and methicillin are different. But, in addition to resistance, *Staph. aureus* can also show the phenomenon of tolerance; the absence or suppression of the normal autolytic enzyme system in the bacterial cell renders the minimal bactericidal concentration (MBC) of bactericidal antibiotics many folds higher than the minimal inhibitory concentration (MIC) (Tomasz, Durack and Tomasz, 1986).

Resistance to some older antistaphylococcal agents such as fusidic acid has remained relatively uncommon over the years. There is a need to re-assess both old and new antistaphylococcal agents in order to deal adequately with methicillin-resistant *Staph. aureus* (MRSA), and to improve the treatment of severe staphylococcal sepsis.

Several studies have been carried out in different countries to assess the problem of staphylococcal infection especially MRSA. This work aims at measuring the size of such a problem in an Egyptian hospital. Also, to determine the sensitivity pattern of staphylococci isolated from hospital-aquired infections and those isolated from community-aquired ones. It also aims to study the effect of treatment of infections due to resistant staphylococci by the suitable antimicrobial agent.

REVIEW OF LITERATURE



STAPHYLOCOCCUS AUREUS

Microbiology

Morphology:

The term Staphylococcus is derived from the Greek expression staphyle (bunch of grapes), and it reflects its characteristic microscopic arrangement in clusters.

Microscopically, *Staph. aureus* is a gram-positive organism, with a diameter of 0.7-1.2 um. These cocci occur singly, in pairs, in short chains, and have a tendency to form clusters, because cell division occurring in the three perpendicular planes does not lead to full separation of the daughter cells. Cluster formation is favoured by culturing the organism on solid media. These properties, although most often present in laboratory strains, sometimes are missing in clinical specimens and can lead to erroneous diagnoses; thus, cells in stationary phase or ingested by phagocytes occasionally appear as gram-negative on smear; clustering can be very limited in liquid media (Waldvogel, 1990). Thus, single cocci, pairs, tetrads, and chains are also seen in liquid cultures. Young cocci stain strongly gram-positive; on ageing, many cells become gram-negative.

Staphylococci are nonmotile, non-spore-forming, facultative anaerobes. They ferment a wide variety of sugars to form acid but no gas. This property permits a differentiation from the large number of avirulent but morphologically similar members of the genus Micrococcus.

All micrococci are obligate aerobes, and, although they oxidize many sugars, they do not produce acid (Volk et al., 1991a).

Macroscopically, *Staph. aureus* is characterized by rapid growth under both aerobic and anaerobic conditions on blood agar and other nonselective solid media. Individual colonies are sharply defined, smooth, and convex, with a diameter of 1-4 mm. The basic golden yellow pigmentation, due to carotenoids, may not be readily apparent under certain conditions (e.g. growth under anaerobic conditions or in liquid medium) and may be visible only as a beige hue. Pigment production can be enhanced by further incubation at room temperature for 24-48 hours. Most strains of *Staph. aureus* produce haemolysis within 24-36 hours on horse, sheep, or human blood agar plates (Waldvogel, 1990).

Staphylococci are relatively resistant to drying, heat (they withstand 50°C for 30 minutes), and 9% sodium chloride but are readily inhibited by certain chemicals, e.g. 3% hexachlorophene (Jawetz et al., 1989a).

Identification:

Within the family Micrococcaceae, the human pathogenic genus Staphylococcus can be separated from the nonpathogenic genus Micrococcus by various tests, including: (1) anaerobic acid production from glucose, (2) sensitivity to 200 ug/ml of lysostaphin, and (3) production of acid from glycerol in the

presence of 0.4 ug/ml of erythromycin, all three tests being positive in the case of staphylococci (Waldvogel, 1990).

Further subclassification into the three main species (associated with humans). *Staph. aureus*, *Staph. epidermidis* and *Staph. saprophyticus* is of clinical importance. Staphylococcus aureus is fully identified by its positive reaction in the following tests :

(1) catalase - a test that differentiates them from the catalase-negative streptococci; (2) coagulase - a test allowing differentation between *Staph. aureus* and *Staph. epidermidis*, which is coagulase-negative. It is based on the action of either a cell-bound bacterial enzyme acting directly on fibrinogen or on the action of an extracellular enzyme on a modified thrombin molecule in rabbit plasma - the complex reacts in turn with fibrinogen to produce a fibrin clot in the absence of Ca^2; (3) mannitol fermentation - which most often allows differentiation between *Staph. aureus* (always positive) and *Staph. epidermidis* (rarely positive). The reaction is based on the property of *Staph. aureus* that degrades the polyhydric alcohol mannitol into acid compounds under anaerobic conditions; and (4) deoxyribonuclease test - most *Staph. aureus* give a positive reaction, as opposed to *Staph. epidermidis*. The test is based on the differential solubilization of whole DNA, or fragments thereof, in acid. Finally, novobiocin resistance allows identification of *Staph. saprophyticus* (Waldvogel, 1990).

Further division of *Staph. aureus* uses phage typing to assign an unknown strain to one of four phage groups. In practice, one places a small drop of each phage group into a plate previously seeded with the unknown strain of *Staph. aureus*. After overnight incubation, clear plaques of lysis allow one to rank the unknown strain in one of the phage groups I-IV. This is difficult and is only done in large diagnostic centers for epidemiologic studies (Volk et al., 1991a).

Microbiologic determinants of infection due to *Staph. aureus*

A. Important Cell Wall Constituents (Antigenic Structure):

Peptidoglycan, a polysaccharide polymer containing linked subunits, provides the rigid exoskeleton of the cell wall. Peptidoglycan is destroyed by strong acid or by exposure to lysozyme. It is important in the pathogenesis of infection: it elicits production of interleukin-1 (endogenous pyrogen and opsonic antibodies by monocytes; and it can be a chemoattractant for polymorphonuclear leucocytes, has endotoxin like activity, produces a localized Shwartzman phenomenon, and activates complement (Jawetz et al., 1989a).

Teichoic acids, which are polymers of glycerol or ribitol phosphate, are linked to the peptidoglycan and are antigenic. Antiteichoic antibodies detectable by gel diffusion may be found in patients with active endocarditis due to *Staph. aureus* (Jawetz et al., 1989a).

Protein A is a cell wall component of many *Staph. aureus* strains that binds to the Fc portion of IgG molecules except IgG3. The Fab portion of IgG bound to protein A is free to combine with a specific antigen. Protein A has become an important reagent in immunology and diagnostic technology; for example, protein A with attached IgG molecules directed against a specific bacterial antigen will agglutinate bacteria that have that antigen ("coagglutination") (Jawetz et al., 1989a).

Most *Staph. aureus* strains also contain a clumping factor or bound coagulase on their outer surface, which binds to fibrinogen by a nonenzymatic reaction and cause the micro-organisms to aggregate.

Some *Staph. aureus* strains are coated with an external polysaccharide layer, meeting the definition of a capsule or loosely associated slime layer. This inhibits phagocytosis by polymorphnuclear leucocytes (PMNL) unless specific antibodies are present (Waldvogel, 1990).

Finally, phage typing is based on the lysis of *Staph. aureus* by one or a series of specific bacteriophages. Such bacteriophage susceptibility is a stable genetic characteristic based on staphylococcal surface receptors (Jawetz et al., 1989a).

B. Important Enzymes and Toxins:

Staphylococci can produce disease both through their ability to multiply and spread widely in tissues and through