STAPHYLOCOCCUS AUREUS FROM SURGICAL DEPARTMENTS OF HOSPITALS IN ADDIS ABABA: STAFF CARRIAGE RATES, ENVIRONMENTAL CONTAMINATION AND ANTIBIOGRAMS

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A B S T R A C T

Six hundred strains of Staphylococcus aureus were isolated from the Black Lion Teaching Hospital Surgical Department environment (353), from the nose, wrist and arm skin of surgical staff of six selected hospitals in Addis Ababa (161) , and a non-hospital population (86).

Out of 454 surgical staff examined, 139 (30.6%) were nasal carriers and 22 (51%) of 43 nasal carriers also carried the organism on their skin. Carrier rates among different categories of surgical staff varied; the highest rate was among surgeons (56%). The non-hospital population comprised 328 students, and 21.6% of them were nasal carriers. Of 55 non-hospital nasal carriers 15 (27%) were found to carry the organism on their wrist skin. The carrier rate of the non-hospital population was significantly lower ($P < 0,01$) than that of the hospital population. The rate was higher in males than in females in hospitals, but not in the non-hospital population.

The environment of operating rooms and surgical wards of Black Lion Teaching Hospital was highly contaminated with Staph. aureus. Over 75% of air samples and 37% of dust samples were positive,

The non-hospital isolates were much more sensitive to antibiotics than the hospital isolates. Over 96% of non-hospital isolates were sensitive to nine antibiotics, but only 37% and 74% were sensitive to penicillin and tetracycline respectively. About 90% of all isolates were sensitive to clindamycin, cephalothin, gentamicin, kanamycin and trimethoprim-sulfamethoxazole, and none was resistant to vancomycin. The majority of hospital staphylococci, 87% of surgical staff isolates and 60% of environmental isolates were resistant to penicillin, and over 60% of both types of isolates resistant to tetracycline.

About 94% and 88% of hospital and non-hospital staphylococci, respectively, were resistant to at least one antibiotic. Multiple resistance among non-hospital staphylococci was remarkably lower (2.3%) than that of hospital isolates (over 37%). Different types of antibiograms were detected: 66 among environmental, 36 among surgical staff, and 6 among non-hospital isolates. These varied between resistance to one and to nine antibiotics. Combined resistance to penicillin and tetracycline was the most frequent pattern.

The findings were compared with other reports from Ethiopia and elsewhere. Based on the present study and other similar recent reports from Addis Ababa, the need for strict antibiotic policy, continued surveillance, assignment of infection officers and maintenance of cleanliness of the hospital environment have been stressed.

CHAPTER 1

I N T R O D U C T I O N
A N D L I T E R A T U R E R E V I E W

1. General Characteristics of Staphylococcus aureus and its Infections

A. Nomenclature and Taxonomy of Staphylococci

Catalase producing Gram positive cocci have been grouped under the family Micrococcaceae. This family includes three genera, Staphylococcus, Micrococcus, and Planococcus. Members of Planococcus are motile and non-pathogenic (Baird-Parker, 1974). However, taxonomic studies have revealed the fundamental genetic and epigenetic differences that exist between staphylococci and micrococci. Taxonomists have questioned the phylogenetic relationship of the members of the family Micrococcaceae (Ludwing et al., 1981).

The difference in guanine and cytosine content of DNA, the composition of the cell wall and oligonucleotide cataloging of 16s rRNA of the two genera strongly support the separation of staphylococci from micrococci (Schleifer and Kandler, 1972; Rosypal et al., 1966; Ludwing et al., 1981). On the bases of oligonucleotide cataloging of 16s rRNA and guanine-cytosine content of DNA, Ludwing et al.

(1981) grouped the staphylococci with the Bacillus cluster and micrococci with the Coryneforms.

In routine laboratory work, several biochemical and physiological tests have been used to differentiate the two genera. The most widely accepted test for identifying staphylococci from micrococci is based on their ability to produce acid from glucose under anaerobic conditions (Baird-Parker, 1963). Schleifer and Kloos (1975) found this single characteristic to be inconsistent and added two more distinguishing characters. Production of acid anaerobically from glycerol in the presence of 0.4 microgram of erythromycin/ml and sensitivity to 200 micro-gram/ml lysostaphin are more accurate and quick tests to differentiate staphylococci from micrococci. Staphylococci are resistant to the action of lysozyme while micrococci are sensitive (Baird-Parker, 1974).

Susceptibility to novobiocin has been used in the taxonomy of Micrococcaceae. Earlier reports indicated that staphylococci were sensitive to 0.25 microgram/ml of novobiocin while micrococci were resistant to this concentration (Mitchel and Baird-Parker, 1967). Later, it was found that susceptibility to novobiocin was inapplicable, since the test was dependent on the exposure of the

organism to the antimicrobial agent. Members of the genus Staphylococcus which were once believed to be sensitive to 0.25 micro-gram/ml novobiocin, were found to resist 1.6 micro-gram/ml (Kloos and Schleifer, 1975).

A new method has been recently introduced by Satta et al. (1978) and Varaldo et al. (1979), and seems to be superior to the other methods. The genus Staphylococcus has the ability to lyse heat-killed Micrococcus luteus, while Planococcus and Micrococcus do not.

Members of Staphylococcus can be identified on the bases of colonial morphology, coagulase production, hemolysis activity, nitrate reduction, phosphatase production, novobiocin and lysostaphin resistance, and anaerobic production of acid from certain carbohydrates (Kloos and Schleifer, 1975; Burn et al., 1978). The chemical composition of the media in which staphylococci grow affects the composition of the cell wall and hence susceptibility to lysostaphin. Therefore the content of the bacteriological media should be specified when lysostaphin susceptibility is used in the classification of staphylococci (Severance et al., 1980). Table 1 shows the different characteristics of human staphylococci.

TABLE 1

Simplified scheme for the identification of staphylococci from human sources, after Kloos and Schleifer, 1975.

Species	Colony diameter 5 days ≥5 mm	Lysostaphin resistance to 50 µg/ml	Coagulase	Hemolysis	Nitrate reduction	Phosphatase	Novobiocin resistance to 1.6 µg/ml	Fructose	Xylose and Arabinose	Ribose	Trehalose	Mannitol	Xylitol	Maltose
Staphylococcus														
aureus	+	−	+	(+)	+	+	S	+	−	+	+	+	−	+
spp.	+	−	+	(±)	+	+	S	+	−	+	+	(±,−)	−	(−)
simulans	(+)	−	−	±,−	+,	(±)	S	+	−	V	(+)	(+)	−	−,±
xylosus	+	−	−	(−)	(+)	(+)	R	+	+,±	V	+	+	(−)	(+)
cohnii	(+)	−	−	(−,±)	−	(−)	R	+	−	−	+	(+,±)	(+,−)	(±,−)
saprophyticus	+	(+)	−	−	−	(−)	R	+	−	−	+	+	(±,+)	+
hemolyticus	(+)	+	−	(+)	(+)	(−)	S	+,−	−	(−)	+	+,−	−	+
warneri	−	+	−	−,+	(−)	(−)	S	+	−	+,−	+	+,−	−	+,±
hominis	−	+	−	(−)	(+,±)	(−)	S	+	−	−	+	−	−	+
epidermidis	−	+	−	−,±	(+,±)	+	S	+	−	−	−	−	−	+
capitis	−	+	−	(−)	(+)	(−)	S	+	−	−	−	+	−	−

Abbreviations and signs:-

+ positive
± weak positive
− negative
V variable
() frequency of 70-89%
S sensitive, but could vary with the exposure to novobiocin
R resistant
two symbols - when either of the character is below 70% but together 80-100%.

Coagulase production has been frequently used to identify Staphylococcus aureus species (Baird-Parker, 1965; Kloos and Smith, 1980). The ability to clot human or mammalian blood plasma is not, however, specific for staph.aureus only and by no means all members of this species clot mammalian plasma (Baird-Parker, 1965; Derviese and Hajke, 1980; Kloos and Schleifer, 1975). In addition to coagulase production other biochemical reaction should be included for critical determination of this species. Members of the genus Staphylococcus, other than Staph. aureus have been found to clot plasma. One such human strain has been isolated by Kloos and Schleifer (1975). This can be differentiated from Staph. aureus by its failure to produce acid aerobically from maltose and mannitol. Two more coagulase positive Staphylococcus species have been recently isolated from animal sources (Derviese and Hajke, 1980). Staphylococcus intermedius and some strains of Staphylococcus hyicus can coagulate rabbit plasma. However both species can not produce acid from mannitol anaerobically.

Some strains of Staph. aureus lack the ability to produce coagulase. Therefore, phosphatase production, mannitol and maltose fermentation should be done before a coagulase negative variant of Staph. aureus is classified with coagulase negative staphylococci (Baird-Parker,

1965; Kloos and Schleifer, 1975; Derviese and Hajke, 1980). Although the coagulase test alone is not reliable for critical identification of Staph. aureus, it still remains the main criterion used in routine isolation of pathogenic staphylococci (Kloos and Smith, 1980).

Staphylococci has been subdivided into lysogroups, by their ability to lyse heat-killed cells of Micrococcus luteus on five different test media and by phosphatase activity (Varaldo et al., 1980), (Table 2). These Workers accepted the lysogroup system as superior to the other methods used for the classification of staphylococci.

Each species of staphylococci can be subdivided into subgroups and types. A variety of techniques have been used, serological, antibiogram and bacteriophage typing (Baird-Parker, 1974; Kloos and Smith, 1980; Cohen, 1972; Smith, 1972).

Serological typing, though promising has been difficult, because of the complexity of the antigenic structure and difficulty in the production of specific antisera for demonstration of a particular antigen (Cohen, 1972; Baird-Parker. 1974; Kloos and Smith, 1980). Continued cultivation in laboratory leads to antigenic changes which complicates the serological picture of staphylococci (Torres Pereira, 1961, 1981).

TABLE 2

Simplified Lysogroup System for the Classification of Staphylococcus (Varaldo et al., 1980).

Lysogroup	Bacteriolytic activity displayed on test media					Phosphatase activity	Species
	Tp_1	Tp_2	T_0	T_1	T_3		Staphylococcus
I	++	+	±	++	-	+	aureus
II	++	+	+	++	-	+	simulans
III	+	±	++	+	-	v	capitis saprophyticus xylosus
IV	++	++	±	++	+	v	cohnii and epidermidis hominis
V	-	+	+	-	±	-	haemolyticus warneri

Signs and abreviation:-

+ large zone of transparency, above 3 mm.
± zone of transparency 1-3 mm:
- zone of transparency below 1 mm.
v variable.

Antibiograms could be valuable tools in the detection of new strains in a region, but it is dependent on the therapeutic practice in the locality and this will cause considerable variations (Cohen, 1972; Kloos and Smith, 1980).

The most established system for epidemiological typing of staphylococci is bacteriophage typing (Baird-Parker, 1974; Smith, 1972; Kloos and Smith, 1980). Staphylococcus aureus is the species of the genus most commonly typed, as it is the most commonly encounterd pathogen. There are 4 phage groups, I, II, III, IV, and untypable strains. Each phage group is further divided into types. The number of such patterns is impractically large, and a basic set of at least 21-22 phages have been recommended for routine work (Smith, 1972; Freeman, 1979).

Group I	29, 52, 52A, 79, 80
Group II	3A, 3B, 3C, 55, 71
Group III	6, 7, 42E, 47, 53, 54, 75, 77, 83A, 84, 85
Group IV	42D
Miscellaneous	81, 187.

This set is by no means complete and additional phages may be necessary in certain localities. Phages 86, 87, and 89 have been used to identify Staph. aureus

hospital strains from USA, Hungary and Denmark respectively (Cohen, 1972). Phage type 94/96 has been an epidemic strain in certain hospitals in America (Kloos and Smith, 1980).

B. Laboratory Characteristics of Staphylococci

Staphylococcus aureus is a Gram-positive, non-motile, non-sporeforming and catalase positive coccus with an average diameter of 0.8 - 1 micron. The cells can be found alone, in pairs, as chains of 2-3 cells or as tetrads and clusters. The most distinguishing characteristic of the organism is the formation of irregular or grape-like clusters (Baird-Parker, 1974). Most of the strains are not capsulated, but certain virulent strains may possess a capsule or slime layer (Wiley, 1972).

They are not fastidious in their nutritive requirments and grow readily on the usual meat extract peptone medium (Kloos and Smith, 1980). They are facultative anaerobes, but grow best in an aerobic condition. On non-selective media they grow abundantly within 18-24 hrs. Colonies of Staph. aureus are usually circular, smooth, raised to slightly convex and glistening, butyrous with an entire

edge (Kloos and Smith, 1980; Baird-Parker, 1974). However under unfavourable conditions, rough, dwarf colonies may be produced (Baird-Parker, 1972). The size of a well-isolated colony of Staph. aureus of 24 hrs growth may be around 1-3 mm in diameter; after a longer incubation period the size may increase (Freeman, 1979).

Colony pigmentation among Staph. aureus is extremely variable: some are white, golden yellow or orange. Certain antibiotic-resistant strains and strains from bovine sources are usually colored yellow (Willis et al., 1964; Baird-Parker, 1974).

Most strains of Staphylococcus aureus are hemolytic on blood agar within 24 hrs (Baird-Parker, 1974; Kloos and Smith, 1980). They can produce acid aerobically and anaerobically from glucose, lactose, maltose and mannitol.

Anaerobic fermentation of mannitol has been used to isolate pathogenic staphylococci (Freeman, 1979; Derviese and Hajke, 1980).

Staphylococci are the most resistant bacteria among the non-spore formers. They can grow in the presence of 15% sodium chloride or 40% bile and resist the lytic activity of lysozyme (Freeman, 1979; Maitland and Matryn,

1948). They are relatively resistant to drying and can stay viable in the air for months (Rountree, 1963).

C. Pathogenicity of Staphylococci and Host Susceptibility to Infection

Pathogenic Factors of Staphylococcus aureus

Staphylococcus aureus is unique among the medically important microorganisms in its ability to produce a variety of enzymes and toxins, and to invade any tissue and organ of the body. The infections may be localized or systemic and either chronic or acute and may be fatal. It is because of these potentialities of Staph. aureus that Ekstedt (1972) gave the title "King of Versatility" to the organism.

Staphylococcus aureus has been one of the microorganisms most extensively studied with respect to the principal factors responsible for its pathogenicity (Noble, 1966; Foster, 1962a,b; 1967; Bartell et al., 1968). Several toxins and enzymes are produced by Staph. aureus, such as the hemolytic toxins, leucocidin, hyaluronidase, coagulase, fibrinolysin, deoxyribonuclease, phosphatase, lysozyme and enterotoxins (Abramson, 1972). These are

by no means the only extracellular products of this organism. Several multivalent extracellular products of an unknown nature have been isolated from Staph. aureus (Baman and Haque, 1970).

Although it seems impossible to quantify virulence due to its multifactorial nature, some of the extracellular products of Staph. aureus are known to be associated with virulent strains. However, there is no general agreement that any of these substances unequivocally represent the major determinant of lethal infection (Abramson, 1972; Ekstedt, 1972; Noble, 1966; Foster, 1963; 1967; Smith, 1962).

The findings of the studies on virulence vary and are conflicting. Several workers correlate the amount of toxins produced to the virulence of the strain (Anderson, 1956; Kedizia, 1963). On the other hand, Noble (1966), Smith (1962) and Foster (1963) found no difference in the biochemical activities of virulent and avirulent strains.

Several workers observed differences in the biochemical activity and virulence of the same Staph. aureus strain grown in vitro and in vivo. Staph. aureus grown in vitro was found to be less virulent than the same

strain grown *in vivo* (Adlam *et al.*, 1968). This indicates loss of some characteristics. The antigenic nature of the organism also differs when grown *in vivo* and *in vitro* (Torres Pereira, 1961; 1981). The majority of *Staph. aureus* strains possess one of the three antigens 13, 17, or 18. On successive subculturing, alteration of antigen 13 to a new one 3, and 17 to 1 was observed by Torres Pereira (1981). Another structural loss observed when strains of *Staph. aureus* were grown *in vitro* was the loss of antiphagocytic antigen, a capsular-like material (Yoshida and Ekstedt, 1968).

Beining and Kennedy (1963) found that cells of *Staph. aureus* grown *in vivo* differ from those grown *in vitro* in several ways. The *in vivo* strain produced quantitatively more deoxyribonuclease, alpha hemolysin, leucocidin and hyaluronidase, than did the *in vitro* grown *Staph. aureus*; it was also more pathogenic in rabbits. The production of toxins by staphylococci is influenced by culture media *in vitro*; therefore the organism may or may not produce the toxins when grown *in vitro* (Foster, E.A., 1965b). There may be qualitative as well as quantitative differences in the production of enzymes by *Staph. aureus in vitro* and *in vivo* (Foster, 1963; Beining and Kennedy, 1963; Bartell *et al.*, 1968).

The Role of Alpha Toxin

Alpha toxin has diversified biological activities such as hemolytic, dermanecrotic, leucocidal, lethal platelet lytic, muscle contracting, lysozyme lytic and cytopathic activity (Bernheimer and Schwartz 1965; Jeljaszewicz, 1972).

Several workers (Smith et al., 1960; Noble, 1966; Foster, E.A., 1965b; 1967) believed that this toxin plays a decisive role in staphylococcal infection. The toxin may have a role in some stages of generalized infection or during the establishment of staphylococcal lesions (Jeljaszewicz, 1972). Noble (1966) indicated that the production of beta lysin and fibrinolysin were associated with the formation of lesions in mice. Foster E.A., (1965; 1967) found alpha toxin to be produced in vivo before the formation of necrosis and exudation, showing its participation in the formation of lesions.

On the contrary, the findings of Foster (1963) and Bartell et al. (1968) did not indicate any importance of alpha toxin in the pathogenicity of staphylococcal infection. Foster (1963) found both alpha hemolysin producers and non-producers to have equal survival chances within

splenic phagocytes. Bartell et al. (1968), studying the role of alpha toxin in the pathogenicity of Staph. aureus, found that the alpha antitoxin protected mice which were injected with the toxin; but the antitoxin failed to protect mice from infection when injected with living cocci. This indicates that interaction with other biologically active staphylococcal products is essential to cause disease (Jeljaszewicz, 1972). The tissue damage due to alpha toxins of Staph. aureus may be indirect, i.e. by the release of histamin and serotonin from mast cells and by platelet degradation (Jelzaszwicz, 1972; Bernheimer and Schwartz, 1965).

The Role of Coagulase

Although the production of coagulase is characteristic of the vast majority of the staphylococci that cause disease, epidemiological as well as experimental studies throw doubt on the importance of the enzyme in causing disease (Foster, 1962a,b; Cawdery et al., 1969; Borchardt and Pierce, 1964; Kediza, 1963).

Davis (1951) and Smith et al. (1947) suggested that coagulase increases a fibrin barrier in lesions, thus

partially protecting the infecting organism from phagocytosis. Borchardt and Pierce (1964); Foster (1962a,b) and Cawdery et al. (1969) disputed the results of Davis (1951) and Smith et al.(1947). They found both coagulase positive and negative strains to be equally phagocytized and no difference was observed in survival time inside the leucocytes. Anticoagulase antisera did not contribute to a decrease in viable intracellular organisms as compared to cocci in the absence of anticoagulase antisera (Borchardt and Pierce; 1964).

The Role of Hyaluronidase and Other Enzymes

McClean (1943) and Duran-Reynals (1942) as cited by Abramson (1972), suggested that microbial hyaluronidase contributes to the infection process by depolymerizing hyaluronic acid present in the intracellular ground substance of connective tissue, there by permitting spread of bacterial toxic metabolic products. Elek, (1959) disagreed and believed that hyaluronidase reduces the infectivity by dilution and makes the invader more susceptible to local defences. The activity of hyaluronidase is limited only to the initial stage of infection, because it is suppressed or antagonized by the inflammatory process

(Abramson, 1972). Choudhuri and Chakrabarty (1969) as quoted by Abramson (1972), found no correlation between hyaluronidase activity and pathogenicity of the organism.

Lysozyme, one of the extracellular products of Staph. aureus, is believed to be related to pathogenicity, although it is produced by non-pathogenic strains as well (Jay, 1966; Grossegbauer et al., 1968).

The roles of phosphatase (Cannon and Hawn, 1963) lipase (Rosendal and Bulow, 1965) and leucocidin (Gladstone, 1965; Woodin, 1972) are still not clearly known.

Although the mechanism of action of staphylococcal enterotoxin in the human body is not clearly known, it is responsible for a variety of abnormalities in the body (Bergdoll, 1972; Bergdoll et al., 1981).

Antiphagocytic Activity of Staph. aureus

Virulent strains of Staph. aureus may not be easily phagocytized. Several extracellular products of staphylococci, such as alpha hemolysin and leucocidin may retard the phagocytic activity of the leucocytes by destroying them (Koening, 1972).

Protein A, a surface antigen of Staph. aureus is capable of blocking the opsonic site on the Fc fragment of immunoglobulin G thus antagonizing the activity of complement and retarding phagocytosis (Dosset et al., 1969).

Capsulated strains of Staph. aureus possess a capsular antigen which is responsible for its resistance to phagocytic destruction and increased virulence (Koening, 1972; Willey, 1972; Yoshida and Ekstedt, 1968; Blackstock et al., 1968; Yoshida et al., 1969).

Virulence and Antimicrobial Resistance

Multiresistant Staph. aureus have been responsible for several epidemics throughout the world, causing severe and lethal infection (Graham et al., 1980; Crawen et al., 1981; McDonald et al., 1981). The increase and severity of staphylococcal infections cannot be due to an increase of antimicrobial resistance (Watt and Okubadejo, 1967; McDonald et al., 1981; Peacock et al., 1980). In fact the biochemical and physiological changes associated with the acquisition of resistance are usually disadvantageous to the cell in the absence of the challenging agent (Koch, 1981; Lacey, 1973, 1975; Noble et al., 1964). Resistant

strains have a decreased rate of growth when compared to their sensitive parents (Lacey, 1975; Bellamy and Klimek, 1948; Sutherland and Rolinson, 1964). Possession of a plasmid could give the cell an extra job to maintain and replicate them. This extra load may suppress other activities of the cell, and may be manifested by decreased virulence (Koch, 1981; Lacey, 1975).

Williams (1971), as quoted by Lacey (1975), noted a decrease in the incidence of staphylococcal primary skin sepsis among healthy individuals in a hospital. The explanation given was the acquisition of resistance to antimicrobial agents. On the contrary, Graham et al.(1980) and Sarovolatz et al. (1982) observed no virulence difference between multiresistant methicillin resistant and sensitive Staph. aureus strains.

Experimental studies have shown that resistance to antimicrobial agents is associated with reduction of virulence of Salmonella enteritidis and Salmonella typhimurium (Kurashige et al., 1975; Koch, 1981; Smith and Tucker, 1976). The virulence of a fosfomycin resistant strain of S. enteritidis was found to be 1000 times less than the sensitive parents (Kurashige et al., 1975).

Route of Entry and Infective Dose

The minimum infective dose in experimental mice was found to be approximately 10^9- 10^{10} bacteria. The outcome of infection also depends on the route of entry. Injection of a similar number of bacterial cells by the intracerebral, intracardiac, intraperitoneal, intrahepatic or intrasplenic route was much more often and rapidly fatal than injection by the intravenous route (Smith et al., 1960).

In general, the pathogenicity of Staph. aureus depends on the presence of living cocci rather than on their extracellular secretions. However, according to Smith et al. (1960) tissue injury is usually caused by the toxic action of the extracellular products of the organisms. The general failure of antibodies against extracellular products of Staph. aureus to prevent infection supports the findings of Smith et al.(1960).

Host Susceptibility to Infection

It is surprising that with all its potentialities to cause severe and dangerous infection, Staph. aureus is a member of the normal flora of some individuals (Williams, 1963; Nahmias and Shulman, 1972). As is also true of

other microbial diseases, host-parasite relationship is important in manifestation of clinical symptoms of Staph. aureus infections. Several factors are known to predispose the body to infection.

The host becomes susceptible to staphylococcal infections when the mechanical barrier, skin, is disrupted (Shulman and Nahmias, 1972). Certain viral infections like influenza and measels are known to predispose the host to staphylococcal infections (Louria et al., 1959). Patients with deficiencies in humoral and cellular immunity are susceptible to Staph. aureus infections (Miller and Nilsson, 1970). Aged and debilitated patients with chronic diseases such as diabetes mellitus, malignant tumors and chronic lung diseases are highly susceptible to staphylococcal infections (Nahmias and Eickhoff, 1961; Shulman and Nahmias, 1972; Cluff, et al., 1968; Mucher and McKenize, 1977; Crawen et al., 1981). The presence of a foreign body, especially in surgical wounds, increases susceptibility to staphylococcal infection (Shulman and Nahmias, 1972; Eickhoff, 1972; Thoburn et al., 1968; McDonald et al., 1981). Suppression of the normal flora of the body by an antimicrobial agent to which the Staph. aureus strain is not sensitive has been found to increase

susceptibility of the host (Shulman and Nahmias, 1972; Tisdale et al., 1960).

Exposure to broad-spectrum antibiotics makes a patient susceptible to multiresistant Staph. aureus infection (Graham et al., 1980). This is especially true for gentamicin and methicillin-resistant Staph.aureus, which are usually resistant to several other antimicrobial agents.

2. Epidemiology of Staphylococcal Infection

A. The Reservoir of Staphylococcus aureus

The epidemiological pattern of Staph. aureus infections is unique in that man himself is a reservoir, transmitter and the target host. The carrier may harbor the organism in the nose, pharynx, umbilicus, axilla, perineum, gastro-intestinal and urogenital tracts and on various areas of the skin including the face, hands and hair. The nasal cavity is the most frequent site of residence (Williams, 1963).

Nasal carriage rates reported by several workers vary. Most of the studies gave rates 20-70% for both hospital

and non-hospital adults (Williams, 1963; Rao and Frederik, 1966). The nasal carrier rate among non-hospital healthy adults reaches 50% (Miles et al., 1944). Reports from several workers show that the hospital community has a higher carriage rate, 40-80% (Williams, 1963; Hofstad and Vogelsang, 1960; Weinstein, 1959; Clarke, 1957). On the other hand, other workers found no such difference between rates among hospital and non-hospital populations (Henning et al., 1979; Maxwell et al., 1969; Sodhi et al., 1968). However, some ecological differences were observed between the hospital and non-hospital carriers. Hospital staff had a more unstable staphylococcal flora, with frequent acquisition and loss of strains (Henning et al., 1979; Maxwell et al., 1969). The explantion is that the hospital population is exposed to a shifting population of patients carrying a variety of Staph. aureus strains.

Several factors have been cited in an attempt to explain the differences observed among individuals in their carrier state: the presence of pre-existing nasal flora, (Davis and Davis, 1965), coincidental respiratory infection (McNamara et al., 1966), the effect of cold weather (Cameron, 1970), genetic factors (Noble et al., 1967) and the immune factors of the host (Ehrenkranz, 1966). Duration of hospital

stay was found to increase the carrier rate (Lindbom, 1964; Williams, 1963; Loh and Street, 1957). In contrast, others believed that the Staph. aureus carrier state depends on the characteristics of the individual rather than on the environment (Henning et al., 1979; Noble et al., 1967; Messinger et al., 1963; Bengtsson et al., 1979).

Carrier rate varies with age. Infants have higher carrier rates, especially in hospitals; upto 90% were found to be carriers in Australia (Laurell and Wallmark, 1953). The rate declines between the age of 6 months to 2 years, the adult carrier rate being detected from the age of 5 (Williams, 1963). On the other hand, Noble et al. (1964) in Europe found carrier rates to be highest in the age group 5-14 year and settled to the adult level during the decade of 15-24 years.

There are few reports comparing the nasal carrier rates among men and women. From the few reports available there seems to be no significant difference between those of adult men and women (Miller et al., 1962), though male infants were found to have higher carrier rates (Nahmias and Shulman, 1972).

There seem to be no significant geographical and racial differences in the carrier rates of Staphylococci in the countries where these are well-studied (Williams, 1963; WHO, 1968). However, Findley and Abraham (1946), as quoted by Davis and Davis (1965), reported a lower rate of nasal and skin carriage among Africans compared with Europeans in Ghana. Although there are few reportes of the carrier rate of Staph. aureus in the tropics, it is some what lower, 15-25% (WHO, 1968) than the 30-50% among people of temperate region, (Williams, 1963). As low as 19% was reported among the people of New Guinea, which was believed to be associated with clothing (Rountree,1956).

In contrast, the people of Nigeria and Ghana have higher carrier rates which lie in the range given by many workers in temperate areas. The mean carrier rate of both hospital and non-hospital populations of Nigeria was 46% (Davis and Davis, 1965). In Ghana the carrier rates of Staph. aureus in persons who had no direct contact with the hospital environment varied between 45.6 and 53.6% and the hospital staff carrier rate ranged from 58-72% (Sodhi et al., 1968). In general, urban dwellers seem to have higher carrier rates than those in rural areas (Williams, 1963).

B. Mode of Transmission

The mode of transmission of staphylococcal infection is complex, involving all possible routes of transmission by direct contact or indirectly through inanimate objects in the environment (Williams, 1966; Hambraeus et al., 1978a, b).

Numerous reports implicate the healthy carrier as a significant "vector" in the transmission of staphylococci (Williams, 1966; Nahmias and Eickhoff, 1961; Nahmias et al., 1960; Payne, 1967; Shulman and Nahmias, 1972; Tanner et al., 1980). Other possible sources of infection are patients with infected lesions (Foster, 1960; Williams et al., 1962).

Bengtsson et al., (1979) found postoperative wound infection to be more frequent among nasal carriers than among the non-carriers. Bassett et al., (1963), however, found no such correlation, but found self-infection to be a less frequent cause of wound sepsis among surgical patients. This difference could be due to differences in the epidemiological properties of the Staph. aureus strains infesting the hospital, methods of pre-operative skin preparation and types of operations (Bassett et al.,1963).

Though the carrier state is one of the peaceful commensalisms that exist in nature (Williams, 1963), the carrier can also be a source of staphylococcal infection to himself (Nahmias and Eickhoff, 1961; Williams, 1963; Weinstein, 1959).

Staph. aureus can be disseminated from the carrier to the environment, the air and any inanimate objects (Nahmias and Shulman, 1972). It has been noted that certain carriers can disseminate their staphylococci much better than others (Noble and Davies, 1965; Sciple et al., 1967; White et al., 1964; Nahmias and Shulman, 1972). The reason for this difference in dispersal of the cocci is not well established. Dispersal rate depends on the number of the organism in a particular carrier site (White, 1961). Tanner et al. (1980), however, found no such relationship. The presence of skin lesions and respiratory viral infections have been found to increase the dispersal rate of staphylococci (Roodyn, 1960; Payne, 1967). Treatment with tetracycline was found to increase the dispersal rate of tetracycline-resistant Staph. aureus as the result of elimination of the sensitive normal flora (Ehrenkanz, 1964; Stokes et al., 1965). Bathing (Williams, 1966; Maxwell, et al., 1969), and increased activities may influence the number

of cocci disseminated by the carriers (Noble and Davies, 1965; Williams, 1966).

Hill et al. (1974) found that the types of clothes influence dispersal rates: well ventilated clothes were found to reduce dissemination of staphyloccocci. Tanner et al. (1980), however, found no such correlation between clothing and dispersion. Some difference has been observed between the dispersal rates of male and female carriers. Ayliffe et al. (1974), and Hill et al. (1974) found male carriers to disseminate more cocci than female carriers. The difference in clothing style of men and women may have influenced the dissemination of the cocci (Hill et al., 1974). Bethune et al. (1965) had also noted higher dissemination rate among men, their explanation was that men are usually more active than women.

Staph. aureus can be transmitted directly from a carrier or a patient to another patient or indirectly through air droplets, or through contaminated inanimate objects (Nahmias and Shulman, 1972). Contamination of the environment with bacteria, more frequently with Staph. aureus, has been found to occur virtually in every area of the hospital (Nahmias and Eickhoff, 1961; Nahmias and Shulman, 1972). Most reports showed that the most impor-

tant mode of transmission is via direct hand transfer (Lidwel et al., 1970; Williams, 1966), and that indirect mode of transmission play a relatively minor role (Oldstine, 1966; Messinger et al., 1963; Shooter et al., 1958).

Staph. aureus can stay viable and maintain its pathogenicity in dry air for several days (Noble, 1962; McDade and Hall, 1963; Rountree, 1963; Colbeck, 1960; Josephson and Butter, 1957). Therefore air contamination has frequently been indicated as an important source of staphylococcal infection (Bourdillon and Colebrook, 1946; Wysham et al., 1957; Ravenholt and Ravenholt 1958; Ravenholt and Laveck, 1956). Blowers and Wallace, (1960) found close association between reduction of Staph. aureus in the air and reduction of wound sepsis in operation rooms.

Several conflicting reports on the importance of aerial contamination in the operation rooms have appeared. Aerial Staph. aureus could play an important role in producing surgical wound sepsis in operation theatres (Blowers et al., 1955; Shooter et al.,1958). Directly or indirectly, sepsis in surgical wounds is most likely to arise from infection in the operation rooms (Bassett et al., 1963; Payne, 1967; Howe and Marston, 1962). The source of Staph. aureus

during operation can be the air (68%), patient carrier sites (50%) and the hands or nasopharynx of the surgical team (20%) (Burke, 1963).

Post-operation wound infections could arise from the puncture of the rubber gloves of the surgeons and leakage of staphylococci through accumulated perspiration (Jepsen, 1972; Nahmias and Eickhoff, 1961). Staph. aureus could be introduced into the body of a patient along with any thing that is introduced into the body, such as tracheal catheters or other types of intubation (Rountree and Beard, 1968; Jensen et al., 1969; Cluff et al., 1968).

C. Types of Infections

Staphylococcal infection can be localized involving the skin, eye, nose and throat, gastrointestinal and urogenital tracts. Metastases may develop in any of the internal organs (Shulman and Nahmias, 1972). Staphylococcus aureus phage group I is usually associated with localized primary skin sepsis, like boils and carbuncles; group II characteristically produce spreading infections of the skin and groupIII are responsible for a variety of infections in hospitals (Lacey, 1975).

Skin staphylococcal infections involves the hair follicles, subcutaneous tissues and sweat glands, causing different types of lesions (Nahmias et al., 1962; Shulman and Nahmias, 1972). Staphylococcus aureus can invade different parts of the eye, with the conjunctiva and sebaceous glands of the eyelids frequently involved (Shulman and Nahmias, 1972). Abscesses can develop in any of the paranasopharyngeal area (Shulman and Nahmias, 1972). Staphylococcus aureus can also cause local infections of the urogenital system (Kunin, 1970; Gati and Toth, 1967).

Systemic or localized foci can be produced in the lungs, causing staphylococcal pneumonia. It is an important cause of morbidity and mortality (Hers et al., 1957) and is frequent in chilren under one year (Eickhoff, 1972; Slim et al., 1965) and in debilitated or elderly patients (Shulman and Nahmias, 1972).

The most severe infection of Staph. aureus is bacteremia which can be fatal within 24 hrs of infection (Shulman and Nahmias,1972). Intravascular coagulation, bleeding disorders, anemia and cardiovascular changes are some of the disorders observed during Staph. aureus septicemia (Lipinski et al., 1969; Shulman and Nahmias, 1972). Once bacteremia is produced, the organisms may invade any of the internal

organs, forming metastatic foci (Cluff et al., 1968) and bacteremia may continually be produced from such metastases. One of the target tissues can be bone, resulting in osteomyelitis. Staphylococcal osteomyelitis is frequent among children under the age of 12. It is a disease of growing bone (Shulman and Nahmias, 1972; Winters and Cohen, 1960). The femur and tibia are most frequently involved (Waldvogel et al., 1970). When the joints are involved, arthritis is likely to develop (Shulman and Nahmias, 1972).

Staphylococcus aureus may involve different parts of cardiovascular system. The most common site of infection is the endocardium. The valve leaflets and valve rings are destroyed quickly, resulting in irreversible damage to the heart (Shulman and Nahmias, 1972). Staphylococcal infection can result in renal failure caused by abscesses in different parts of the kidney (Nydahl and Hall, 1965) and by the deposition of immune complex (Tu et al., 1969).

Muscle abscess due to Staph. aureus is rare in temperate regions (Shulman and Nahmias, 1972). However Staph. aureus pyomyositis is responsible for 1-2% of hospital admissions in some tropical countries (Foster, W.D., 1965; Levin et al., 1971). Staphylococcus aureus is capable of invading the central nervous system resulting in abscesses

and meningitis (Kislak et al., 1962; Jones et al., 1969). Abscesses can develop in almost any intra-abdominal organ; in particular the liver, pancreas and spleen (Shulman and Nahmias, 1972).

D. Magnitude of Staphylococcal Infections

Despite the introduction of numerous antimicrobial agents, staphylococcal infections remained a major cause of morbidity and mortality (Musher and McKenize, 1977; Benner and Kayser, 1968; Nahmias and Shulman, 1972). It is one of the main problems among hospital-acquired infections. It has been reported that 15-50% of all nosocomial infections are due to Staph. aureus (Nahmias and Shulman, 1972; McGowan and Finland, 1974). Staphylococcal infections accounted for 34% of all bacterial infections in some hospitals (Kislak et al., 1964). It has been estimated that several million dollars are lost annually due to such nosocomial infections in developed countries like the USA (Shulman and Nahmias, 1972).

In Ethiopia, Staph. aureus infections accounted for 16% of all bacterial infections in the Black Lion Hospital in Addis Ababa. Forty-four percent, of all Staph. aureus isolated during 1976-1980 in this hospital came from the

Surgical Department (Gedebou, 1982a). The work of Perine et al., (1975) showed that Staph. aureus was the most common bacterial pathogen isolated from wounds and pus in St. Paul Hospital in Addis Ababa.

It has been reported that 5-9% of the population in Great Britain suffer from staphylococcal skin diseases and 1.5 million cases of skin infections occur each year in the USA (Nahmias et al., 1962; Gould and Cruickshank, 1957; WHO, 1968). The situation may be worse in tropical countries, related to the increased humidity and to hypersensitivity of sweat glands (Cruickshank, 1953). The disease known as scalde-skin syndrome is common among children, with 20% mortality if no appropriate therapy is given (Howells and Jones, 1961; Lyell et al., 1969; Melish and Glasgow, 1970; Nahmias and Shulman, 1972).

Sepsis of surgical wounds due to Staph. aureus among surgical patients has been estimated as around 40% (WHO, 1968). Up to 23.2% of surgical wound-healing failure has been reported due to Staph. aureus (Weinstein, 1959). Postoperative infection due to Staph. aureus may result in complications causing delay in the patients discharge from the hospital and even death in severe case (WHO, 1968; Payne, 1967).

During non-epidemic periods in Great Britain, Staph. aureus infections accounted for 5% of all hospitalized bacterial infections of the lung (Grist et al., 1952; Hausmann and Karlish, 1956). More than half of the deaths during the Asiatic influenza epidemic in the Netherlands were due to Staph. aureus (Hers et al., 1957). Mortality rate due to staphylococcal pneumonia in the USA remained as high as 20-25% even after the use of antimicrobial agents (Fisher et al., 1958).

The most dangerous and fatal complication caused by Staph. aureus is systemic bacteremia. It can be fatal within 24 hrs of infection and accounts for 23% of all bacteremia found in some hospitals (Watt and Okubadejo, 1967; Waisbren and Abbound, 1960). Mortality rates as high as 40-50% have been reported even after the antimicrobial era (Spink, 1954; Jensen et al., 1969; Finland, 1970). During the year 1969, 25% of food-borne diseases were due to staphylococcal enterotoxins in the United States of America (Bergdoll, 1972).

Staphylococcal epidemics in maternity wards as well as surgical wards have been reported from many parts of the world, forcing some wards to be closed because of uncontrollable epidemic conditions (Blowers et al., 1955; Temple and

Blackburn, 1963). Twelve percent of the neonatal mortality has been due to Staph. aureus infections in some American hospitals (Ravenholt et al., 1957). Skin sepsis in infants in maternity wards has reached 4-28% during non-epidemic periods and 40% during epidemics (WHO, 1968). Breast abscess due to Staph. aureus among hospital-delivered mothers was estimated as 10-19% (WHO, 1968).

3. Antimicrobial Resistance of Staphylococcus aureus

A. Nature of Antimicrobial Resistance

Not more than one percent of the staphylococcal population are known to be naturally resistant to any antimicrobial agent (Lacey, 1975). Penicillinase-producing strains have been isolated from people who have never taken the antimicrobial agent (Rountree, 1956). Methicillin resistant strains also occur naturally (Parker and Hewitt, 1970; Jevons 1961).

The general belief at present is that resistant strains arise from rare naturally-occurring resistant strains, as a result of selection by an antimicrobial agent and from spontaneous gene mutation (Spink, 1954; Lacey, 1973;1975;

Macfarlane et al., 1960; Koch, 1981). However, mutation and selection alone could not account for the high rate of appearance of resistant strains (Lacey, 1973). Transfer of certain genes by transduction, especially plasmid genes between strains of Staph. aureus is mainly responsible for the acquisition of antimicrobial resistance and for the wide and rapid distribution of multiple resistance (Lacey et al., 1970; Lacey, 1973, 1975; Richmond, 1972; Noble and Naidoo, 1978).

Resistance to an antimicrobial agent is determined by a gene located on the chromosome or on an extrachromosomal element, the plasmid (Lacey, 1973, 1975; Koch, 1981). Table 3 shows the location of the genes determining resistance to various antimicrobial agents in Staph. aureus . Staphylococcal plasmids occur usually in multiple copies (Lacey, 1975; Chopra et al., 1973; Inoue et al., 1975). The plasmid carrying tetracycline resistance can be found in 31-47 copies per organism, that for penicillinase in 15 and the plasmid for neomycin resistance in 16 copies per organism (Chopra et al., 1973).

Staphylococcal plasmids usually carry a single gene determining a single character, resistance to an antimicrobial agent (Murray and Moellering, 1978; Inoue et al.,

TABLE 3

Location of Genes Coding for Antimicrobial Resistance in *Staphylococcus aureus*

Antimicrobial agent	Site of gene	Reference
Penicillin	Plasmid (90%)	Koch, 1981; Novick, 1963; Lacey, 1975
	Chromosome	Asheshov, 1966b; Sweeney, and Cohen, 1968; Poston, 1966
Methicillin	Plasmid (unusual)	Lacey, 1975; Dornbusch *et al.*, 1969
	Chromosome	Lacey, 1975; Annear and Grubb, 1973
Gentamicin	Plasmid	Faden et al., 1979; Porthouse *et al.*, 1976
Kanamycin, Neomycin	Plasmid	Lacey *et al.*, 1970; Ayliffe 1970; Lacey 1973
Chloramphenicol	Plasmid	Novick, 1963; Inoue *et al.*, 1975; Kayser *et al.*, 1972
Tetracycline	Plasmid	May et al., 1964; Lacey, 1972
	Chromosome (rarely)	Poston, 1966; Lacey 1971; 1975
Streptomycin	Plasmid	Grinsted and Lacey, 1973; Ayliffe, 1970; Lacey, 1975 Poston, 1966
	Chromosome (70%)	Harwood and Smith, 1969
Erythromycin	Plasmid	Lacey, 1972; 1975 Murray & Moellering, 1978

1975). However in certain strains, genes determining the resistance for erythromycin (Mitsuhashi et al., 1965; Annesr and Grubb, 1973), fusidic acid (Lacey and Grinsted, 1972), ethidium bromide (Johnston and Dyke, 1969) and inorganic salts like cadmium, arsenate, arsenite, bismuth and lead (Peyru et al., 1969) were found on the penicillinase plasmid. Kanamycin and neomycin resistance was also found to be genetically linked (Lacey et al., 1970; Ayliffe,1970; Lacey, 1973).

Possession of a plasmid affords significant flexibility to the organism. Antimicrobial resistance genes can be amplified when needed and deamplified when not needed (Koch, 1981; Noble and Naidoo, 1978). Most plasmids are lost in the absence of the challenging agent (Lacey, 1975; Koch, 1981).

Inter-strain gene transfer among staphylococci is mainly mediated by phages and can take place in vitro as well as in vivo (Lacey, 1975; 1972; Noble and Naidoo,1978; Koch, 1981). Unlike the plasmids in Gram negative bacilli, which are usually transfered by conjugation, the staphylococcal plasmids appear to be transferred exclusively by phage transduction (Lacey, 1975; Murray and Moellering, 1978). However, Lacey (1980) reported 'Phage-mediated

conjugation in Staph. aureus which required cell-to-cell contact. Transformation is unlikely to occur in nature, since it requires a high concentration of calcium ions and the absence of DNAase (Lacey, 1980). Staphylococcal plasmids carrying DNA coding for antimicrobial resistance can be transfered to other bacterial species, e.g. Bacillus subtilis (Feitelson and Lederberg, 1980).

Plasmids carrying the gene for resistance to penicillin, tetracycline, neomycin, kanamycin, chloramphenicol, erythromycin, and fusidic acid are known to be transferred between strains of Staph. aureus (Lacey, 1975). Chromosomal gene transfer is less frequent, but joint transduction of resistance to tetracycline, streptomycin and sulfanilamide has been reported by Kasuga et al. (1968). However, there is presently no evidence on the possibility of transfer of plasmids carrying resistance to methicillin, trimethoprim and novobiocin; therefore, resistance may probably arise by mutation and selection (Lacey, 1975; Dornbusch et al., 1969; Cohen and Sweeney, 1970; Noble and Naidoo, 1978).

Plasmid fragmentation within the cell (Lacey and Grinsted 1972; Novick and Richmond, 1965) may be an important factor in the spread of antibiotic resistance between strains, since the smaller the plasmid size the higher

its frequency of transfer between mixed cultures *in vitro* (Chopra *et al.*, 1973; Porthouse *et al.*, 1976).

Plasmids play an important role in the rearrangement of genes, both between and within organisms (Asheshov, 1966a; Lacey, 1975; Koch, 1981). Asheshov (1966a) reported the recombination of the chromosomal gene responsible for penicillinase production with an existing plasmid. Under experimental condition; several plasmids may become integrated to form a single larger plasmid (Richmond, 1969, Lacey and Grinsted, 1972; Novick and Richmond, 1965). The gene determining resistance for erythromycin, initially part of the penicillinase plasmid, was found to be integrated into the chromosome (Lacey, 1975). Therefore gene interchange may take place between plasmids, or between plasmid and chromosome, among strains of staphylococci.

B. Mechanisms of Antimicrobial Resistance

Most microbial mechanisms of resistance to antimicrobial agents appear to be based on genetic changes that alter some of the cellular components: the antimicrobial agent may not reach its goal, or the target site is altered so that it does not react with the antimicrobial agent, or an enzyme may be produced to inactivate the

antimicrobial agent (Koch, 1981; Benveniste and Davies, 1973). Presently several mechanisms of resistance are known and more than one may be responsible for resistance to a single antimicrobial agent (Murray and Moellering, 1978).

Decreased Permiability

Resistance to antimicrobial agents due to permeability barrier changes can be natural or acquired (Murray and Moellering, 1978). Resistance to penicillin G among Gram negative bacilli is due to the impermeable nature of the cell envelope, especially the outermost phospholipid membrane (Suginaka et al., 1975; Zimmermann and Rosselet, 1977). Enterococci resistant to aminoglycosides possess natural permeability barriers, which can be destroyed when the organism grows in the presence of agents inhibiting cell-wall synthesis, like penicillin (Moellering et al., 1971; Moellering and Weinberg, 1971). Another example of a naturally occurring permeability barrier is that of Proteus mirabilis to that of polymxin B (Sud and Feingold, 1970).

Resistance due to an acquired permeability barrier has been reported among Gram-negative bacilli and Staph.

aureus. Resistance to tetracycline in Escherichia coli and Staphylococcus aureus is due to decreased uptake of the antimicrobial agents (Izaki and Arima, 1963). Mutants of methicillin-resistant Staph. aureus showed reduced uptake of the antimicrobial agent because of an altered cell wall (Sabath et al., 1970). Some clinical isolates of chloramphenicol-resistant E.coli and Pseudomonas aeruginosa possess an inducible permiability block to the antimicrobial agent (Nagai and Mitsuhashi, 1972; Mitsuhashi et al., 1975). Watanabe (1963) has reported decreased permeability to sulfathiazole in E. coli carrying resistance factor. Resistance to fusidic acid in Staph. aureus mediated by chromosomal mutation was found to be due to reduced uptake of the antimicrobial agent. Chemical alteration of the cell wall of such mutants has been reported (Chopra, 1978).

Enzymatic Inactivation

Some resistant bacteria are capable of producing enzymes that can inactivate antimicrobial agents by preventing their action on the target site. Resistance to penicillins and cephalosporins is due to the inducible production of penicillinase, which hydrolyze the beta-lactam ring (Murray and Moellering, 1978; Koch, 1981).

Chloramphenicol-resistant Shigella and Staph. aureus inactivate chloramphenicol by acetylation of hydroxyl groups (Okamoto et al., 1967; Shaw and Brodsky, 1968; Shaw, 1967; Suzuki et al., 1966). Enzymatic reduction of the nitro group of chloramphenicol has been reported for a number of different microorganisms (O'Brein and Morris, 1971). Escherichia coli resistant to sulfonamide are capable of inactivation of the antimicrobial agent (Benveniste and Davies, 1973).

There are three different mechanisms by which amino-glycosides are inactivated: acetylation of the amino group, phosphorylation of the hydroxyl group, or adenylation of the hydroxyl group (Benveniste and Davies, 1973; Murray and Moellering, 1978; Doi et al., 1968).

Alteration of the Target Site

Microbial resistance to antimicrobial agents due to an altered site can be grouped as follows:

1. genetic alteration of the target site
2. increased production of competing substance
3. over-production of sensitive protein
4. diversion to an alternative metabolic pathway
5. by-pass to a blocked metabolic step.

Genetic alteration of target site

Genetic modification of the sensitive enzyme has been employed by certain bacteria to overcome the action of antimicrobial agents. The enzyme is chemically altered and thus no longer has affinity for the agent. Resistance to psicofuranine (Benvinste and Davies, 1973) and sulfonamide (Ortiz, 1970) in E. coli isolates have been due to the possession of an altered enzyme. The penicillin-binding proteins of methicillin-resistant Staph. aureus appear to have a decreased affinity for methicillin when compared to that of methicillin-sensitive Staph.aureus (Hartman and Tomasz, 1981). The genetic alteration of these penicillin-binding proteins, which are involved in cell wall synthesis, may have resulted in a difference of the amino acid sequence in the cell wall of these methicillin-resistant Staph. aureus from that of their sensitive parents. Such changes in the fine structure of the cell wall may affect the permeability of the cell wall to the antimicrobial agent observed by Sabath et al., (1970) (Hartman and Tomasz, 1981).

The target site of certain antimicrobial agents in the cell have been the protein synthetic machinery (Freeman, 1979). In resistant bacteria, alterations of these sites have been reported. Streptomycin resistance has been characterized as mutation affecting the 30S ribosome (Brige and

Furland, 1969). Neomycin/Kanamycin resistance is also due to genetic alteration of the 30S or the 50S subunit of the ribosome of E. coli (Benveniste and Davies, 1973). Erythromycin-resistant strains are believed to have an altered ribosomal protein in E. coli (Koch, 1981; Tanaka et al., 1968).

Increased Production of Competing Substances

Sulfonamides are inhibitors of folic acid synthesis by competing with para-aminobenzoic acid for the enzyme dihydropteroate synthetase. Sulfonamide-resistant Staph. aureus produce upto 20 times as much para-aminobenzoic acid as their sensitive counterparts and by doing so they overcome the competetive inhibition of sulfonamides (Koch,1981; White and Woods, 1965; Murray and Moellering, 1978).

Over-Production of Sensitive Proteins

Trimethoprim inhibits the function of dihydrofolate reductase in microorganisms (Koch, 1981). E. coli resistant to trimethoprim were found to produce at least 300-fold more dihydrofolate reductase than the wild type, to overcome the diverse action of the antimicrobial agent (Baccanari et al., 1975).

Diversion to an Alternate Metabolic Pathways

Trimethoprim-resistant E. coli can grow in the presence of 50 or 100 micrograms trimethoprim/ml, if the low molecular weight products of folate metabolism or their precursors, thymidine, purines, methionine, glycine and pantothenate were supplied in the medium (Harvey, 1973; Bertino and Slacey, 1966; Koch, 1981). Streptococcus faecalis was found to grow in the presence of trimethoprim in media free of folate, but still containing serine, methionine, thymine, adenine and guanine (Samuel, et al., 1970).

By-pass to a Blocked Metabolic Step

Introduction of plasmids carrying genes coding for a by-pass to a blocked metabolic step accounts for resistance in certain bacteria. In trimethoprim-resistant Enterobacteriaceae, a qualitatively different dihydrofolate reductase, coded by a plasmid-carried gene, supplements the chromosomally produced enzyme and is less sensitive, or completely insensitive, to the action of trimethoprim (Datta and Hedges, 1972).

C. Antimicrobial Resistance of Hospital Strains

The increase in antimicrobial resistance of Staph. aureus in hospitals is well known. Soon after the introduction of many types of antimicrobial agents, mortality and morbidity rates due to staphylococcal infections were sharply reduced. Before 1937, the mortality rate due to Staph. aureus septicemia in several clinics of Minnesota was greater than 80%, but when penicillin was introduced, the mortality rate decreased to 28% in 1942-1944 (Hall and Spink, 1945). Most of the other antimicrobial agents lost their effectiveness against hospital strains of Staph. aureus a few years after their introduction (Jevons, 1961; Spink, 1954; Dowling et al., 1953; Forber, 1949; Jevons et al., 1966; Wills et al., 1966).

Staphylococcal resistance to penicillin is still increasing in may hospitals of the world. In Great Britain, in 1946, 14% of hospital strains were resistant to penicillin; in 1947 the rate increased to 38%, and to 59% in 1949 (Barber and Whithead, 1949). It is more surprising that in 1957, in one general hospital of Newfoundland, up to 93% of hospital strains of Staph. aureus were resistant to penicillin (Josephson and Butler, 1957). In London in 1955, 59% of the isolates were found to be resistant to penicillin,

30% to streptomycin, 26% to tetracycline and 3% to chloramphenicol. After 3 years, the situation got worse and multiply-resistant strains emerged. Sixty-two percent of the isolates were resistant to penicillin, 20% to penicillin and tetracycline and 18% to penicillin, streptomycin, tetracycline and erythromycin (Barber and Burston, 1955). Before 1946, in Boston City hospital, 86% of 120 isolates tested were susceptible to penicillin, by 1947 only 25% were sensitive (Finland et al., 1950).

The ability of staphylococci to develop resistance quickly is not limited to penicillin only. Resistance of Staph. aureus to erythromycin rose from 0 to 70% within 5 months and to chloramphenicol rose from 10-20% to 55% within 6 months. (Lepper et al., 1954; Gibson and Thompson, 1956; as quoted by Murray and Moellering, 1978). Recent reports show that hospital strains are multiply-resistant to common antimicrobial agents (Schaefler et al., 1981; Semel et al., 1980; Crawen et al., 1981; McDonald et al., 1981). In Addis Ababa, Ethiopia, 75% of 820 hospital Staph. aureus isolated between 1976-1980 were multiresistant (Gedebou, 1982a).

Methicillin resistance was reported 2 years after its introduction (Jevons, 1961). Since then there have been several epidemics in many hospitals in Europe, USA and

Australia due to such strains (Finland, 1970; Parker and Hewitt, 1970; Klimek et al., 1976; Graham et al., 1980; Peacock et al., 1980; McDonald et al, 1981). There has been a tenfold increase in the frequency of isolation of methicillin-resistant strains during a period of 5 years, 1966-1970, (Ridley et al., 1970).

In 1967 there were major infections due to methicillin-resistant strains in several cities of the United States, France and Switzerland (Benner and Morthland, 1967). In Switzerland, methicillin-resistant Staph. aureus strains were responsible for 9.7%, 17.3%, 16.1% of all staphylococcal infections in 1965, 1966, 1967 respectively (Benner and Kayser, 1968).

Most of the teaching hospitals in Australia had experienced infection with multiresistant methicillin-resistant Staph. aureus. In Melbourne Hospital 53% of all staphylococcal bacteremia were due to methicillin-resistant Staph. aureus (McDonald et al., 1981). In Sydney, in 1976, there were 13 methicillin-resistant isolates; in 1980 and 1981 the number rose to 124 and 181, respectively (Graham et al., 1981).

The practical significance of methicillin resistant strains lies in their multiresistance to other antimicrobial agents (Finland, 1970; Parker and Hewitt, 1970; Klimek et al., 1976; Graham et al., 1981; Peacock et al., 1980). It is believed that prior exposure to antimicrobial agents is essential to acquire methicillin resistance (Peacock et al., 1980; Graham et al., 1981). However, Klimek et al., (1976) isolated methicillin-resistant strains of Staph. aureus from patients who have not received any treatment with antimicrobial agents, and concluded that no prior exposure to antimicrobial agents is required to acquire methicillin resistance. The incidence of methicillin-resistant staphylococci may not be directly associated with the usage of the antimicrobial agent since other penicillins and cephalosporins may select for methicillin-resistant staphylococci (Parker and Hewitt, 1970).

Methicillin-resistant Staph. aureus have the ability to colonize patients, especially debilitated patients, and spread within hospitals (Jensen et al., 1969; Benner and Kayser, 1968; McDonald et al., 1981; Peacock et al., 1980). Since methicillin-resistant Staph. aureus usually infect debilitated patients, treatment is difficult and infections are often fatal (Klimek et al., 1976; O'Toole et al., 1970; Colley et al., 1965; McDonald et al., 1981).

Gentamicin-resistant Staph. aureus were rare until 1975 (Porthouse et al., 1976; Faden et al., 1979) and was first reported 12 years after its introduction (Noble and Naidoo, 1978). Recent reports showed that gentamicin-resistant strains are increasing in hospitals due to the widespread use of the antimicrobial agent (Schaefler et al., 1981; Semel, 1980; Faden et al., 1979). Topical use of gentamicin has been the source of gentamicin-resistant staphylococci (Graham et al., 1980). There were several outbreaks of staphylococcal infections due to such strains in many hospitals (Speller et al., 1976; Shanson et al., 1976; Porthouse et al., 1976). Upto 96% of all isolates during an epidemic were found to be resistant to gentamicin in America in 1977 (Faden et al., 1979). In patients with persistent bacteremia, the mortality rate was 33% during an extensive outbreak of gentamicin-resistant Staph. aureus infection in 1978-1980 (Crawen et al., 1981).

Gentamicin-resistant strains are usually multiresistant and strains resistant to both gentamicin and methicillin are causing epidemics throughout the world. Such strains were first isolated from England (Klimek et al., 1976). Since then there has been a marked increase in the frequency of gentamicin and methicillin-resistant Staph. aureus in the

USA and Australia (Schaefler et al., 1981; Graham et al., 1981; McDonald et al., 1981; Peacock et al., 1980). In America, 30% of isolates were resistant to both gentamicin and methicillin in 1979-1980 (Schaefler et al., 1981). In Australia, at the Royal Melbourne Hospital, 6% of methicillin-resistant strains were also resistant to gentamicin in 1978; but by the end of 1979 more than 70% of methicillin resistant Staph. aureus were also gentamicin-resistant (McDonald et al., 1981). In Sydney Teaching Hospital, until 1980, not more than 32% of methicillin-resistant isolates were resistant to gentamicin, but in 1981 the rate increased sharply to 96% (Graham et al., 1981).

The multiresistant nature of methicillin-resistant Staph. aureus strains has led investigators to assume that there may be a genetic linkage . Richmond (1972) believed that the gene for methicillin resistance could be found on the plasmid determining penicillinase production. Graham et al., (1980), based on their observation of coexistence of methicillin and gentamicin-resistant Staph. aureus, suggested that the two characteristics may be found on the same plasmid. This, however, has not been further confirmed. On the other hand, Montefiore et al., (1973) and Gedebou (1982a) isolated penicillin-sensitive, methicillin-

resistant Staph.aureus strains. Acriflavin, a mutagen can eliminate methicillin resistance without affecting penicillin resistance, showing that these two characteristics are not genetically linked (Dornbusch et al., 1969). Egan (1972) believed that the coexistance of methicillin and penicillin resistance to be based not on genetic resistance, but rather on an ecological association resulting from the selective advantage of survival from antimicrobial agents. The genes coding for methicillin and penicillin resistance may not be found on the same plasmids, since independent loss was observed in a Staph. aureus strain stored at room-temperature (Anner and Grubb, 1973).

Staph. aureus resistant to clindamycin were rare until 1972 (Eickhoff, 1972). However, recent reports show that multiresistant staphylococci responsible for several outbreaks were clindamycin-resistant (McDonald et al., 1981; Graham et al., 1981). Semel et al. (1980) have reported that an epidemic strain of Staph. aureus resistant to gentamicin and clindamycin, was responsible for a large outbreak in America. The mortality rate was 73%, in contrast to 28% due to strains sensitive to both agents.

Kanamycin has been shown to be effective against staphylococcal infection . However, in some centers over 40% of

Staph. aureus isolates were found to be resistant to kanamycin a few years following its introduction (Eickhoff, 1972).

Except for its toxic nature, vancomycin is effective against Staph. aureus and resistance is extremely rare (Eickhoff, 1972). Even the multiresistant gentamicin-and methicillin-resistant Staph. aureus strains that caused epidemics in USA, England and Australia were found to be sensitive to vancomycin; treatment with this antimicrobial agent was effective in at least 80% of the patients (Klimek et al., 1976; Graham et al., 1980; McDonald et al., 1981; Peacock et al., 1980; Crawen et al., 1981).

There is ample evidence on the occurrence of resistant strains of Staph. aureus to the common antimicrobial agents such as tetracycline, erythromycin, chloramphenicol, and streptomycin. Such strains have been responsible for many outbreaks in varions parts of the world (Benner and Kayser, 1968; Eickhoff, 1972; Wallmark and Finland 1961; Graham et al., 1981; Peacock et al., 1980; Faden et al., 1979).

D. Antimicrobial Resistance of Community Strains

The problem of resistance is not limited to the hospital environment. The hospital personnel do not confine

their activities to the hospital environment only. They carry and spread the resistant Staph. aureus strains in their homes and among their family members (Spink, 1954; Nahmias and Shulman, 1972; WHO, 1968). Patients on discharge from hospitals will certainly carry the hospital strain and disseminate it to the community (Ravenholt et al., 1957; Messinger et al., 1963; Macfarlane et al.,1960).

Antimicrobial resistance among non-hospital Staph. aureus has remained low (Maxwell et al., 1969; Goldie et al., 1971). However, recent reports from several workers show a steady increase of resistance of community Staph. aureus to the common antimicrobial agents (Fallon ., 1968; Price et al., 1968; Goldie et al., 1971). In England, the ratio of penicillin-resistant Staph. aureus in 1949, 1952, 1955, 1957, 1960 was 6%, 16%, 21%, 25% and 39%, respectively; it has doubled within 5 years (Macfarlane et al., 1960). In Bristol, the level of penicillin resistance stayed below 5% until 1952, then rose to 12% by 1955 and by 1967 it had increased sharply and exceeded 50% (Goldie et al., 1971). In America in 1971, up to 83% community strains of Staph. aureus were penicillin-resistant, in 1972, 84% (Rose et al., 1974) and in 1974 up to 85% (Hughes et al., 1976) were resistant to penicillin.

The wide and indiscriminate use of penicillin in the clinic and hospital is responsible for the increase of penicillin resistance among Staph. aureus strains in the community (Nahmias and Shulman, 1972; Nahmias et al., 1962; Macfarlane et al., 1960).

In contrast to single resistance to penicillin, multi-resistance remained low among community staphylococci (Goldie et al., 1971; Lacey et al., 1970). Non-hospital strains usually belong to phage groups I and II and are resistant to few antimicrobial agents (Price et al., 1968; Lacey, 1975; Goldie et al., 1971), while most hospital strains belong to phage group III and are multiply-resistant (Jevons et al., 1966; Lacey, 1975; Henning et al., 1979). Combined resistance to penicillin and tetracycline was rare in a British community where these two antimicrobial agents were widely used (Lacey et al., 1970).

Several explanations were given for the low incidence of multiple-resistnace among non-hospital strains of Staph. aureus. The genetic changes that accompany the development of resistance was found to be disadvantageous, except in the presence of the challenging agent (Lacey, 1973; Noble et al., 1964; Koch, 1981). Carriage of some of the plasmids mediating penicillinase production diminish the growth of staphy-

lococcal colonies (Richmond, 1972). Bellamy and Klimek (1948) found that penicillin-resistant variants of Staph. aureus grew less rapidly than its sensitive parent culture and also lost their ability to grow anaerobically. Penicillin and methicillin-resistant strains have a decreased rate of growth compared to the sensitive ones (Seligman, 1966). Methicillin-resistant variants of Staph. aureus grow slowly and are generally at a disadvantage when compared to sensitive strains (Sutherland and Rolinson, 1964; Lacey, 1972, 1975).

Therefore in the absence of the antimicrobial agent, the bacteria lose their plasmid and become sensitive (Lacey, 1973; Koch, 1981). Multiresistant phage group III were found to be killed by desiccation more quickly than the other groups (Lacey et al., 1970). Therefore their chance of survival outside the hospital among healthy people, is very low (Goldie et al., 1971).

4. Objectives of the Study

The epidemiology of staphylococcal infections and antibiograms of Staph. aureus strains in developed countries have been well-studied. Although staphylococcal infection is also a problem of developing countries (WHO, 1968;

Williams 1966; Levin et al.,1971; Hemnessey and Milles, 1958; Sodhi et al., 1968), there are only a few studies on the carrier rates and incidence of Staph. aureus infections in such countries (Rountree, 1956, Sodhi et al.,1958; Foster W.A., 1965; Davis and Davis, 1965; Cocard, 1959).

The incidence of staphylococcal infections and the extent of this problem in Ethiopia are reflected only by the limited reports from Addis Ababa by Ploride et al. (1970), Perine et al.(1975) and Gedebou (1982a). However, there has not been any full report on the carrier rates of Staph. aureus among hospital or non-hospital populations in this country. Although the antimicrobial susceptibilities of strains from patients from 2 hospitals have been studied (Ploride et al., 1970; Gedebou, 1982a), there is not such information on strains from healthy carriers. This study, the first of its kind, was initiated:

1. to determine the carrier rate of surgical staff of different hospitals in Addis Ababa.

2. to compare the carrier rate of surgical staff with that of the non-hospital population.

3. to detect environmental contamination of the wards and operation rooms of one selected hospital.

4. to study the antibiograms of the isolates and obtain useful information on the *in vitro* effectiveness of the antimicrobial agents commonly used for the treatment of staphylococcal infections.

5. to provide such information as a guide for the empirical choice of antibiotics for treatment when culture and sensitivity facilities are not available.

CHAPTER 2

MATERIALS AND METHODS

1. Collection of Specimens

A. Sources

Four hundred and fifty four nasal swabs of surgical staff of all categories (Table 5) from 6 different hospitals (Table 6) in Addis Ababa were collected during a period of 7 months. The first date of collection was December 23, 1980, and the last date was July 14, 1981. Out of these 454 surgical staff, 290 (63.9%) were females and 164 (36.1%) males (Table 7). The non-hospital population comprised 328 biology students of first, second and third year of the 1980-1981 academic year of Addis Ababa University. Most of them were males, 279 (85.1%) and only 49 (14.9%) were females (Table 7).

Table 8 shows the sources of environmental samples. A total of 544 environmental samples, mainly of air and dust from the floor, were collected on January 1 and 30 and February 10, 18, and 26, 1981.

On the first date of sampling, 50% of rooms of two surgical wards B_3 and C_3 of the Black Lion Hospital, and the corridors between the rooms, were sampled. Operation rooms and the central sterilizing unit, as well as the corridors between the rooms, were sampled during the remaining 4 days. Other sites of collection were the scrub rooms located infront of each operation room. The tea room, washing room, dressing rooms, shoe-changing room as well as rooms of the central sterilizing unit, were also included in the study.

B. Methods

Nasal swabs were collected using sterile cotton swabs moistened with sterile saline solution. Each individual was asked to make circular sweeps in each nostril if he/she was willing; otherwise swabs were taken from one nostril. Skin swabs were collected by swabbing the wrist skin and the anterior part of the forearm of the nasal carriers.

Air samples were collected by leaving three plates (of 5% sheep-blood agar containing 7.5% sodium chloride) open in each room for 4-5 hrs in the morning during the busy hours. Corridors between the rooms were also sampled

by placing open plates of blood agar at several sites.

Floor dust samples were taken at the same time as the air sampling; and an equivalent number of samples were taken. An area of approximatly 25 sq. cm was swabbed several times using sterile cotton swab moistened with sterile saline solution. Each floor swab was then inoculated into plates of 5% sheep blood agar containing 7.5% sodium chloride.

2. Isolation of Staphylococcus aureus

Mannitol salt agar and 5% sheep-blood agar with 7.5% sodium chloride were prepared from Difco dehydrated powders.

Each nasal and skin swab was inoculated onto the sheep blood agar and mannitol salt agar. All plates were incubated at 35-37°C for 24 hrs and examined for typical colonies of Staph. aureus. Plates which required further incubation were reincubated for an additional 24 hrs. Any colony surrounded by zone of hemolysis on the blood agar and a yellow zone of fermentation on the mannitol salt agar (and sometimes a colony with typical golden yellow pigment) was presumptively considered to be Staph. aureus. Such a

colony was Gram-stained and its cellular morphology was microscopically examined. Those isolates showing typical staphylococcal characteristics, i.e. Gram-positive cocci forming clusters, were subcultured on a non-selective medium, nutrient agar.

As a quality control, (a) uninoculated plates were incubated for 24 hrs at 35-37°C, and (b) a plate was inoculated with a standard Staph. aureus strain with known hemolytic activity as a check for any deficiency of the media prepared.

3. Identification of Staphylococcus aureus

A. Catalase Test

The catalase test was performed by taking a loopful of the culture from the agar and mixing it with a drop of 3% hydrogen peroxide on a slide. Formation of gas bubbles indicated the production of catalase. All catalase-positive cultures were further tested for the production of coagulase.

B. Coagulase Test

undiluted human plasma was used for testing coagulase production of the isolates. At the beginning of the study

heparinized human plasma was obtained from the Blood Bank in the Black Lion Hospital. Later on, fresh human plasma containing 0.1% ethylenediaminetetraacetic acid (EDTA)as an anticlotting agent, was obtained from the Central Laboratory and Research Institute. An overnight old culture from the nutrient agar was used for the coagulase test, carried out on slides or in tubes, as described below.

Slide Test

A loopful of the 18-24 hrs culture from the nutrient agar was mixed with a drop of water on a clean slide. If any autoagglutination (false-positive reaction) was detected, the isolate was reserved for the tube test. If there was no autoagglutination a drop of undiluted human plasma was added and mixed using a sterilized wire loop. Immediate formation of white clumps indicated coagulase production. All isolates negative in the slide test, and those that showed autoagglutination, were checked using the tube test.

Tube Test

A loopful of an 18-24 culture from the nonselective media was added to a test-tube containing 0.5 ml of

undiluted human plasma and was incubated at 35-37°c. Any degree of clotting was checked at intervals of 30 minutes for 3 hrs. If no clotting was observed within the three hours, the tubes were left in the incubator and examined for clotting after 24 hrs.

As quality controls, a known coagulase-positive Staph. aureus strain and a test-tube containing only plasma were routinely tested.

All coagulase-positive isolates were maintained on nutrient agar slants and tested for their susceptibility to 12 different antimicrobial agents.

4. Antimicrobial Susceptibility Testing

The agar disc diffusion technique recommended by Bauer et al. (1966) was followed strictly in this study. Table 4 shows the types of the antimicrobial agents and their concentrations used in the susceptibility testing of the isolates.

From the nutrient agar slant of each isolate a broth suspension was inoculated on to sheep blood agar. After an incubation of 18-24 hrs at 35-37°C, 5-6 well-isolated colonies were selected. Using a sterile straight wire,

the top of each colony was touched and was inoculated into a tube containing about 5 ml of trypticase soy yeast (TSY) broth. Inoculated tubes were then incubated at 35-37°C for 2-3 hrs until a moderate cloudiness was formed. The suspension was then diluted using sterile TSY broth to obtain turbidity visually equivalent to that of 0.5 Mcfarland (0.5 ml of 1.175% $BaCl_2 \cdot 2H_2O$ plus 99.5 ml of 0.36N H_2SO_4).

Using a sterile cotton swab, the entire surface of a Mueller-Hinton agar (Oxoid) in 15 cm diameter glass petri dish was swabbed evenly with the TSY broth culture of the isolates. Such plates were left at room temperature for 3-5 minutes. Twelve different discs (Table 4) were placed on the surface of inoculated Mueller-Hinton agar plates using an automatic dispenser and the plates were first left at room temperature for 30 minutes and then incubated at 35-37°C. After 18-24 hrs, the zone of growth inhibition around each disc was measured in millimeters, using a metal caliper, and interpreted according to Bauer et al. (1966), as resistant, intermediate or sensitive.

Staphylococcus aureus (ATCC25923), sensitive to all the antimicrobial agent, was routinely tested as a control.

TABLE 4

Antimicrobial Agents Used in Susceptibility Test of Staph. aureus

Antimicrobial agent	Abbreviation	Concentration of disc in ug
Penicillin G.	Pen	10 (units)
Tetracycline	Tet	30
Streptomycin	Str	10
Chloramphenicol	Chl	30
Erythromycin	Ery	15
Methicillin	Met	5
Kanamycin	Kan	30
Trimethoprim-sulfamethoxazole	Sxt	25
Gentamicin	Gen	10
Cephalothin	Cep	30
Clindamycin	Cli	2
Vancomycin	Van	30

CHAPTER 3

R E S U L T S

Six hundred Staph. aureus strains were isolated from different sources. Three hundred fifty three isolates were from the Black Lion Hospital environment of surgical wards and operation rooms. One hundred thirty nine strains were from the nares of surgical staff of six different hospitals in Addis Ababa; 22 strains were from the wrist skin of the nasal carriers. Eighty-six strains were isolated from the non-hospital population: 71 from anterior nares and 15 from the wrist skin of nasal carriers.

1. Carrier Rates

Out of 454 surgical staff examined, 139 (30.6%) were found to be carriers of Staph. aureus (Table 5). The carrier rates among different categories of surgical staff varied between 26.3-55.8%. The surgeons had the highest rate. (55.8%) , followed by resident doctors (37.5%), other surgery supporting staff (31.0%), health assistants and cleaners (28%), and nurses (26.3%).

The carrier rates of surgical staff of the different hospitals are presented in Table 6: 38% in Yekatit 12 Hospital, 34% in Menelik Hospital, 31% in St. Paûl Hospital,

27% in Black Lion Hospital, 26% in Zewditu Memorial Hospital and 25% in Ras Desta Hospital.

Table 7 shows the carrier rates among the sexes. Females comprised 290 and 27.2% were found to carry the organism in their anterior nares. The number of males examined was relatively lower, 164, and 36% of them were carriers. Out of the 139 nasal carriers, 43 were examined and 22 (51%) were found to carry the organism on their wrist skin.

The carrier rate among the non-hospital population, 21.6%, was found to be significantly lower ($P < 0.01$) than the hospital population Table 5 and 7. Out of 49 females and 279 males examined 11(22%) and 60(21.5%), respectively were found to be carriers of Staph. aureus. Of 55 nasal carriers, 15(27%) were found to harbor the organism also on their wrist skin.

2. Environmental Contamination

The number of Staph. aureus isolates and their environmental source in Black Lion Hospital are shown in Table 8. Staphylococcus aureus was isolated from 75% of the air and 37% of the dust samples, or 56% of both samples. Two

samples from floor dust and 32 samples from air had more than one isolate each. Eight of the 32 plates had 3 different strains; 3 plates had 4 strains each and the remaining samples had two strains each, as recognized by their different antibiograms. Therefore the total of environmental isolates was 353: 104 from dust and 249 from air samples.

TABLE 5

Carriage Rates among Surgical Staff

Specimen		Strains isolated	
Source	No.	No.	%
* Surgeons	34	19	55.5
Resident Doctors	8	3	37.5
* Nurses	91	24	26.3
Health Assistants	121	34	28.0
Cleaners	139	40	28.7
** Other surgery supporting staff	61	19	31.1
Total	454	139	30.6

* 25 of the surgeons, 8 of other surgery supporting staff and 1 of the nurses were not Ethiopians.

**Includes medical students of different years and interns attached to the Department of Surgery during the survey, anaesthetists, etc.

TABLE 6

Carriage Rates of Surgical Staff of Different Hospitals

Hospital	No. of Specimens	Strains isolated	
		No.	%
Black Lion	170	47	27
Zewditu Memorial	23	6	26
Yekatit 12	34	13	38
Menelik II	113	39	34
Ras Desta	24	6	25
St. Paul	90	28	31
Total	454	139	30.6

TABLE 7

Carriage Rates Among Hospital and Non-hospital Males and Females

Source of Specimen	Males			Females		
	No. of specimens	Strains isolated No.	%	No. of specimens	Strains isolated No.	%
Hospital	164	60	36.5	290	79	27.2
Non-Hospital	279	60	21.5	49	11	22
Total	443	120	27.1	339	90	26.5

TABLE 8

Sources of Environmental Isolates

Source	No. of specimens	Strains isolated	
		No.	%
Air	272	204*	75
Dust	272	101*	37
Total	544	305	56

*More than one type of isolate (strains) were isolated from 34 of the 305 specimens. In eight and three of these, 3 strains and 4 strains were isolated, respectively. Thus total environmental isolates were 353: 104 from dust and 249 from air specimens.

3. Antimicrobial Susceptibility and Resistance of Strains

Over 90% of all isolates were sensitive to clindamycin, cephalothin, gentamicin, kanamycin and trimethoprim-sulfamethoxazole. Vancomycin was effective against all isolates. Nine of the antimicrobial agents, excluding penicillin and tetracycline, were effective against the majority (97%) of non-hospital isolates.

Few isolates, less than 7%, were intermediate in their resistance to methicillin, streptomycin, and trimethoprim-sulfamethoxazole. Eleven percent of the non-hospital, and less than 3% of hospital isolates, were also of intermediate resistance to penicillin.

Antimicrobial resistance of Staph. aureus isolated from nostril and wrist skin of surgical staff and from non-hospital sources is presented in Table 9. Resistance rates to tetracycline, streptomycin, methicillin or chloramphenicol of non-hospital isolates were relatively lower than those of the hospital isolates. Most of the hospital isolates (86.9%) and 74.4% of non-hospital isolates were resistant to penicillin. The hospital isolates were resistant to 4 other antimicrobial agents, to which the non-hospital isolates were susceptible. These were erythromycin (20.4%),

trimethoprim-sulfamethoxazole (10.5%), kanamycin (6.2%) and gentamicin (0.6%). (Table 9).

The rates of resistnace of strains from surgical staff and the environment to a number of antimicrobial agents were similar (Table 10): tetracycline (65.2% and 63.4%), streptomycin (30.8% and 38.5%), methicillin (16.7% and 18.6%), kanamycin (9.8% and 6.2%), gentamicin (3.8% and 0.6%) and trimethoprim-sulfamethoxazole (6.7% and 10.5%). However, the rate of penicillin resistance of strains from surgical staff was higher, 86.9%, than that of the environment isolates, 59.4%. (Table 10).

Only 5.6% of surgical staff isolates, 11.6% of non-hospital isolates, and 11.8% of environmental isolates were found to be susceptible (sensitive and intermediate) to all the antimicrobial agents tested. Multiple resistance (resistance to 3 or more antimicrobial agents) among non-hospital isolates was considerably lower, 2.3%, (Table 11), than that of surgical staff isolates 40.3% and of environmental isolates, 34.3% (Table 12).

Evironmental isolates had 66 different antibiograms (resistance patterns). These varied between resistance to one and to nine antimicrobial agents, (Table 13). Forty one

strains (11.6%) showed single resistance to penicillin, 42 strains (11.9%) to tetracycline, 8 strains (2.2%) to erythromycin and 5 strains (1.4%) to methicillin. Less than one percent of the isolates were resistant only to streptomycin or chloramphenicol.

Among 8 antimicrobial agents, fouteen different combinations of double resistance were detected. The most frequent was Pen-Tet, demonstrated with 46 strains (13%). Triple resistance was observed in 11 different combinations of 9 different antimicrobial agents. The pattern Pen-Tet-Chl accounted for 2.8% and Pen-Tet-Str for 2.2%. Twelve types of combinations of 9 antimicrobial agents were observed in resistance to four antimicrobial agents. Eight (2.2%) of the isolates had the Pen-Tet-Str-Chl pattern, which was the most common within the 12 combinations. Resistance to 5 antimicrobial agents had 9 different combinations of 11 antimicrobial agents. Of these the patterns Pen-Tet-Str-Ery-Chl (1.1%) and Pen-Tet-Str-Chl-Met (1.1%) were more frequent.

There were 6 different patterns of resistance to 6 of 9 antimicrobial agents. The most frequent was Pen-Tet-Str-Ery-Chl-Met, observed in 6 (1.7%) isolates. Twelve isolates were resistant to 7 antimicrobial agents and there were 4

different combinations. Nine isolates were resistant to 8 antimicrobial agents, and only three types of patterns were demonstrated. Resistance to nine antimicrobial agents was detected only in 3 isolates, with the pattern of Pen-Tet-Str-Chl-Ery-Met-Kan-Gen-Sxt.

Thirty six antibiograms were detected among the isolates from the surgical staff (Table 14). These varied between resistance to one and to 8 antimicrobial agents. The patterns observed were similar to those observed among the environmental isolates, except that the number of combinations in each group was lower. In addition, single resistance to erythromycin or methicillin was not observed among the staff isolates.

Only 6 resistance patterns were detected within the non-hospital isolates: 4 of single resistance, 1 of double, and 1 of triple resistance. Resistance only to penicillin accounted for 48.8%, to tetracycline for 11.6%, to methicillin for 1.6% and to chloramphenicol for 1.6% of the isolates. The pattern Pen-Tet was demonstrated in 20(23.2%) of the isolates and Pen-Tet-Str in only 2(2.3%) of the isolates.

TABLE 9

Antimicrobial Resistance of Hospital and Non-hospital Staph. aureus Nasal and Skin Strains

Antimicrobial agent	Hospital strain (161)		Non-hospital strains (86)	
	Resistance		Resistance	
	No.	%	No.	%
Pen	140	86.9	64	74.4
Tet	105	65.2	32	37.0
Str	62	38.5	2	2.3
Met	30	18.6	1	1.6
Chl	48	29.8	1	1.6
Ery	33	20.4	-	-
Sxt	17	10.5	-	-
Kan	10	6.2	-	-
Gen	1	0.6	-	-
Van	-	-	-	-
Cli	-	-	-	-
Cep	-	-	-	-

Figures in parentheses indicate the respective number of isolates

- resistant strains were not detected.

TABLE 10

Antimicrobial Resistance of Staph. aureus isolated from Hospital staff and Environment

Antimicrobial agent	Environmental strains (353)		Staff strains (161)	
	Resistance		Resistance	
	No.	%	No.	%
Pen	210	59.4	140	86.9
Tet	224	63.4	105	65.2
Str	109	30.8	62	38.5
Chl	88	24.9	48	29.8
Ery	84	23.1	33	20.4
Met	59	16.7	30	18.6
Kan	35	9.8	10	6.2
Sxt	24	6.7	17	10.5
Gen	14	3.8	1	0.6
Cep	3	0.8	-	-
Cli	3	0.8	-	-
Van	-	-	-	-

Figures in parentheses represent the respective numbers of isolates tested

- resistant strains were not detected.

TABLE 11

Single and Multiple Resistance of Hospital and Non-hospital Strains of Staph. aureus isolated from nose and skin.

Resistat to:	Hospital strains (161)		Non-hospital strains (86)	
	No.	%	No.	%
None*	9	5.6	10	11.6
One	43	26.7	54	62.7
Two	44	27.3	20	23.2
Three	18	11.1	2	2.3
Four	10	6.2	-	-
Five	10	6.2	-	-
Six	17	10.5	-	-
Seven	8	4.9	-	-
Eight	2	1.2	-	-

Figures in parentheses represent the respective number of isolates tested

* Includes sensitive and intermediates.

\- Resistant strains were not detected.

TABLE 12

Single and Multiple Resistance of Hospital Staff and Environmental Isolates of Staph. aureus

Resistant to	Environmental strains (353)		Staff strains (161)	
	No.	%	No.	%
None*	42	11.8	9	5.6
One	101	28.5	43	26.7
Two	89	25.2	44	27.3
Three	38	10.6	18	11.1
Four	29	8.2	10	6.2
Five	16	4.5	10	6.2
Six	14	3.9	17	10.5
Seven	12	3.4	8	4.9
Eight	9	2.5	2	1.2
Nine	3	0.85	-	-

Figures in parentheses represent the respective number of isolates

* Includes sensitive and intermediates.

\- Resistant strains were not detected.

TABLE 13

Antibiograms of 353 Staph. aureus environmental isolates

Resistance Pattern	Strains No. (353)	%
*Pen	41	11.6
Tet	42	11.9
Str	3	0.8
Chl	2	0.56
Ery	8	2.2
Met	5	1.4
Pen Tet	46	13.0
Pen Str	8	2.2
Tet Str	7	1.9
Tet Chl	5	1.4
Pen Ery	2	0.5
Pen Chl	1	0.3
Pen Met	1	0.3
Tet Met	1	0.3
Pen Sxt	2	0.5
Ery Chl	2	0.5
Met Sxt	1	0.3
Ery Tet	1	0.3
Kan Chl	1	0.3
Ery Str	1	0.3
Pen Chl Tet	10	2.8
Pen Tet Str	8	2.2
Pen Tet Ery	4	1.1
Pen Tet Kan	3	0.8
Pen Tet Met	1	0.3
Pen Chl Ery	1	0.3
Pen Str Ery	1	0.3
Pen Str Chl	3	0.8
Pen Str Met	3	0.8
Tet Str Chl	3	0.8
Tet Kan Gen	1	0.3
Pen Tet Str Chl	8	2.2
Pen Tet Chl Sxt	1	0.3
Pen Str Chl Ery	1	0.3
Pen Tet Ery Str	2	0.5
Pen Tet Met Sxt	1	0.3
Pen Tet Str Met	6	1.7
Pen Tet Str Kan	1	0.3
Pen Tet Chl Ery	1	0.3
Pen Tet Ery Kan	4	1.1
Pen Tet Chl Met	1	0.3
Tet Str Chl Kan	2	0.5
Tet Str Chl Ery	1	0.3

Table 13 (Cont'd)

Resistance pattern	Strains No.(353)	%
*Pen Tet Str Ery Chl	4	1.1
Pen Tet Str Kan Gen	1	0.3
Pen Tet Str Chl Met	4	1.1
Pen Tet Str Ery Met	2	0.5
Pen Tet Str Met Sxt	2	0.5
Pen Tet Chl Ery Sxt	1	0.3
Tet Str Chl Ery Cli	1	0.3
Pen Ery Met Cli Cep	1	0.3
Pen Tet Str Chl Kan	1	0.3
Pen Tet Str Ery Met Kan	1	0.3
Pen Tet Str Ery Met Sxt	2	0.5
Pen Tet Str Ery Chl Met	6	1.7
Pen Tet Str Chl Met Sxt	1	0.3
Pen Tet Chl Ery Kan Gen	1	0.3
Pen Tet Str Chl Ery Kan	3	0.8
Pen Tet Str Chl Ery Kan Met	5	1.4
Pen Tet Str Ery Chl Gen Kan	1.	0.3
Pen Tet Str Chl Ery Met Sxt	4	1.1
Pen Tet Str Chl Ery Met Cep	2	0.5
Pen Tet Str Ery Chl Met Kan Sxt	1	0.3
Pen Tet Str Ery Chl Kan Gen Sxt	3	0.8
Pen Tet Str Ery Chl Met Kan Gen	5	1.4
Pen Tet Str Chl Ery Met Kan Gen Sxt	3	0.8

Figure in parenthesis indicates the number of isolates tested

* See Table 4 for abbreviations of antimicrobial agents.

TABLE 14

Antibiograms of 161 Hospital staff Staph. aureus isolates

Resistance pattern	Strains No. (161)	%
*Pen	35	21.6
Tet	7	4.3
Chl	1	0.6
Pen Tet	35	21.6
Pen Chl	2	1.2
Pen Str	4	2.2
Pen Ery	1	0.6
Tet Str	2	1.2
Tet Chl	1	0.6
Met Str	1	0.6
Pen Tet Str	8	4.9
Pen Tet Chl	3	1.8
Pen Tet Ery	3	1.8
Pen Chl Str	2	1.2
Pen Ery Sxt	1	0.6
Pen Tet Str Chl	7	4.3
Pen Tet Met Str	2	1.2
Pen Tet Str Ery	1	0.6
Pen Chl Tet Met Str	2	1.2
Pen Tet Chl Str Ery	1	0.6
Pen Tet Str Met Kan	1	0.6
Pen Tet Chl Str Kan	1	0.6
Pen Tet Str Ery Sxt	1	0.6
Pen Tet Str Chl Sxt	1	0.6
Pen Tet Str Met Sxt	1	0.6
Pen Tet Str Ery Met	2	1.2
Pen Tet Chl Str Ery Met	9	5.5
Pen Tet Chl Str Ery Sxt	2	1.2
Pen Tet Str Ery Met Sxt	2	1.2
Pen Tet Str Ery Chl Kan	2	1.2
Pen Tet Str Chl Met Kan	2	1.2
Pen Tet Str Chl Ery Met Sxt	5	3.0
Pen Tet Str Chl Met Kan Sxt	2	1.2
Pen Tet Str Chl Ery Met Kan	1	0.6
Pen Tet Str Chl Ery Met Kan Sxt	1	0.6
Pen Tet Str Chl Ery Met Kan Gen	1	0.6

Figure in the parenthesis indicates the total number of isolates tested.

* See Table 4 for abbreviations of antimicrobial agents,

TABLE 15

Antibiograms of 86 Non-hospital *Staph . aureus*

Resistance pattern	Strains	
	No. (86)	%
*Pen	42	48.8
Tet	10	11.6
Met	1	1.6
Chl	1	1.6
Pet Tet	20	23.2
Pen Tet Str	2	2.3

Figure in parenthesis represents the total number of non-hospital isolates tested.

* See Table 4 for abbreviations of antimicrobial agents.

CHAPTER 4

D I S C U S S I O N

The carrier rates of both non-hospital population (21.6%) and of hospital staff (30.6%) reported in this study were actually lower than the 46% reported in Nigeria (Davis and Davis, 1965) and 46%-72% in Ghana (Sodhi et al., 1968). However, it still lies within the range given by several workers (Williams, 1963;Rao and Frederik, 1966).

The carrier rate of surgical staff (30.6%), was found to be significantly higher ($P < 0.01$) than that of the non-hospital population (21.6%). Several workers reported higher carrier rates among hospital staff (Williams, 1963; Hofstad and Vogelsang, 1960; Weinstein, 1959). Others did not find any significant difference in the carrier rates among hospital and non-hospital populations (Henning et al., 1979; Maxwell et al., 1969; Sodhi et al., 1968). However, it is generally accepted that carrier rates are higher among hospital staff (Nahmias and Eickhoff, 1961; Williams, 1963).

In this study the carrier rates among surgical staff varied between 26.3 and 55.5%. Surgeons had the highest

carrier rate, 55.5% followed by resident doctors, 37.5% and other surgery supporting staff, including medical students, 31.3%. Bengtsson et al. (1979) had observed a similar difference in Sweden. Doctors and medical students had the highest carrier rate and nurses and assistant nurses, the lowest. On the other hand, the study of Josephson and Butler (1957) in Newfoundland showed very little difference in the nasal carrier rates among the different categories of surgical staff.

In general there is no difference in the carrier rates among males and females (Miller et al., 1962). But in this study (Table 7) the carrier rate was significantly higher ($P < 0.05$) among hospital males (36.5%) than in females (27.2%). In contrast to this, Davis and Davis (1965) reported a higher carrier rate among females (60%) than in males (35%) in Lagos, Nigeria. In the non-hospital population, no difference was observed in the carrier rates among males and females (21.5% and 22%, respectively).

Most of the time nasal carriers are also skin carriers, though the organism is usually present in much smaller numbers on the skin than in the nose (WHO, 1968; Williams, 1963; Naidoo, 1981). It is reported elsewhere that 20% of nasal carriers also carry the organism on their arm

skin (Miles et al., 1944; Williams, 1946). The skin carrier rate here among the nasal carriers was again remarkably higher among the surgical staff (51%) than among the non-hospital population (27%). Since the hospital population is exposed to heavily-contaminated hospital air and to fomites, it is not surprising that Staph. aureus was isolated in higher frequency from the exposed skin of surgical staff than from that of the non-hospital population.

Staphylococcus aureus was isolated from 75% of hospital air samples and 37% of floor dust samples. Since environmental contamination in hospitals depends on several factors, comparision of results obtained by different workers from various places may not be possible. The presence or absence of heavy disperser (Williams, 1966), traffic (Noble and Davis, 1965) and different sampling techinique (O'Toole et al., 1970), may greatly influence the number of variety of bacteria in the air of hospitals. Seasonal variation has been observed in the concentration of air-borne bacteria. In Sweden, during the winter months, Staph.aureus was isolated from 50% of exposed plates and only from 10-20% during spring months (Laurell and Wallmark, 1953).

Floor dust contamination (37%) reported in this study was relatively lower than that reported from a similar study, in Sweden, by Laurell and Wallmark, (1953). They found that almost all dust samples tested were positive for Staph. aureus. The floor, especially of operation theatres of the Black Lion Hospital, were frequently washed with formalin during the period of collection of dust samples. The low incidence of Staph. aureus in the floor dust may be due to the regular washing of the floor.

The air of the Surgical Department of the Black Lion Hospital was heavily contaminated (75%) with Staph. aureus In Denmark, (Jepsen, 1972) only 25% of the exposed plates in wards and 5% of those exposed in operation theatres were positive for Staph. aureus. The high incidence of Staph. aureus in the air of the Surgical Department of the Black Lion Hospital may be due to the particular overcrowded situation in the hospital and infrequent sterilization of operation theatres. About 44% of all Staph. aureus isolated from patients during the years 1976-1980 from the Black Lion Hospital came from the Surgical Department (Gedebou, 1982a).

As can be seen from Table 16, the antibiograms of nasal and skin isolates from the same person vary.

TABLE 16

Antibiograms of Skin and Nasal Isolates of Staph. aureus tested.

Antibiograms of hospital isolates*		Antibiograms of non-hospital isolates*	
Nasal	Skin	Nasal	Skin
-	-		
-	Pen Tet Str Met Sxt	-	Met
Pen	Pen Kan Sxt Chl Tet Met Str	Tet	Pen
Tet	Pen Chl Tet	Pen	Pen
Pen	Pen Kan Gen Chl Met Ery Str	-	Pen
Pen Tet	Pen Tet Met Ery Str	Tet	Pen
Pen Str	Pen Sxt Tet Met Str	Pen Tet	Pen Tet
Pen Str	Pen Sxt Str Tet Chl Met Ery	Pen	Pen
Pen Tet	Pen Sxt Tet Chl Met Ery Str	Pen	Pen
Pen Tet Ery	Pen Kan Chl Tet Met Ery Str	Tet	Tet
Chl	Pen	Tet	Tet Pen
Pen Tet	Pen Tet	Pen Tet	Pen
Pen Tet	Pen Tet	Chl	Pen
Pen Chl Tet Met Ery Str	Pen Chl Tet Met Ery Str	Tet	Tet Pen
Pen Str Chl	Pen Tet Str	Pen	Pen Tet
Pen Tet Met Chl	Pen Tet	Tet	Tet
Pen Tet Met Ery Str	-		
Pen Tet Ery Str	Pen Sxt Ery		
Pen Sxt Chl Tet Ery Str	Pen Tet Met Str		
Pen Tet Ery	Pen		
Pen Sxt Tet Met Ery Str	Pen Chl Tet Str		
Pen Chl Tet Met	-		

* The respective antibiograms listed horizontally are for nasal and skin isolates from the same individual.

\- Indicate that the strain isolated was sensitive to all the antimicrobial agents tested.

Usually, staphylococci from different skin areas of a person are found to be of one phage type, often with the same antibiograms and most of the time it is the same with the type present in the nose (Ridley, 1959; Williams, 1946; Noble, 1977). However, the antibiograms of Staph. aureus strains isolated from the nares and skin of an individual may differ due to the chemical differences that exist between the two sites (Naidoo, 1981; Greenway and Dyke, 1979). The skin was found to select resistant variants (Noble, 1977). The nasal and skin isolates from the same individual may not be the same strain, since the wrist skin is exposed to the heavily-contaminated hospital environment (Williams, 1946, 1963). Therefore, the origin of the cocci on the arm skin of the carriers is diverse, and may not be the same as in their nasal flora.

The majority of hospital (86.9%) and non-hospital isolates (74.4%) were resistant to penicillin (Table 9). It was reported by several workers that the ratio of penicillin resistance of non-hospital Staph. aureus is low compared to that of hospital isolates (Maxwell et al., 1969; Josephson and Butler, 1957; Sodhi et al., 1968). However reports by Cocard (1959) in Nigeria and Semel et al., (1980) in America showed a high incidence of

pencillin resistant strains among non-hospital staphylococci.

In agreement with the literature (Montefiore et al., 1973; Cocard, 1959; Goldie et al., 1971; Price et al., 1968), resistance to the remaining 11 antimicrobial agents among non-hospital isolates was relatively low. Single resistance, as well as combined resistance to penicillin and tetracycline, were high among both hospital and non-hospital Staph. aureus in the present study. These patterns were also the most frequent among the Black Lion Hospital isolates of staphylococci during the years 1976-1980 (Gedebou, 1982a). This reflects the extent of the use of these antimicrobial agents in the hospitals of Addis Ababa.

Unlike the isolates from out-patients from the Black Lion Hospital (Gedebou, 1982a), non-hospital staphylococci from healthy individuals in this study were much more drug-sensitive. This may be explained by the fact that, the non-hospital individuals surveyed in this study were young students who were not as much exposed to the indiscriminate chemotherapy widely practiced outside hospitals in this country.

The percentage of resistance to tetracycline, chloramphenicol and erythromycin reported in this study, and from the Black Lion Hospital reported by Gedebou (1982a), was higher than that reported about 11 years ago by Ploride et al., (1970) from the same city. This shows the usual trend (of increase in the incidence of resistant staphylococci) reported in many parts of the world (Cocard, 1959; Montefiore et al., 1973; Hughes, et al., 1976; Ridley et al., 1970: Finland, 1955).

Generally, the frequency of isolation of antibiotic-resistant Staph. aureus from the non-hospital population in this study was relatively lower than reports of similar studies in Ghana (Sodhi et al, 1968) and Nigeria (Montefiore et al., 1973). Only 2.3% of the isolates in Addis Ababa were resistant to streptomycin, in contrast to over 70% of similar isolates in Ibadan, Nigeria. Combined resistance to penicillin and tetracycline was demonstrated in 23% of the non-hospital isolates as compared to 42% of the Nigerian Isolates.

Multiple resistance (resistance to 3 or more antimicrobial agents) was remarkably low (2.3%) in non-hospital staphylococci, compared with the hospital staff isolates (40%) and environmental isolates (34%) (Tables 11 and 12).

The incidence of multiply-resistant staphylococci outside hospitals remained relatively low (Goldie et al., 1971) Price et al., 1968; Lacey et al., 1970). Hospital staphylococci are usually multiresistant and belong to phage group III (Barber and Burston, 1955; Josephson and Butler, 1957). Again, multiresistance was higher among the isolates from out-patients of the Black Lion Hospital (Gedebou, 1982a) than among the strains in this study.

The rate of penicillin resistance among environmental isolates (59.5%) was lower than that of staff isolates (86.9%). This difference may have been due to the different in the nature of the strains. Some strains of Staph. aureus are known to be more resistant to environmental factors than others, especially phage groups I and II (Lacey et al., 1970). Carriers are not the only source of staphylococci in the environment and not all of them disseminate the cocci equally (Noble and Davies, 1965; Sciple et al., 1967; White et al., 1964).

In this study 18.6% and 1.6% of the surgical and non-hospital isolates, respectively, were resistant to methicillin. Although methicillin-resistant Staph. aureus were found to be poor colonizers of the environment (Bulger et al, 1972; O'Toole et al., 1970), 16.7% of the environmental

isolates from the Black Lion Hospital were resistant to methicillin. Methicillin resistance has been reported since 1961 with varying frequencies (McDonald et al., 1981; Peacock et al., 1980; Shanson et al., 1976; Montefiore et al., 1973; colley et al., 1965; Benner and Kayser,1968). The rate reported here is generally consistent with the literature except during epidemics due to such strains (Graham et al., 1981; McDonald et al., 1981).

Most of the methicillin-resistant strains were multi-resistant; in agreement to the reports by others (Ridley et al., 1970; Lacey, 1973; Klimek et al., 1976; Graham et al., 1980). Five environmental isolates and 2 of non-hospital isolates were resistant to methicillin but sensitive to the remaining 11 antimicrobial agents. On the other hand, 2 environmental isolates and 1 hospital staff isolate were penicillin sensitive, but methicillin-resistant. This was similar to the findings of Parker and Hewitt (1970), who reported penicillinase-negative methicillin- resistant strains of Staph. aureus; and of Montefiore et al., (1973) and Gedebou (1982a), who also isolated similar variants, respectively from Ibadan and Addis Ababa.

Staphylococci resistant to methicillin belong to phage group III and are rare among non-hospital staphylococci (Montefiore et al., 1973; O'Toole et al., 1970; Klimek et al., 1976; Goldie et al., 1971; Lacey et al,, 1970). Consistent with this, only 1.6% of non-hospital staphylococci were methicillin-resistant in this study. The incidence of methicillin-resistant staphylococci among the isolates from out-patients of the Black Lion Hospital reported by Gedebou (1982a) was remarkably higher (35%) than 6% of isolates from St. Paul Hospital (Ploride et al. (1970) and the finding in this study; 18.6% of isolates from non-diseased individuals.

The rate of methicillin resistance reported in this study should be taken as the minimum for various reasons.

1. The techniques recommended for the detection of methicillin resistance were not followed in this study. To ensure maximum isolation of methicillin-resistant Staph. aureus, the cultures should be incubated at 30°C overnight or at 37°C for 48 hrs. Addition of 5% sodium chloride to the media was found to enhance the expression of methicillin resistance in staphylococci (Sutherland and Rolinson, 1964; Parker and Hewitt, 1970).

2. It has been reported that methicillin resistance is unstable at room temperature (Annear and Grubb, 1973; Grubb and Annear, 1972). In the present study the staphylococci were stored at room temperature for 2-3 months before the susceptibility test was done.

3. Methicillin-resistant Staph. aureus strains consist of mixed populations in which the majority of cells are sensitive (Sutherland and Rolinson, 1964). Methicillin-resistant cells, which are present in smaller numbers, may have been easily missed in the in vitro tests.

Gentamicin resistance among the isolates in the present study was low: 3.8% of the environmental isolates and 0.6% of surgical staff isolates. This was comparable to the rate (2%) among isolates from the Black Lion Hospital out-patients (Gedebou, 1982a). Gentamicin-resistant Staph. aureus strains were rare until 1976 (Shanson et al., 1976). Since then it has been reported at low rates, except during outbreaks due to such strains (Graham et al., 1981; Peacock et al., 1980; Faden et al, 1979; Shanson et al., 1976).

Staphylococcus aureus resistant to both gentamicin and methicillin have caused epidemics in Australia (Graham et al., 1981; McDonald et al., 1981), England (Shanson et al., 1976; Klimek and Quintiliani, 1977; Speller et al., 1976), and in the United States (Peacock et al., 1980; Faden. et al., 1979; Graham et al., 1980). It should be noted that 75% of all the gentamicin resistant strains in this study were also resistant to methicillin and to at least other 5 antimicrobial agents. Consistent with the reports of Graham et al., (1981) Peacock et al.(1980), and McDonald et al. (1981) all gentamicin and methicillin resistant-strains of Staph. aureus were sensitive to vancomycin. However, in contrast to their findings, most of the methicillin and all of the gentamicin-resistant strains were sensitive to cephalothin.

CHAPTER 5

C O N C L U S I O N S A N D R E C O M M E N D A T I O N S

This study showed that staphylococci were fairly widely distributed in and outside hospitals in Addis Ababa. Carrier rates were found to be higher among surgical staff than among the non-hospital population. The non-hospital staphylococci were sensitive to most antimicrobial agents tested, while the hospital isolates were resistant to the antimicrobial agents most commonly used in the treatment of staphylococcal infections. About 40% of the hospital Staph. aureus isolates, from healthy carriers, in this study and 75% of Staph. aureus isolates from out-patients of the Black Lion Hospital (Gedebou, 1982a) were multiply-resistant. Over 80% of common pathogens, Gram-negative bacilli, from Addis Ababa were multiply-resistant (Gedebou, 1982b, 1983; Gedebou and Tassew 1982). Gradual increases in resistance to common antimicrobial agents have been reported among Escherichia coli in the Black Lion Hospital in the last 5 years (Gedebou and Tassew, 1983). Based on the findings of the present study, and on similar reports from Addis Ababa, the following recommendations are made.

1. Strict Policy on antimicrobial usage.

To avoid emergence of resistant staphylococci, intelligent and conservative use of antimicrobial agents is desirable in Ethiopia. As much as possible, chemotherapy should be based on case laboratory reports. Prophylactic use of antibiotics must be minimized. The antimicrobial agents to which the majority of staphylococci are still sensitive, in this study, cephalothin, clindamycin, vancomycin, gentamicin, trimethoprim-sulfamethoxazole and methicillin, must be used discriminately, if they are to remain effective against life-threatening staphylococci infections.

The unlimited use of certain drugs without prescription has been partially responsible for the present level of resistance of Staph. aureus. The easy availability of such antibiotics must be controlled.

2. Infection control officer.

It is suggested that every hospital assign one infection control officer. The duties of such an officer would be to collect information within the hospital, from other hospitals and other related institutions and clinics.

The data collected from laboratory reports, and clinical records of patients, should be evaluated and appropriate measures be taken.

3. Reduction of cross-infections in hospitals.

Once the source of infection is detected, preventive measures must follow. To reduce cross-infection, the patient who may be a possible source of infection must be isolated. The carrier state of the staff must be checked regularly, and individuals carrying multiresistant and highly virulent strains should, where possible, not have close contact with susceptible patients. If such a person is a member of the clinical staff, the individual should be treated to eradicate the organism.

4. Surveillance.

Since the immunologic means of control of staphylococal infection is not available at present, control must be based on careful use of available drugs and on the results of epidemiologic studies. There have not been such studies in Ethiopia. Thus, regular surveillance in and outside hospitals should be made. Such studies are absolutely essential in detecting the emergence of new strains resistant

to certain antibiotics and in providing information to clinicians to choose the most suitable antimicrobial agents in the absence of laboratory reports.

5. Maintenance of cleanliness of the hospital environment.

The examination of dust and air samples in one of the main hospitals in Addis Ababa showed that the environment was highly contaminated. Therefore, it is recommended that the environment of wards, as well as of the operation theatres, should be kept clean. Regular assessment of the environmental contamination should be made to control the efficiency of any preventive measures taken.

6. Research.

To enrich our knowledge on the epidemiology of staphylococcal infections in Ethiopia, extensive surveys should be conducted in and outside hospitals.

To counteract the notorious nature of the organism, the mechanisms of antibiotic resistance in Staph. aureus should be fully understood.

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