CHARACTERISATION OF STAPHYLOCOCCI ASSOCIATED WITH ATOPIC ECZEMA AND CHRONIC PLAQUE PSORIASIS.

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SUMMARY.

Though well characterised diseases of the skin, the causes of atopic eczema and chronic plaque psoriasis have yet to be elucidated. The association of *S.aureus* in eczema and group A beta-haemolytic streptococci in psoriasis, have been well documented, however, the role of coagulase-negative staphylococci (CNS) has been largely understudied.

In this investigation, the skin flora of ten patients with atopic eczema and ten patients with chronic plaque psoriasis was quantitatively assessed. The staphylococci isolated were identified by conventional techniques and by the molecular technique of pulsed-field gel electrophoresis (PFGE).

Blood samples were obtained from each patient and ten control patients to test for antibody levels to gram-positive organisms by means of a novel ELISA which incorporates a short chain lipoteichoic acid, lipid S. This test has been of value in detecting patients with sepsis due to Gram-positive cocci and was used in this study to determine whether the organisms on the skin surface produce an immune response.

Psoriatic skin consisted of many different species and genotypes of CNS, with no one strain being associated with the condition. Many different genotypes of CNS were also identified from eczematous skin but *S.aureus* was by far, the most predominant organism. Each patient had their own unique strain of *S.aureus,* which could be reisolated from different areas at different times and thus in contrast to the CNS from both diseases, was a constant entity. The *S.aureus* isolates from eczematous skin were tested for superantigen production and certain enzymes, however, the results were inconclusive and as with the PFGE results, no common strain was found for all.

With regards to the ELISA, 80% of the eczema patients and 40% of the psoriasis patients had positive titres, however, at this stage it is not possible to say what organism the raised titres were due to.

From the results of this study it was concluded that due to the heterogeneity of the CNS from both skin diseases, these organisms do not appear to play a role in the exacerbation of either condition. However, further work is needed to improve the usefulness of this ELISA for these patients, for example ability to differentiate between antibodies to *S.aureus* and CNS, and testing on much larger patient groups during exacerbations and stable periods of the diseases.

Key words: eczema, psoriasis, PFGE, CNS, *S.aureus,* ELISA

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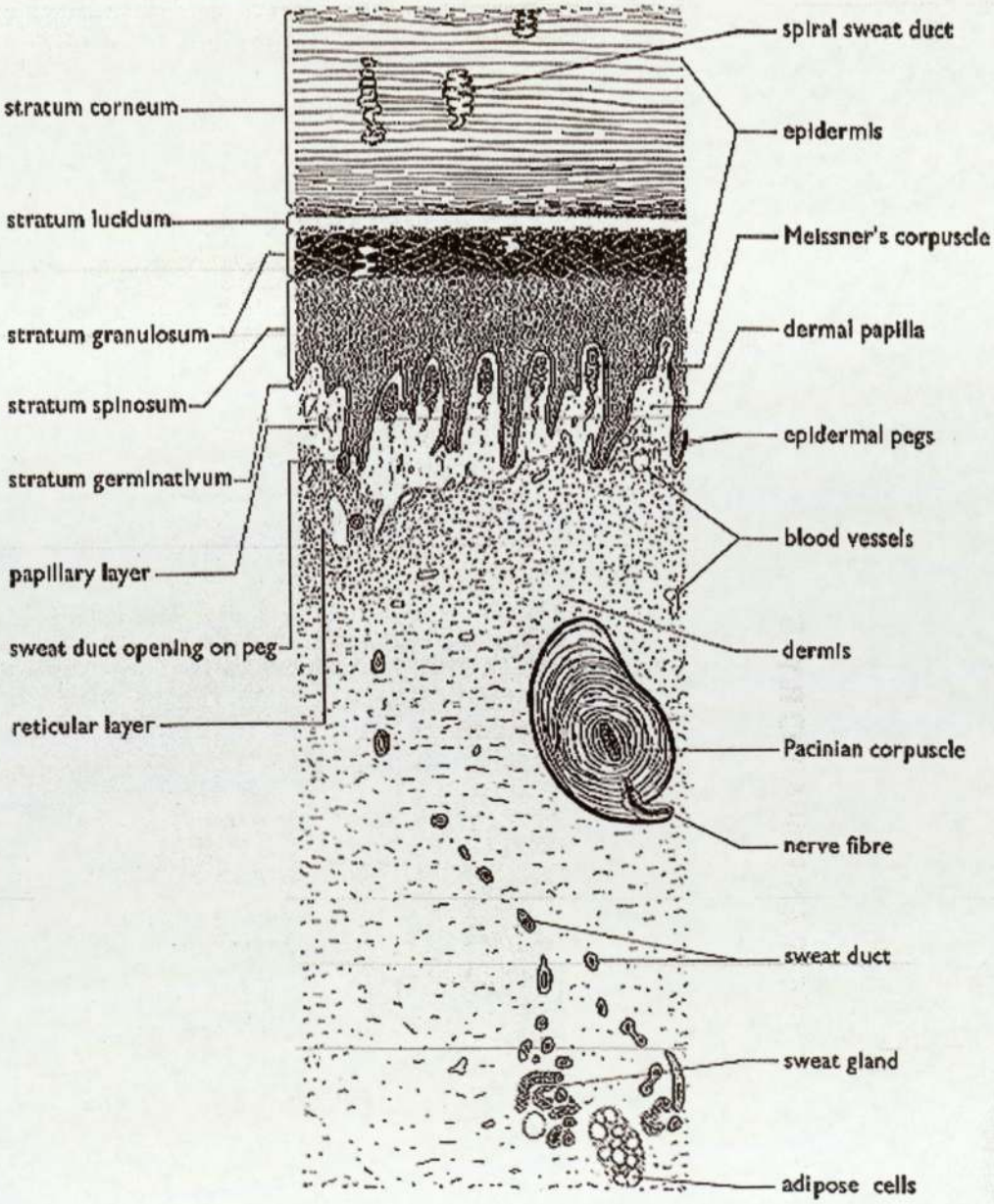
1.0 INTRODUCTION.

1.1 Structure and function of skin.

In addition to the internal immune defence system, the body is equipped with external defence mechanisms designed to prevent penetration by microbes wherever body tissues are exposed to the external environment, the most obvious is the skin, the largest organ of the body. The skin is divided into two main layers, the epidermis and the dermis, as illustrated in figure 1.

Figure 1. Drawing to illustrate the different layers of the skin (Freeman and Bracegirdle, 1988).



1.1.1 The epidermis.

In non-hairy areas such as the sole of the foot the following layers can be identified: stratum basale/germinativum (closest to the dermis), stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum. In such instances the total thickness of this layer can be 0.4-0.6 mm. However, where hair is present, the stratum lucidum is generally absent and the stratum granulosum if present, is usually found as a single, often intermittent layer, the stratum spinosum is much thinner. Epidermal thickness therefore varies inversely with how dense the hair is, that is, the thicker the hair the thinner the epidermis (Noble,1993).

The epidermis is formed by mitotic division of cells in the **stratum basale/ germinativum**, which then migrate outwards, in a columnar formation. The **stratum spinosum** is composed of keratinocytes which are firmly attached to each other by small interlocking cytoplasmic processes, abundant desmosomes and an intercellular 'cement' of glycoproteins and lipoproteins. Many lamellar bodies are seen in the superficial keratinocytes of this layer. They contain polysaccharides, free sterols, lipids and hydrolytic enzymes, which are discharged into the intercellular space of the **stratum granulosum.** Lipids and sterol sulphates form electrochemical bonds, the rupture of which, by sterol sulphatase, present in the horny layer, may account for shedding of keratinised squames.

Cells continue to differentiate in the **stratum granulosum,** which usually has two or three layers of cells, which are flatter than those in the stratum spinosum. Granular layer cells contain lamellar bodies and large irregular basophilic granules of keratohyalin. Hydrolytic and degrading enzymes found in the lysosomes of granular cells, destroy the cells nuclei and intracytoplasmic organelles, as the granular cells become horny cells.

The **stratum corneum,** or horny layer is made up of piled up layers of flattened dead cells. Their cytoplasm is packed with keratin filaments, embedded in a matrix, probably derived from the keratohyalin granules. The horny cell membrane is much thicker than that of the underlying keratinocytes. Cells of the horny layer stick tightly together but flake off at the surface, resulting, as epidermal production continues, in the shedding of cells into the environment. These cells act as 'flying saucers', for attached microbes, aiding in their dissemination (Hunter *et al.*,1989, Noble,1993).

Keratins in the horny layer contain cystine, which is responsible for the intra and interchain disulphide cross-links, which give the epidermis strength to withstand tearing. The horny layer is impervious to most substances, it serves to resist passage in both directions between the body and the external environment, that is, loss of water and other vital constituents from the body (Hunter *et al.*,1989, Noble,1993).

The rate of epidermal turnover is variable and relatively slow, taking about 12-14 days for complete transfer of a cell from the basal layer to the granular layer of the healthy epidermis. The processes of cell production, differentiation and keratinisation are influenced by a number of factors such as circulating and local humoral agents (Noble,1993).

1.1.2 The dermis.

This interdigitates with the epidermis so that upward projections of the dermis, the upper stratum papillare, interlock with downward ridges of the lower stratum reticulare. This interdigitation is responsible for the ridges seen most readily on the fingertips, that is fingerprints, and is important in the adhesion between the epidermis and dermis, increasing the area of contact between them.

The dermis, is a connective tissue matrix that contains elastin fibres for stretch and collagen fibres for strength, providing a supple framework for the vascular supply

which transports the nutrients for maintenance and growth and the lymphatic system, which aids drainage. The volume of blood flowing through these vessels is subject to control by specialised nerve endings, to vary the amount of heat exchange between these skin surface vessels and the external environment.

The amorphous ground substance of the dermis consists largely of two glycosaminoglycans: hyaluronic acid and dermatan sulphate, with smaller amounts of heparan sulphate and chrondroitin sulphate.

The important functions it performs are :

(a) it binds water, allowing nutrients, hormones and waste products to pass through the dermis

(b) it is a lubricant between the collagen and elastin fibre networks during skin movement

(c) it provides bulk allowing the dermis to act as a shock absorber.

The main cells of the dermis are fibroblasts which synthesise collagen, reticulin, elastin, fibronectin, glycosaminoglycans and collagenase (Noble,1993).

Special infoldings of the epithelium, into the underlying dermis, form the skin's exocrine glands, the sweat and sebaceous glands, as well as the hair follicles.

1.1.2.1 Sweat glands.

There are two types of sweat glands, eccrine and apocrine:

Eccrine glands are distributed in all areas of the skin, although they are more concentrated on the palms of the hands and the soles of the feet, the forehead and the axillae. This gland is composed of a secretory coil where the sweat is initially formed,

and an excretory portion, the distal duct. In the secretory coil, sweat is isotonic with a pH close to neutrality, but as it passes through the distal duct, sodium, potassium and chloride ions are selectively reabsorbed, this then results in a hypotonic solution and a lower pH of approximately 5.0.

Apocrine glands are concentrated in the hairy parts of the body, the axillae and groin in particular. They remain underdeveloped until puberty. They comprise a secretory coil which is embedded deep in the dermis and a straight tubular duct through which sweat is carried to the hair follicle and from there to the skin surface. When first secreted, apocrine sweat is thought to be a colourless, odourless liquid, which is then enzymatically altered by skin microrganisms to compounds that are responsible for body odour. Lysozyme in apocrine ducts may be relevant to protection against microbial invasion (Noble, 1993).

1.1.2.2 Sebaceous glands.

These are found on most of the skin surface, with the exception of the palms of the hands and the soles of the feet. They predominate on the face and scalp. The clusters of acini that make up the bulk of the gland are responsible for sebum production, they are derived from renewing epithelial cells formed along a basement membrane, which gradually differentiate and produce lipid forming globules that can be seen in the cytoplasm.

When fully differentiated the cells appear to consist of lipid, including essential fatty acids such as linoleic, as the cells disintegrate the lipid is forced into the duct. The ducts are lined with stratified squamous epithelium that is continuous with the epithelium of the hair follicle and is constantly shedding keratinous cells. The duct

therefore, contains a mixture of lipid and cornified cells which is forced into the hair follicle, from here the oily sebum flows to the surface of the skin, oiling the hairs and the outer keratinised layers, to help waterproof them and prevent them from drying and cracking e.g chapped hands or lips (Noble,1993).

Disorders of the sebaceous glands.

Acne vulgaris.

This is a disorder of the pilosebaceous unit (hair follicle and its associated sebaceous gland), peaking in adolescence and characterised by comedones, papules, pustules, cysts and scars. Many factors combine to cause acne, these are:

(a) Increased sebum excretion, with an excess of free fatty acids.

(b) Sebaceous glands which respond excessively to steroid hormones.

(c) Increased and abnormal keratinisation within the follicle obstructs the pilosebaceous ducts fluid retention.

(d) *Propionibacterium acnes*, a normal skin commensal, colonises the pilosebaceous ducts, breaks down triglycerides and initiates the inflammatory reaction.

Lesions are confined to the face, shoulders, upper chest and back. The skin is greasy and comedones, due to the plugging by keratin and sebum of the pilosebaceous orifice, or whiteheads due to accretions of keratin and sebum deeper in the pilosebaceous duct are always seen.

Local treatments are enough for most patients these include regular cleansing with soap, use of benzoyl peroxide, retinoic acid and topical antibiotics such as tetracycline, clindamycin and erythromycin (Hunter *et al.*,1989).

1.1.2.3 Hair follicles.

With the exception of the palms of the hands and soles of the feet, hair follicles are distributed over the entire body surface. They are derived from cells at the base or bulb, of the follicles, which also divide by mitosis, however, in contrast to the epidermis, they grow outwards in a single column rather than a sheet. These cells then differentiate and keratinise to produce hairs, that is, cylinders of dead cells composed of keratin (Noble,1993, Jawetz *et al.*,1989).

1.1.3 The hypodermis.

Also known as subcutaneous tissue, is a loose layer of connective tissue, which anchors the skin to the underlying tissue (muscle or bone). Most fat cells in the body are found here, these subcutaneous fat deposits throughout the body are collectively known as adipose tissue (Noble,1993, Sherwood,1989).

1.2 The skin immune system.

There are several specialised cells of the epidermis which participate in immune defence, these include:

Keratinocytes.

These cells make up about 85% of the cells in the epidermis, as well as forming the outer protective keratinised layer of skin, they are also responsible for generating hair and nails. These cells are important immunologically in that they secrete interleukin 1, which influences the maturation of T-cells that localise in the skin (Noble,1993).

Langerhans cells.

These cells migrate to the skin from the bone marrow. They take up exogenous antigen, process it and present it to T-lymphocytes, either in the skin or local lymph nodes. Loss of these cells as a result of exposure to ultraviolet radiation, may induce skin tumours not only by causing mutations in the epidermal cells, but also by damaging Langerhans cells, so that cells bearing altered antigens are not destroyed by the immune system. The Langerhan cell is the principal cell in skin allografts to which the T-lymphocyte of the host reacts during rejection (Noble,1993, Hunter *et al.*,1989).

Granstein cells.

Interact with suppressor T cells, serving as a 'brake' on skin activated immune responses (Sherwood,1989, Noble,1993).

1.3 Normal flora of the skin.

Although the skin acts as a barrier to colonisation from the environment, it provides in its outer layers and hair follicles, a protected region with nutrients capable of supporting growth and development for resident microorganisms. These organisms are on a continual 'moving staircase', due to the cell division of the epidermis which restricts their inward spread.

Resident microorganisms are distributed as mixed colonies on or between layers of the outer stratum corneum and hair follicles. These observations have been illustrated by certain techniques, which include:

1.3.1 Skin scrubbing.

Williamson and Kligman, (1965), devised a method which utilised a buffered non-ionic detergent, Triton X-100, as a sampling fluid, in order to assure complete removal of bacteria from the skin and to disperse the removed organisms so that subsequent colony counts reflected single bacterial cells rather than aggregates. In their study, 85% of the organisms were removed from the skin surface, in a single one minute scrub, and 97-98% in two.

1.3.2 Skin stripping with cellulose tape.

95% of the organisms were removed after the application of four strips of cellulose tape to the outermost horny layer, ten strips were no more effective. These findings indicated that the major portion of the horny layer is a coherent membrane, which does not provide crevices for the accumulation of bacteria (Williamson and Kligman, 1965).

1.3.3 Scanning electron microscopy of skin biopsies.

In a study carried out by Malcolm and Hughes, (1980) biopsies were taken by either, sectioning, or by removing the stratum corneum with cyanoacrylate ester adhesive. The biopsies were analysed using a Jeol JSM-T_{20} scanning electron microscope, which revealed that bacteria on the surface of the skin, were scattered widely in small colonies (usually containing less than ten bacteria). Bacteria were also found as relatively large colonies within the stratum corneum, but these were usually associated with sweat ducts or the underside furrows in the skin surface.

The largest concentration of microorganisms has been found in hair follicles, though not all are colonised (Montes and Wilborn,1969, Leeming *et al.*,1984, Kearney *et al.*,1984).

In a study by Leeming *et al.*, (1984), pilosebaceous units were isolated from punch biopsies of unaffected skin on the upper back of patients with acne vulgaris. The biopsies were immersed in 1M $CaCl_2$ at 4^0C for two hours to enable the peeling of the epidermis and attached follicles from the dermis. Once detached from the epidermis, each follicle was homogenised using Triton X-100 (Williamson and Kligman,1965). The homogenates were then diluted and viable counts made.

The most commonly isolated organisms were staphylococci and propionibacterium. Both genera were present in high densities, in all parts of the follicular canal. Of the staphylococci 50% were *Staphylococcus epidermidis*, the rest were other species of coagulase negative staphylococci (CNS). All propionibacterium were *P.acnes*. *Pityrosporum spp.*, was the third major microbial group, suggesting that the pilosebaceous unit, that is the hair follicle and its related sebaceous gland, were the primary sites of growth of these organisms.

Factors responsible for follicular colonisation are poorly understood, the follicle provides a specialised physicochemical environment, with changes in oxygen tension and pH, which contribute to microbial proliferation. Abnormal keratinisation in the hair follicle, leading to occlusion and subsequent inflammation, has been postulated as a critical step in the development of acne lesions, although as previously mentioned, this is a multifactorial disease (Noble,1993).

The predominant microorganisms recovered from the stratum corneum therefore, are *Corynebacterium, Propionibacterium* and CNS. Other species are also found and include :

(a) *Staphylococcus aureus* and *Peptococcus species.*

(b) Gram-positive, aerobic, spore-forming bacilli, that are ubiquitous in air, water and soil.

(c) alpha haemolytic streptococci e.g *Streptococcus viridans, Enterococcus faecalis*

(d) Gram-negative coliform bacilli and *Acinetobacter species*

(e) fungi and yeasts, often present in skin folds

(f) acid-fast, nonpathogenic mycobacteria, which occur in areas rich in sebaceous secretions such as genitalia and the external ear (Jawetz *et al.*, 1989, Noble,1993).

1.4 Infections of the skin.

Breach of the skin increases the susceptibility to infection, by pathogens and also opportunistic normal skin flora (Noble,1993).

A full account of every skin infection is beyond the scope of this study, however, table 1 summarises some of the more common types of bacterial skin infection relevant to this current investigation.

Table 1. Some bacterial infections of skin (Hunter *et al.*, 1989).

NAME OF INFECTION	CAUSATIVE ORGANISM	PRESENTATION
Trichomycosis axillaris	Commensal diphtheroids	Axillary hair becomes beaded with yellow concretions.
Pitted keratolysis	Commensal diphtheroids	Organisms digest keratin resulting in a pattern of fine punched out depressions on the soles accompanied by an unpleasant smell.
Erythrasma	Commensal diphtheroids	Macular wrinkled, slightly scaly, pink, brown or macerated white areas most commonly found in the armpits, groin or between the toes.
Impetigo	Bullous type = *S.aureus* Crusted ulcer type = *S.pyogenes*	A thin walled blister forms and ruptures rapidly leaving an extending area of exudation and yellowish crusting. Multiple lesions occur, often around the face.
Boils	Usually *S.aureus*	Acute pustular infection of the hair follicle. A tender red nodule enlarges and later discharges pus, before healing to leave a scar. This is accompanied by fever.
Carbuncle	*S.aureus*	A group of adjacent hair follicles become deeply infected, leading to a swollen, painful, suppurating area discharging pus from several points.

Table 1 continued.

NAME OF INFECTION	CAUSATIVE ORGANISM	PRESENTATION
Scalded skin syndrome.	Toxin of *S.aureus*	Skin splits at the stratum granulosum so that layers can be rubbed away by gentle sideways pressure.
Erysipelas	*S.pyogenes*	Malaise, shivering and fever. Affected area of skin becomes red after a few hours and the eruption spreads with a well defined edge.
Cellulitis	Predominantly *S.aureus* and *S.pyogenes*	Subcutaneous tissues are involved and the affected area becomes raised and swollen, though the oedema is less marginated than in erysipelas.
Folliculitis	*S.aureus, S.pyogenes* and *Malassezia furfur.*	Occlusion of the follicular opening by keratinaceous material and proliferation of a critical mass of organisms.

For the purpose of this present study, two of the most common skin conditions, chronic plaque psoriasis and atopic eczema, and the pathogens associated with them, are reviewed.

1.5 Psoriasis.

1.5.1 Cause and Pathogenesis.

Psoriasis affects approximately 2% of the UK population and usually occurs in adult life. Though a well-defined disease of the skin, the exact cause of this condition has yet to be elucidated. Genetic factors are important in the expression of the disease and certain environmental factors such as trauma to the skin, streptococcal infection, stress and certain drugs may precipitate or aggravate the condition. Various biochemical abnormalities also exist and are thought to interact. However, psoriasis is currently considered to be primarily an immunological disease (Baker and Fry,1992).

1.5.1.1 Genetics.

A child with one affected parent has a 25% chance of developing the disease, this rises to 60% if both parents are affected. Inheritance may be polygenic but an autosomal dominant trait with incomplete penetrance is also possible. The idea of 'psoriatic tendency', gained scientific merit when a strong association between the disease and certain antigens of the major histocompatibility complex was found including HLA-CW6 and those HLA (human leucocyte) antigens linked with CW6 e.g B13, B17, B27, B37 and DR7.

Three possible sites for genetic defects in psoriasis are: the T-lymphocyte, antigen-presenting cell or the keratinocyte or a combination of these because as mentioned above, psoriasis may be a polygenic disease (Baker and Fry, 1992, Hunter *et al.*,1989).

1.5.1.2 Increased epidermal proliferation.

In psoriasis, due to an excessive number of germinative cells entering the cell cycle and a decrease in cell cycle time, there is increased epidermal proliferation. The transit time

of cells through the viable epidermis is shortened to two days as compared to four days in normal skin and the epidermal turnover time falls from twelve to fourteen days to five or six days. This epidermal hyperproliferation accounts for many of the metabolic abnormalities associated with psoriasis (Hunter *et al.*, 1989).

1.5.1.3 Altered epidermal maturation.

During normal keratinisation, the molecular weights (MW) of keratins change as a cell moves from the basal layer towards the surface, that is MW 55 and 60 kDa to 70 kDa. 30% of the keratin in a normal epidermis is of MW 70 kDa but this falls to about 7% in psoriasis, as a result of increased epidermal proliferation.

1.5.1.4 Biochemical abnormalities.

- Arachidonic acid metabolism.

Arachidonic acid is bound to cell membranes and released from them by the activity of phopholipase A_2. Levels of this acid and of its metabolites, prostaglandin E_2, leukotriene B_4, 12-hydroxyeicosatetrenoic acid (12-HETE) and 15-HETE are elevated in the lesional skin of psoriasis, though it is uncertain how these chemicals influence cell proliferation. Intermediates including 12-HETE, may inhibit adenylate cyclase and lower intracellular cyclic AMP (cAMP). Leukotriene B_4 also strongly attracts polymorphs and maybe responsible for the microabscesses which are a feature of the histology.

- Polyamines

The biosynthesis of the polyamines: putrescine, spermidine and spermine, is intimately associated with cell proliferation. In the lesions of psoriasis, the levels of ornithine

decarboxylase, which catalyses the reaction ornithine to putrescine, are raised, therefore the levels of the polyamines mentioned are also elevated (Hunter *et al.*, 1989).

- Proteases and antiproteases

Proteases and certain protease inhibitors regulate cell proliferation. In psoriatic lesions plasminogen activator, a protease, is increased and parallels the mitotic rate, this may be due to the deficiency of an inhibitor. Plasminogen activator is normally released following experimental injury and in psoriasis its release could lead to the Koebner phenomenon (Hunter *et al.*, 1989).

Koebner showed that injury-induced psoriasis was not dependent upon the nature of the injury; a tattoo, excorations, suppurative skin lesions and a horse-fly bite all induced psoriasis in the same patient. Epidermal injury is thus, the initiating factor in trauma-induced psoriasis, but not all patients with psoriasis respond to injury in this way (Baker and Fry, 1992).

- Calmodulin

In the lesions of psoriasis, the levels of calmodulin, a specific receptor protein for calcium, are raised. The calcium-calmodulin complex by influencing the activity of phospholipase A_2 and phosphodiesterase, which catalyses cAMP to AMP, may regulate cell proliferation abnormally in psoriasis. With successful treatment, levels of calmodulin fall.

1.5.1.5 Immunopathology.

The linkage between certain HLA antigens and psoriasis, the persistence of the disease throughout life once it has manifested, suggests the existence of a 'memory'. Furthermore, the spontaneous exacerbations and remissions of disease activity characteristic of the disease, involving a chronic immune response, strongly support an

ongoing immune response in psoriasis. Indeed both cellular and non-cellular components of the immune system have been implicated in the psoriatic process (Baker and Fry,1992).

It has been proposed that psoriasis is an immunological disease which requires the presentation of a specific antigen for example streptococcal, by class II positive antigen-presenting cells to CD4 T-cells, in the epidermis for the initiation of the disease. This in turn leads to the release of cytokines such as IL-2, IL-6 and IL-8, by activated CD4 T-cells, which directly stimulate keratinocyte proliferation and γ-interferon. This can then induce ICAM-1 expression (intercellular adhesion molecule for lymphocytes and monocytes) by both keratinocytes and endothelial cells, enabling T-cells to enter the dermis.

Tumour necrosis factor (TNF-α) and IL-1 secreted by keratinocytes and macrophages, induce increased expression of the intercellular adhesion molecules, ELAM-1 (endothelial leucocyte adhesion molecule-1) and VCAM-1 (vascular cell adhesion molecule-1) on endothelial cells which force further leucocytes , including memory T-cells, expressing the cutaneous lymphocyte-associated antigen (HECA-452), from the blood into the lesion. In turn, the psoriatic keratinocytes are stimulated by interaction with T-cell derived cytokines to synthesise their own cytokines, to make the process self-perpetuating.

The T-cell derived cytokines are probably required throughout the psoriatic process and not just for initiation, therefore it is possible that different cytokines and hence different T-cell subpopulations are involved at different stages of the maturation and resolution of a lesion (Baker and Fry,1992).

1.5.1.6 Environmental factors associated with initiation of psoriasis.

- **Trauma** – lesions appear in skin damaged by scratches or surgical wounds in active psoriasis (Koebner phenomenon).
- **Infection** – Beta-haemolytic streptococcal tonsillitis, often triggers guttate psoriasis.
- **Sunlight** – most psoriatics improve, but 10% become worse.
- **Drugs** – antimalarials, β-blockers and lithium may worsen psoriasis. Psoriasis may also 'rebound' after treatment with systemic steroids or potent topical steroids.
- **Emotion or Stress** – can bring on exacerbations of psoriasis.

1.5.2 Histology.

The main changes in psoriatic skin are shown in figure 2, these include:

(1) The retention of nuclei in the horny layer, in contrast to normal skin.

(2) Irregular thickening of the epidermis; but thinning over dermal papillae, this is apparent clinically when bleeding is caused by scratching and the removal of scales.

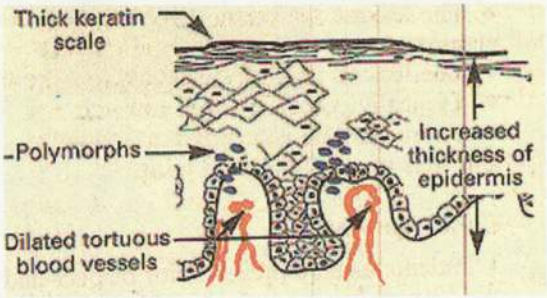
(3) Formation of polymorphonuclear leucocyte microabscesses – polymorphs that migrate into the epidermis form sterile pustules, these are most commonly seen on the palms and soles.

(4) Dilated and tortuous capillary loops in the dermal papillae, giving the clinical picture of intense erythema.

(5) T-lymphocyte infiltrate in the upper dermis.

(Hunter *et al.*, 1989, Buxton,1993).

Figure 2. Changes in psoriatic skin (Buxton,1993).



1.5.3 Presentation.

Clinically recognised types of psoriasis include: scalp, nail, flexures and palms but the most common are plaque and guttate psoriasis.

- **Plaque psoriasis** – this is a chronic, non-infectious inflammatory disease.

Lesions are well demarcated and range from a few millimetres to several centimetres in diameter. The lesions are pink or red with large dry silvery white scales. Plaque psoriasis has a predilection for the elbows, knees, lower back and scalp. See figures 3 and 4 (Hunter *et al.*,1989, Buxton,1993, MacKie,1997).

Figure 3. Psoriasis of the back (MacKie,1997).

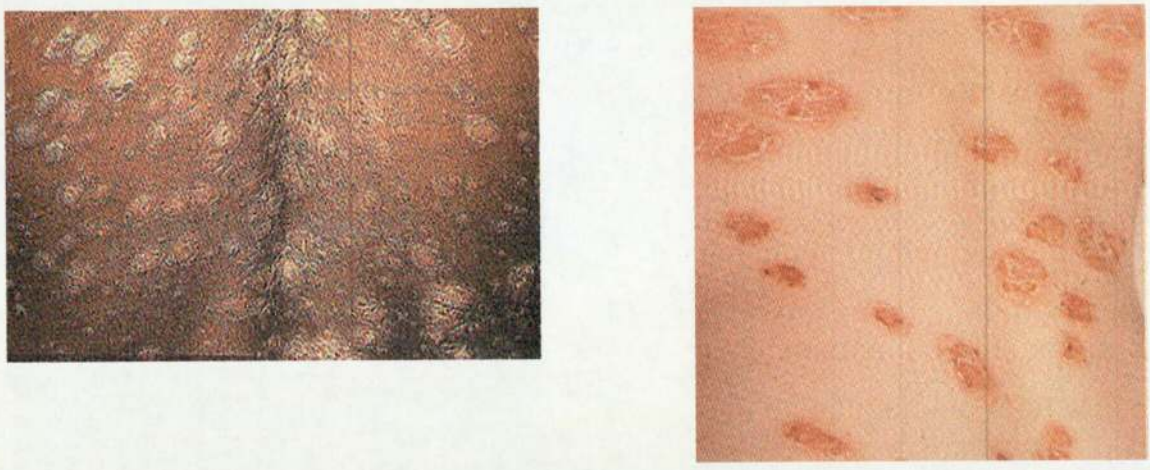
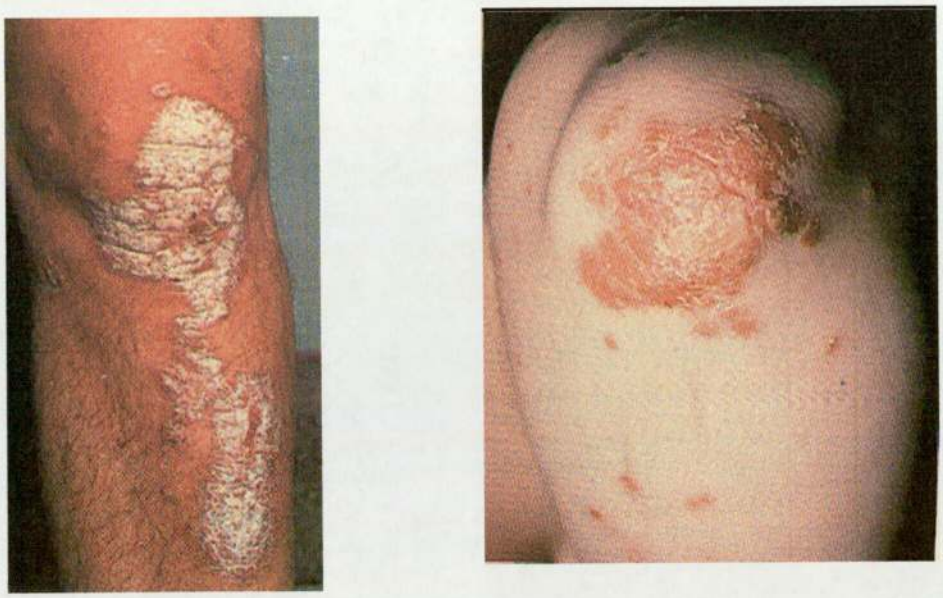
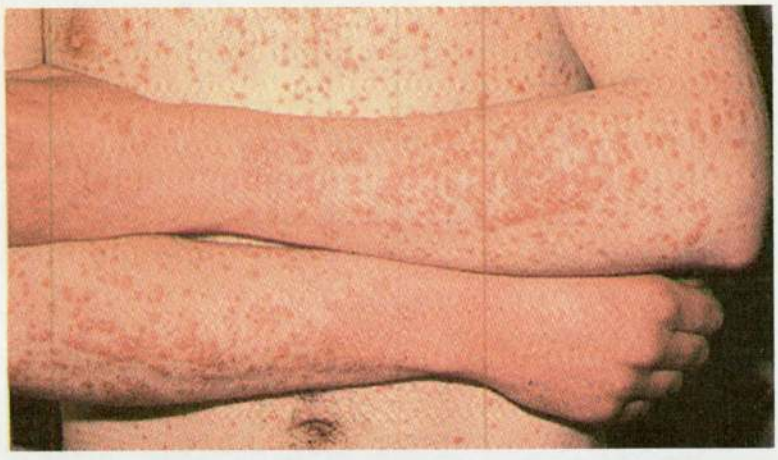


Figure 4. Psoriasis plaques of the knee and elbow (MacKie,1997).



- **Guttate psoriasis** – this is usually seen in children and adolescents and is frequently preceded by a streptococcal throat infection. Numerous small round red macules develop acutely on the trunk and rapidly become scaly. The rash often clears in a few months but plaque psoriasis may develop later. See figure 5. (Hunter *et al.*,1989, Buxton,1993).

Figure 5. Extensive guttate psoriasis (MacKie,1997).



1.5.4 Pathogens associated with psoriasis.

Marples *et al.*, (1973), found that 42 of the 92 psoriatic lesions they sampled were colonised by *S.aureus*, where the density ranged from 20 to 30,000 per square centimetre. Normal skin yielded *S.aureus* in only 14 of the 52 sites sampled and all samples of diseased and normal skin contained moderate numbers of coagulase negative staphylococci (CNS).

The relationship of psoriasis and beta haemolytic streptococci has been well documented, (Baker and Fry,1992, Lewis *et al.*,1993, Hunter *et al.*,1989, Buxton,1993 and MacKie,1997)). In patients with guttate psoriasis, the eruption of skin lesions has

been strongly associated with throat infections and raised antibody titres to streptococcal antigens. Furthermore group A streptococcal antigen-specific T-cell lines have been consistently isolated from skin lesions of guttate psoriasis, but less frequently from those of other inflammatory dermatoses.

It has been estimated that over two thirds of patients with a history of guttate psoriasis subsequently develop chronic plaque psoriasis. Exacerbation of chronic plaque psoriasis following streptococcal tonsillitis has also been reported. Removal of the tonsils or treatment with rifampicin combined with penicillin or erythromycin, can lead to resolution of psoriatic plaques. *In vitro*, both guttate and chronic plaque psoriasis patients have been shown to have enhanced lymphocyte responses to group A streptococcal antigens (Lewis *et al.*,1993, Baker and Fry,1992).

1.5.5 Treatment options.

Table 2, shows the most commonly used treatments for psoriasis.

Due to the nature of the disease, permanent cures are never promised, instead encouragement and a treatment that is not more troublesome than the disease itself, are carefully selected.

Local treatments : (Hunter *et al.*,1989, Buxton,1993)

- **Coal tar preparations.**

Crude coal tar and its distillation products have been used to treat psoriasis for many years. Though they inhibit DNA synthesis their precise mode of action is uncertain.

- **Dithranol.**

Like coal tar, Dithranol inhibits DNA synthesis, but some of its benefits may be due to the formation of free radicals of oxygen.

Table 2. Treatments used for psoriasis (adapted from Buxton,1993).

TYPE OF PSORIASIS	TREATMENT OF CHOICE	ALTERNATIVE TREATMENTS
Stable plaque	Vitamin D analogues	Tar Short-contact dithranol
Extensive stable plaque (>20% surface area)	PUVA (Photochemotherapy with Psoralen and Ultraviolet A)	Short-contact dithranol UVB Tar Systemic treatment
Widespread small plaque	UVB	Tar.
Eruptive/unstable psoriasis	Bland emollients	Consider Systemic treatments
Guttate	Emollients whilst erupting then UVB. Also a course of penicillin V or erythromycin, for any associated beta haemolytic streptococcal infection.	Weak tar preparation. Mild local steroids.

- **Topical steroids.**

Topical steroids are initially effective however their use is limited to 'difficult' sites such as face and flexures where other topical treatment may irritate. Long term use of these agents is not recommended as on withdrawal, psoriasis may relapse rapidly.

- **Vitamin D analogues.**

Topical vitamin D analogues normalise keratinocyte maturation and proliferation and are the treatment of choice for mild to moderate chronic plaque psoriasis.

- **Ultraviolet radiation – UVB**

Most patients improve with natural sunlight and courses of artificial UVB may also be used. This treatment is particularly indicated for widespread lesions with guttate or small plaque psoriasis.

Systemic treatments (Hunter *et al.*, 1989, Buxton, 1993).

These are needed if >20% of the body surface is affected and not responding to prolonged courses of tar or dithranol.

- **PUVA – Photochemotherapy with Psoralen and Ultraviolet A.**

An oral dose of 8-methoxypsoralen is taken 1-2 hours before exposure to a bank of UVA tubes (320-400nm). The psoralen becomes active in the presence of UVA and interacts with the DNA in the basal cells of the psoriatic plaques, slowing their growth rate to normal. Treatment is given 2 to 3 times a week with increasing doses of UVA depending on erythema production and the therapeutic response. Clearance takes 5-6 weeks. PUVA is an effective treatment for a flare up of psoriasis which is too extensive to manage with local applications.

- **Retinoids.**

Acitretin, is a recently introduced analogue of vitamin A, which is used as an adjuvant to treat pustular psoriasis and to reduce hyperkeratotic plaques.

Retinoids and PUVA act synergistically and they clear plaque psoriasis more rapidly than PUVA alone.

- **Methotrexate.**

This is a folic acid antagonist and inhibits DNA synthesis during the 'S' phase of mitosis. An oral dose (10mg) for adults, given once a week is often adequate to control the most aggressive psoriasis.

- **Low dose Cyclosporin A.**

Cyclosporin A is used at low dose to treat extensive chronic plaque psoriasis. It works by suppressing T lymphocytes. Long term use may be limited by nephrotoxicity.

- **Other possible future therapies.**

Photodynamic therapy, (Stables,1999) and use of fumaric acid esters (Ameen and Russell-Jones,1999) may be future treatments for psoriasis, following the necessary clinical trials.

1.6 Atopic eczema.

1.6.1 Cause.

As with psoriasis, the precise cause of atopic eczema remains to be elucidated. Genetic factors are important and hypersensitivity to a range of allergens and non-specific irritants may be implicated.

- **Genetics** - most patients with atopic dermatitis have a positive family history of dermatitis, asthma and allergic rhinitis, the atopic triad. Although the exact mode of inheritance is obscure, autosomal dominance with variable penetration has been postulated (MacKie, 1997).
- **Hypersensitivity** - serum IgE levels are elevated in 80% of patients, but within the normal range in the remaining 20%, of these patients. However, about 5% of the normal population also have elevated IgE levels and are clinically normal, hence elevated levels of IgE are not sufficient to diagnose atopic eczema.

  Radioallergosorbent testing (RAST), is used to determine the specific antigens against which IgE is synthesised, this was found to differ in infants and older children. In infants, high RAST levels were found against foods such as eggs, dairy products and fish. At the age of one, the pattern changes, and most of the elevated IgE is directed against inhaled allergens such as the house dust mite, animal hair and pollens (MacKie,1997).
- **Immunological abnormalities** – the current understanding of these complex abnormalities, is that there is a disorder of immune regulation involving an imbalance in number and function of T cell subsets. T helper cells can be divided into TH1 and TH2 subsets and in atopic dermatitis there is over activity of the TH2 subset. These lymphocytes produce IL-4 and IL-5, which in turn stimulate IgE production by B lymphocytes. In contrast the numbers and activity of TH1

lymphocytes is low. TH1 cells stimulate production of γ-interferon. Babies born to atopic mothers subsequently develop atopic dermatitis. Their cord blood levels of γ-interferon are low, suggesting that this could be a primary rather than a secondary immunological event in the development of atopic dermatitis. Reduction in levels of γ-interferon may be due to increased activity of cAMP phophodiesterase, which causes increased synthesis of prostaglandin E2 which in turn inhibits γ-interferon production, perpetuating a cycle of over-activity (MacKie,1997).

1.6.2 Histology.

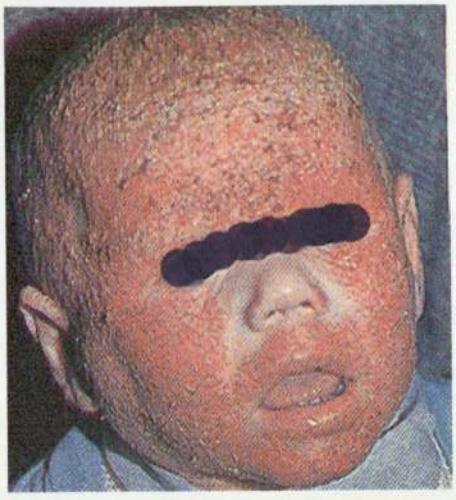
In the acute stage, oedema in the epidermis progresses to the formation of intra-epidermal vesicles, which may combine into large blisters or rupture. The chronic stages of eczema show less oedema and vesiculation in the epidermis, but more thickening of the innermost and horny layers. These changes are accompanied by a variable degree of vasodilation and infiltration with lymphocytes (Hunter *et al.*, 1989).

1.6.3 Presentation and course.

This condition usually begins before the age of six months, affecting at least 3% of infants. Its onset may be delayed until childhood or adult life. The distribution and character of the lesions vary with age, but a general dryness of the skin may persist throughout life.

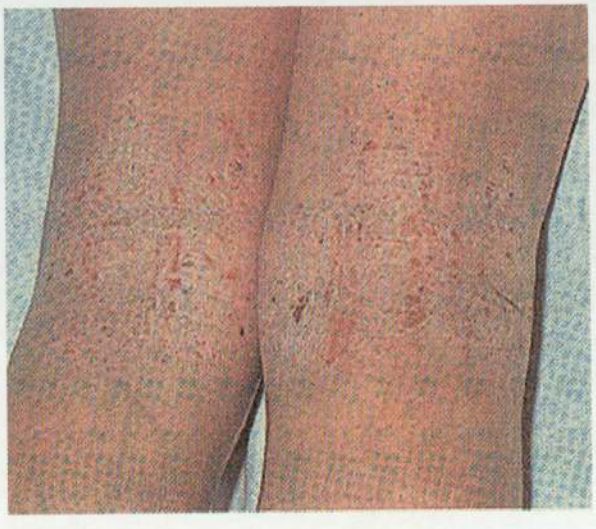
- **Infancy** – atopic eczema tends to be vesicular and weeping. It often starts on the face, see figure 6, with a non-specific distribution elsewhere, commonly sparing the nappy area.

Figure 6. Atopic eczema in infancy (MacKie,1997).



- **Childhood** – the eczema becomes dry, leathery and excoriated, affecting mainly the elbow and knee flexures, wrists and ankles. A stubborn 'reverse' pattern affecting the extensor aspects of the limbs is also recognised (Hunter *et al.*, 1989). See figure 7.

Figure 7. Atopic dermatitis of the knee flexures, lichenification and recent excoriations (MacKie,1997).



- **Adults** – the distribution is as in childhood with a marked tendency towards lichenification and a more widespread but low-grade involvement of the trunk, face and hands.

The central feature of atopic eczema is itching; and scratching may account for most of the clinical picture. In at least two thirds of affected children, the condition remits spontaneously before the age of 10, though it may relapse with stress. Eczema and asthma may see-saw, so that as one is improving, the other may deteriorate (Hunter *et al.*,1989, MacKie,1997).

1.6.4 Pathogens associated with atopic eczema.

Exposure to ***Herpes simplex type-1*** causes a severe infection with the development of small blisters in areas of dermatitis. This can cause severe systemic upset which prior to the use of acyclovir, could be fatal (Mackie,1997).

Staphylococcus aureus, is the pathogen most commonly associated with atopic eczema (Leyden *et al.*, 1974, Aly *et al.*, 1977, Cole, 1979, Hanifin and Rajka, 1980, Dahl, 1983, White and Noble, 1986, Ring *et al.*, 1992, McFadden *et al.*, 1993, Campbell and Kemp, 1998). *S.aureus* colonises both involved and uninvolved skin, with the density of the organism increasing with the severity of the atopic lesions (Williams *et al.*, 1990). The consequences of such colonisation, and how colonisation differs from infection, is unclear. McFadden *et al.*, (1993), have suggested that exotoxins produced by this organism have superantigenic properties, which may explain in part, the exacerbations of atopic eczema. That is, sufficient superantigen (a protein that produces a much larger immune response than an ordinary antigen) is released by the *S.aureus* in

the lesions of atopic eczema, to cause T-lymphocyte activation, cytokine release and mast cell degranulation.

1.6.5 Treatment (MacKie,1997).

The following are used for treating patients with **mild and moderate dermatitis**:

- **Emollients** – these reduce the discomfort and itch of dry atopic skin.
- **Topical steroids** – topical steroids are used in the management of this condition.
- **Antibiotics** – the combination of a topical steroid and antistaphylococcal antibiotic is often useful as staphylococcal infection can aggravate atopic dermatitis. Treating the staphylococcal colonisation is frequently associated with clinical improvement.
- **Antihistamines** – sedating antihistamines help control the itch of atopic dermatitis and prevent night time scratching e.g promethazine and trimeprazine which are used alternately as patients tend to become used to one preparation after 3-4 weeks.
- **Wet wraps** – can include an antiseptic, such as aluminium acetate (Burrows solution) or a steroid.
- **Occlusive bandaging** – ichthyol impregnated bandages over a topical steroid can also be used.
- **Ultraviolet B radiation** – as discussed previously for psoriasis.
- **Removal of allergen exposure**

Severe atopic dermatitis- when the more established methods have failed, the following treatments are used however, many of these have serious side-effects.

- **PUVA** – use is as for psoriasis.
- **Cyclosporin** – this maybe extremely effective in controlling severe atopic dermatitis.
- **Aziathioprine** – this is a cytotoxic drug which may also control the condition.

1.7 The staphylococci.

Staphylococci are non-motile, generally aerobic, catalase positive, gram-positive cocci, which appear in irregular grape-like clusters under the microscope, single and paired cells are more common in fluid culture. The basis of the current taxonomy was founded in 1975 by Kloos, which differentiated ten staphylococcal species. There are currently 31 species recognised in this genus. Fifteen species of which are indigenous to humans and include: *S.aureus* which is coagulase-positive, and *S.epidermidis, S.haemolyticus, S.saprophyticus, S.cohnii, S.xylosus, S.capitis, S.warneri, S.hominis, S.simulans, S.saccharolyticus, S.auricularis, S.caprae, S.lugdunensis* and *S.schleiferi* all of which are coagulase-negative. Eight subspecies have also been described and include *S.capitis* subsp. *ureolyticus,* and *S.cohnii* subsp. *urealyticum,* which are indigenous to humans (Kloos and Bannerman,1994).

The staphylococci are a major cause of human infection, both in the hospital and in the community.

1.7.1 *Staphylococcus aureus.*

This organism demonstrates a preference for the anterior nares in adults and is somewhat more widely distributed in preadolescent children. This species is especially adapted to damaged or traumatised tissue or skin (Kloos and Bannerman,1994). *S.aureus* differs considerably from the coagulase negative staphylococci (CNS) with respect to its pathogenicity, in that it elaborates a large number of extracellular virulence factors and other proteins, whilst CNS rely more on their ability to colonise and infect foreign bodies such as catheters and other indwelling medical devices from which they may spread systemically. Table 3, summarises the virulence factors of *S.aureus* (Crossley and Archer,1997).

Table 3. Virulence factors of *Staphylococcus aureus*

VIRULENCE FACTORS INVOLVED IN ATTACHMENT	VIRULENCE FACTORS INVOLVED IN EVASION OF HOST DEFENCES	VIRULENCE FACTORS INVOLVED IN INVASION/ TISSUE PENETRATION.
Clumping factor	Enterotoxins A,B, C1-3, D, E, H	α-Toxin
Fibrinogen binding protein	Toxic shock syndrome toxin-1	β-Haemolysin
Fibronectin binding protein A	Exfoliative toxins A,B	γ-Haemolysin
Fibronectin binding protein B	Protein A	δ-Haemolysin
Collagen binding protein	Lipase	Phospholipase C
Coagulase	V8 protease	Metalloprotease (elastase)
Polysaccharide/adhesin	Fatty acid modifying enzyme (FAME)	Hyaluronidase
Polysaccharide intracellular adhesin	Panton-Valentine leukocidin	
	Leukocidin R	
	Capsular polysaccharide types 1,5,8	
	Staphylokinase	

1.7.2 Coagulase negative staphylococci.

These microorganisms are ubiquitous commensals of the human skin and mucous membranes, however, they are also responsible for causing an increasingly important number of infections. These infections are usually associated with implanted foreign material including intravascular catheters, shunts and heart valves (Gemmell,1986, Geary *et al.*,1997, Livesley *et al.*,1998). The pathogenesis is related to the ability of these staphylococci to adhere to and grow on polymer surfaces and to produce an extracellular slime substance. This slime interferes with various host-protective mechanisms and with the action of antistaphylococcal antibiotics (Peters,1988).

1.7.3 Typing methods used for staphylococci.

Standard methods of strain typing include biotyping, bacteriophage typing and serotyping. More recently, molecular methods have been developed and include: plasmid fingerprinting (Burnie *et al.*,1988), ribotyping (Cookson *et al.*,1992, Weide-Botjes *et al.*,1996), PCR-based methods and analysis of chromosomal DNA restriction patterns by pulsed-field gel electrophoresis (PFGE) (Shi *et al.*,1997, Livesley *et al.*,1998, Aaresstrup *et al.*,1997). Although bacteriophage typing is still widely used for epidemiologic studies of *S.aureus,* methods such as pulsed-field gel electrophoresis can be used to type a broader array of bacterial species (Tenover *et al.*,1995).

1.7.3.1 Pulsed-field gel electrophoresis.

This technique involves embedding the organisms in agarose, lysing the organisms *in situ,* and digesting the chromosomal DNA with restriction endonucleases. Slices of agarose containing the chromosomal DNA fragments are then inserted into the wells of an agarose gel and the restriction fragments are resolved into a pattern of discrete bands in the gel by an apparatus that switches the direction of current according to a predetermined pattern. The DNA restriction patterns of the isolates are than compared with one another to determine their relatedness (Tenover *et al.*,1995).

1.8 AIMS AND OBJECTIVES OF STUDY.

Until now the role of coagulase-negative staphylococci in patients with psoriasis and atopic eczema, has been largely understudied. The aims of this study were:

(1) To assess quantitatively the staphylococcal flora associated with eczematous and psoriatic lesions and to identify fully the isolates obtained.

(2) To investigate various virulence factors including esterase, non-specific protease, lipase, elastase and extracellular slime production associated with *S.aureus* isolates from eczematous skin, as well as detection of the exotoxin TSST-1 and enterotoxins A,B,C and D, with regards to potential superantigenic properties.

(3) To determine whether the microorganisms associated with psoriasis and eczema produce an immune response, by use of a novel ELISA, which incorporates a short chain lipoteichoic acid, lipid S, to measure circulating antibodies to Gram-positive organisms.

2.0 METHODS AND MATERIALS.

2.1 PATIENT GROUPS AND SAMPLE SIZE.

Approval from the South Birmingham Local Research Ethical Committee was obtained to swab the skin of ten patients with chronic plaque psoriasis, ten with atopic eczema and collect blood from each. Blood samples were also taken from a further ten patients with healthy skin, to serve as controls for the serology aspect of the work. All patients attended the Dermatology department at Selly Oak Hospital.

The age group of the patients ranged from 21 to 71 years. Criteria for recruitment included:

(a) No oral antibiotics for a month or topical antibiotics for 2 weeks preceding the study.

(b) No PUVA or second line treatment.

(c) No sore throat six months prior to testing.

Any positive microbiology for the patients from the preceding three years, obtained from computer records, was also recorded.

The severity of psoriasis was graded by the dermatologist using the Psoriasis Area and Severity Index (PASI) scoring system and for eczema using a modified scoring system (Appendix 1).

2.2 QUANTIFICATION OF BACTERIAL FLORA.

The bacterial flora of the patients, was quantitatively assessed by swabbing the edge, the centre of the plaque and uninvolved skin for the psoriatics, figure 8 and an exudative area, dry area and uninvolved skin for the eczema patients, figure 9.

Figure 8. Psoriatic lesion (Marks *et al.*, 2000). Areas swabbed are labelled.

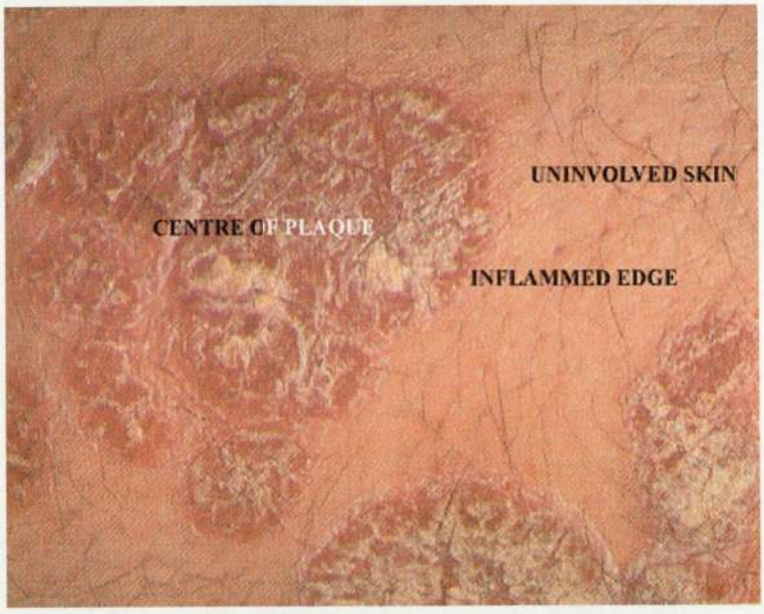
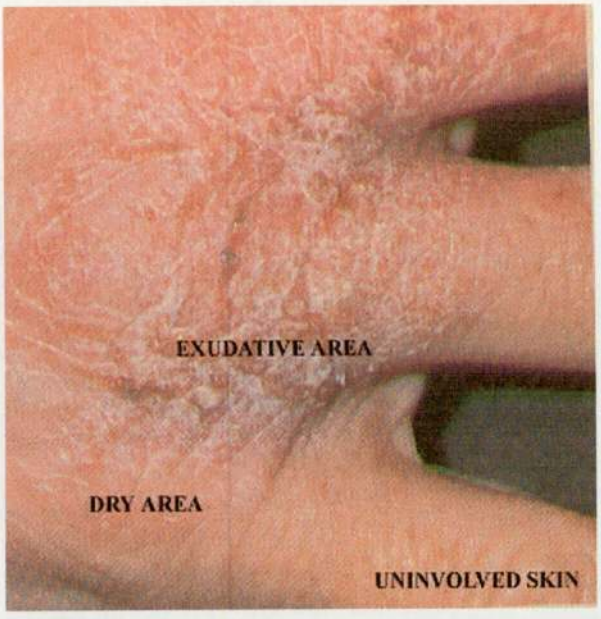


Figure 9. Lesion from eczematous skin (Chu *et al.*,2000).



A sterile cotton swab was moistened with phosphate buffered saline (PBS) containing 0.1% Triton-X-100, for maximum removal and recovery of organisms (Williamson and Kligman,1965). An area of 2 cm^2 was swabbed in an even manner and pressure, ten times in one direction and ten times at 90^0 to the first direction. The swabs were

transferred to 2 ml of Stuarts transport medium (STM) in sterile bottles and vortex mixed for 30 seconds. Three dilutions of each swab were made in phosphate buffered saline (PBS), 1:10, 1:100 and 1:1000. 100µl of each dilution, from each area was inoculated onto 7% horse blood agar plates (Oxoid,UK), and incubated at 37^0C for 48 hours in air. The number and type of organisms were counted and recorded, and the plates were then left for another 48 hours at room temperature to allow further morphological differentiation.

Five of the patients, three eczema and two psoriasis, had repeat swabs taken at a later date from three different parts of the body. Isolates were stored on Microbank™ beads (Pro-lab diagnostics) at -20^0C.

2.3 STRAIN CHARACTERISATION.

Isolates were identified using conventional techniques such as the Gram stain and catalase reaction. Staphylococci were further discriminated by their biochemical reactions using the API Staph 32 (bioMerieux, France), detection of clumping factor, protein A and surface antigens by use of Staphaurex plus (Murex, France) and production of DNase using DNA agar plates (Oxoid,UK). Each method was used in accordance with the manufacturer's instructions. Antibiograms of the staphylococci isolated were also produced using the modified method of Stokes (Stokes and Ridgeway,1980). The technique of pulsed-field gel electrophoresis (PFGE) was used to further characterise the staphylococci by analysis of macrorestriction fragment patterns of genomic DNA.

2.3.1 PULSED-FIELD GEL ELECTROPHORESIS.

- **Standard stock solutions.**

(a) **1M Tris-HCl** (Tris-Hydrochloric acid)

Two solutions of 1.0M Tris-HCl were made, one with a pH of 7.6 the other with a pH of 8.0. 12.11g of Tris base (Sigma,UK), was dissolved in 90 ml of distilled water and the pH adjusted using concentrated hydrochloric acid. The volume was made up to 100 ml with distilled water and autoclaved at 121^0C for 15 minutes. The solutions were stored at 4^0C.

(b) **0.5M EDTA** (Ethylenediamine tetra-acetic acid)

Two solutions of EDTA were also made, pH 8.0 and pH 9.0. 18.61g of EDTA (Sigma, UK), was added to 80 ml of distilled water and the pH adjusted using sodium hydroxide pellets (Sigma,UK). The volume was made up to 100 ml using distilled water and autoclaved as above. The solutions were stored at room temperature.

(c) **TE** (Tris-HCl and EDTA)

5ml of 1M Tris-HCl pH 8.0 and 1ml of 0.5M EDTA pH 8.0 were mixed together and made up to 500ml with distilled water and autoclaved as above, and stored at room temperature.

(d) **10 X TBE** (Ten times concentrated Tris, Boric acid and EDTA electrophoresis buffer).

60.55g of Tris base, 27.5g of boric acid (Sigma,UK) and 20 ml 0.5M EDTA pH 8.0, were mixed and made up to 500 ml with distilled water. The solution was autoclaved as above and stored at room temperature.

- **In-use solutions for PFGE.**

(a) **NET-100** (Sodium chloride, EDTA and Tris-HCl-100)

20 ml of 0.5M EDTA pH 8.0, 1 ml of 1M Tris-HCl pH 8.0 and 0.58g of sodium chloride (Sigma,UK), were mixed together and made up to a volume of 100ml with distilled water. The solution was autoclaved as previously mentioned and stored at room temperature.

(b) 0.9% chromosomal grade agarose

0.45g of chromosomal grade agarose (BioRad, Hercules), was added to 50 ml of NET-100 and heated in steam at 100^0C until the agarose was completely dissolved. When required the solidified agarose was resteamed and kept in a 50^0C waterbath.

(c) 0.5% molecular biology grade agarose

0.25g of molecular biology grade agarose (BioRad, Hercules), was added to 50 ml TBE and dissolved and used in the same way as above.

(d) Lysis solution

0.6 ml 1M Tris-HCl pH 7.6 was added to 20 ml 0.5M EDTA pH 8.0 and 5.8g of sodium hydrochloride. The solution was made up to 100 ml with distilled water and autoclaved as previously mentioned. For use, 0.5% sarcosyl (Sigma,UK) and 1mg/ml lysozyme (Sigma, UK) were added to the autoclaved solution. For example, for 20 ml of lysis solution, 0.02g of lysozyme and 0.1g of sarcosyl were added.

(e) **ESP** (EDTA, sarcosyl and proteinase K)

This solution consisted of 0.5M EDTA pH 9.0, 1% sarcosyl and 1.5mg/ml proteinase K (Sigma,UK). For example, 20 ml ESP consisted of, 0.2g of sarcosyl, 0.03g of proteinase K and 20 ml of EDTA pH 9.0.

(f) **ES** (EDTA and Sarcosyl)

ES was prepared as for ESP, but without the proteinase K.

2.3.1.1 Preparation of chromosomal DNA.

Staphylococci were subcultured from microbank beads, in batches of eight strains, onto 7% horse blood agar (Oxoid,UK) and incubated overnight at 37^0C in air. One colony of each organism was then inoculated into brain heart infusion broth (Oxoid, UK) and incubated at 37^0C in air, for 18 hours.

1 ml of each of these broths was dispensed into a pre-weighed sterile eppendorf tube, and centrifuged in a microfuge at 13,000 rpm for 5 minutes, to pellet the cells. All of the fluid was removed and the tube reweighed. A wet weight of 20 mg of cells was required and achieved by a series of calculations leading to resuspension of each pellet in a set volume of NET-100. Whilst warming the cells in a 50^0C waterbath, the 0.9% chromosomal grade agarose was heated to 100^0C, cooled and added to the cell suspension in an equal volume to that of the NET-100. Once mixed, this suspension was dispensed into a perspex mould (BioRad, Hercules) on ice. Three blocks were made for each isolate. Once set, the blocks were put into 3 ml of lysis solution with the addition of 40µl of lysostaphin (6.6 units / ml (Sigma, UK)), and incubated in a waterbath at 37^0C for 24 hours.

The lysis solution was replaced with 3 ml ESP and incubated in a waterbath at 50^0C for 48 hours. The ESP was then replaced with 3ml TE and the tubes were rolled on a slow roller for two 2 hour sessions and two 1 hour sessions, with a change of TE for each, to wash the blocks and remove products that inhibit the activity of the restriction enzyme. The DNA containing blocks were stored at 4^0C until required.

2.3.1.2 Digestion of chromosomal DNA.

A 1 mm sliver of each block was cut using a glass coverslip, and put into an eppendorf tube together with 180µl of sterile water and 20µl of restriction enzyme buffer A (Boehringer Mannheim,Germany). This was incubated on ice for 15 minutes. This solution was then replaced with 175 µl of sterile water, 20 µl of restriction enzyme buffer A and 5 µl of the restriction enzyme, *Sma I* (Boehringer Mannheim,Germany), and incubated overnight in a waterbath at 37^0C.

The enzyme solution was replaced with 200 µl of ES and incubated at 50^0C for 15 minutes. 1ml of TE replaced the ES and was left at room temperature for 15 minutes, after which, this solution was removed and the digested slivers stored at 4^0C for a maximum of 48 hours.

2.3.1.3 Casting and loading the gel.

1g of molecular biology grade agarose was added to 5 ml TBE and made up to 100 ml with distilled water and heated to dissolve the agarose. The gel was cast on a platform in a separate casting stand (BioRad, Hercules), which was assembled in accordance with the manufacturer's instructions. The 'comb' was placed into position, allowing

for a 2-3 mm gap between the comb and the platform. A spirit level was used to ensure that the surface was level and once cooled to approximately 50^0C, the agarose was poured onto the platform and allowed to set.

The comb was then removed, and the slivers loaded into each well. A Lambda Ladder, that is, a DNA size standard (BioRad), was put into the last well of each gel, in order to determine the band sizes of the test DNA, when analysed. Once in the gel, the wells were sealed with 0.5% agarose.

2.3.1.4 Electrophoresis of digested fragments using the CHEF-DR® III System.

Once washed with 2 litres of distilled water, the surface of the tank was checked to ensure it was level. 1900ml of distilled water was then added to 100 ml of ten times TBE put into the tank and cooled to 10^0C by a chiller unit and recirculation pump. The gel was removed from the casting stand and placed in the centre of the tank. The gel was submerged under cooled (10^0C) recirculated buffer, (as the migration of DNA molecules is sensitive to temperature), and electrophoresed horizontally with the following parameters:

Initial pulse time: 1 second

Final pulse time: 50 seconds

Run time: 24 hours

Voltage: 6V/cm

Angle:120^0C

2.3.1.5 Staining of the DNA fragments.

Following electrophoresis the gel was immersed in 500 ml of distilled water containing 2.5 µl of 10mg/ml ethidium bromide. This was left to shake gently for thirty minutes.

The fluid was then drained off and replaced with distilled water for at least one hour, to destain the gel.

The DNA was visualised using a UV light scanner (UVP products, UK) and the images obtained saved to disc.

2.3.1.6 Analysis of the PFGE gels.

The Phoretix ID Advanced gel analysis computer programme (Phoretix International,UK) was used to interpret the fragment profiles. The size of the fragment bands were determined by comparison with the DNA standard bands (range 48.5 – approx 1,000 kb). Only bands >30 kb were included in the analysis. On completion, the gel analysis was transferred to the Phoretix ID database programme to enable strain comparison by calculation of the Dice correlation coefficient. A dendrogram was constructed to show clustering of the isolates by the unweighted pair group method of arithmetic averages (UPGMA).

2.4 VIRULENCE FACTORS OF *S.aureus.*

2.4.1 Plate assays.

For each assay the *S.aureus* isolates recovered from microbank beads on 7% horse blood agar plates, were inoculated into 10mls of brain heart infusion broth and incubated overnight at 37^0C in air. All isolates were tested in duplicate. **All media constituents were obtained from Oxoid,UK, unless stated otherwise.**

2.4.1.1 Lipase activity.

- Olive oil agar (Kouker *et al.*,1987).

10g/L Tryptone
5g/L Yeast extract
5g/L Sodium chloride (Sigma,UK)
13g/L Agar number 1
1 L distilled water

Once mixed, the agar was autoclaved at 121^0C for 15 minutes. On cooling, 2.5% dry heat sterilised olive oil (Sainsbury Plc,UK), and a 0.001% (w/v) filter sterilised aqueous solution of rhodamine B (Sigma,UK) was added, whilst stirring the autoclaved agar vigorously.

The bacterial cultures were diluted 1:100 using sterile BHI and streaked onto an olive oil agar plate and incubated at 37^0C in air for 18-20 hours. Any negative strains were incubated for a further 18-20 hours. *Pseudomonas aeruginosa* PA01 was used as a positive control and *E.coli* as a negative control.

2.4.1.2 Esterase assay.

- Tween-80 agar (Barrow *et al.*, 1991).

Tryptone soya agar (TSA) supplemented with 1% (v/v) Tween 80 (BDH, Poole).
5g/L Sodium chloride (Sigma,UK)
0.01g/L Calcium chloride (Sigma,UK)
1L distilled water.

After mixing, the agar was autoclaved as above.

The bacterial cultures, were diluted 1:100 with sterile BHI, and then inoculated onto Tween 80 plates and incubated at 37^0C in air for 2 days, followed by 12 days at room temperature. The plates were examined for esterase activity around the bacterial streak on days 1,2,3,5,7,11 and 14. *Ps.aeruginosa* PAO1, was used as a positive control and sterile BHI as a negative control.

2.4.1.3 Extracellular polysaccharide slime layer.

- Congo red agar (Heilmann and Gotz,1998).

30g/L Tryptone soya broth
10g/L Glucose (BDH, Poole)
15g/L Agar number 1
0.8g/L Congo Red (BDH, Poole)
1L distilled water

After mixing the agar constituents were autoclaved as previously described. The congo red was prepared as a concentrated aqueous solution and autoclaved separately and added to the agar after cooling to approximately 50^0C. A slime producing *S.epidermidis* (ATCC 35984), and non-slime producing *S.hominis,* (ATCC 35982), were included as controls.

The bacterial cultures were diluted 1:100 with sterile BHI and spread onto a quarter of a congo red agar plate. The plates were incubated as 37^0C in air for 18-20 hours.

2.4.1.4 Non-specific protease activity.

- Skim milk agar.

1% w/v Agar number 1
1% w/v Skim milk
1L distilled water

After mixing the agar was autoclaved as previously described.

The large bore end of a sterile glass pasteur pipette was used to cut six equidistant wells into the skim milk agar plates. 1 ml of each bacterial culture was then centrifuged at 13,000 rpm for 5 minutes from which 75µl of culture supernatant was inoculated into each well and incubated at 37^0C for 18-20 hours, in air. *Ps.aeruginosa* PA01 was used as a positive control and BHI as a negative control.

2.4.1.5 Elastase activity by qualitative plate method.

- Elastin agar (Janda,1986).

Brain heart infusion agar supplemented with 0.3% bovine neck ligament elastin.
1L distilled water

The constituents were mixed and autoclaved as previously described.

The bacterial cultures were diluted 1:100 and inoculated onto an elastin agar plate and incubated at 37^0C in air for 2 days, followed by 19 days at room temperature. Cultures were observed for elastin degradation on days 3,5,7,11,14 and 21.

Ps.aeruginosa PA01 was used as a positive control and BHI as a negative control.

2.4.2 Toxin detection.

Enterotoxins A, B, C and D, and exotoxin TSST-1 were assayed using the SET and TST, reversed passive latex agglutination method (Oxoid, UK). These kits were used in accordance with the manufacturer's instructions but briefly *S.aureus* was inoculated into 10ml of tryptone soya broth for SET and brain heart infusion for TST, and incubated at 37^0C in air for 18-24 hours. The broths were then filtered using a low protein binding filter 0.2μm – 0.45μm, the filtrates were used to assay the toxin content. V-bottomed microtitre wells were used for both assays.

With regards to **enterotoxin** detection, five rows of eight wells were needed for each sample. In each well of the five rows 25μl of diluent was added and into the first well of each row 25μl of test was added. Starting with the first well of each row, doubling dilutions were performed by transferring 25μl along each of the five rows stopping at well seven, so that well eight contained the diluent only. To each well in the first row 25μl of latex sensitised with anti-enterotoxin A was added, to the second row anti-enterotoxin B, to the third anti-enterotoxin C, to the fourth anti-enterotoxin D and to the fifth row latex control. The contents were mixed, the plate sealed and left undisturbed for 20-24 hours at room temperature.

With regards to **TSST-1** detection, two rows of eight wells were needed for each test. Wells two to eight were inoculated with 25μl of diluent and wells one and two of each row were inoculated with 25μl of each test sample. From well two, doubling dilutions were performed by transferring 25μl to each well stopping at well seven so that well eight contained diluent only. To each well of the first row, 25μl of sensitised latex was added and to each well of the second row, 25μl of the control latex. The contents were mixed, the plate sealed and left undisturbed at room temperature for 20-24 hours.

2.5 SEROLOGY.

2.5.1 AST and ASOT.

All serum samples were tested for antistaphylolysin (AST) and antistreptolysin (ASOT) titres by latex slide agglutination (Behring, Germany) in accordance with the manufacturer's instructions, but briefly 40µl of each serum sample was inoculated onto a slide and mixed with 40µl of antistaphylolysin (AST)/ antistreptolysin (ASOT) latex for three to five minutes (AST) and two minutes (ASOT), positive and negative controls were also tested in the same manner. If agglutination was observed for the tests, the patients samples were diluted and then tested as above, to estimate the ASOT and AST concentration.

2.5.2 Lipid S ELISA.

All serum samples were tested for the presence of IgG to lipid S by ELISA, using essentially the method described by Elliott *et al.,* (2000), with the further modification of a 4 hour as opposed to a 24 hour incubation period (Worthington *et al.,* in press). Briefly, antigen (a purified preparation of seven strains of coagulase negative staphylococci obtained from patients with proven catheter related sepsis) (Lambert *et al.,* 2000) was diluted with 100 volumes of sodium/bicarbonate buffer (0.05M, pH 9.6). 100 µl of diluted antigen was dispensed into each well of a microtitre plate and incubated at 4^0C for 18 hours. The plates were then washed with TBS-Tween (0.01M Tris-HCl pH 7.4, NaCl 0.9% w/v and Tween-20 0.3% v/v). To block unbound antigen sites, 200 µl of the same buffer was put into each well and incubated at 4^0C for 1 hour. Following incubation the buffer was removed from the wells and the plates stored at – 20^0C until required. Prior to use, the plates were allowed to reach room temperature.

Patients sera were diluted 1: 6400 by the addition of 5µl of patients serum to 2 mls of TBS-Tween buffer, and then 100 µl of this diluent into a further 1.5 mls of buffer.

100 µl of TBS-Tween was added to the first well in each row of a microtitre plate. Starting at well 2, 100 µl of diluted test sera was added. Two wells were used for each sample so as to test in duplicate. The last column was used for the controls: a high positive, a low positive and a negative control, again in duplicate. The plates were then incubated at 37^0C for 2 hours in air. The plates were then washed three times in TBS-Tween to remove the excess serum. To detect IgG, 100 µl of anti-human IgG conjugate (50 µl of anti-human conjugate in 50 mls of TBS-Tween buffer) was added to all wells and incubated at 37^0C for 1 hour in air. The plates were washed three times with TBS-Tween to remove unbound conjugate. 100 µl of chromogenic substrate was added to all wells and incubated at 37^0C for 25 minutes in air. The substrate contained 0.01g of 3,3',5,5'-tetramethylbenzidine (Sigma) dissolved in 1 ml dimethyl sulphoxide and diluted in 100 mls of citrate acetate/citrate buffer (0.1M, pH 6.0) containing 50 µl of H_2O_2 (5% v/v). The reaction was stopped by the addition of 100 µl of sulphuric acid (1M) and the optical density at 450 nm was measured. Blank control wells containing TBS-Tween only, produced no colour.

Calculation of IgG titres was done using the following equation:

$$\frac{Ab_{450}\text{ test sample} - Ab_{450}\text{ negative control}}{Ab_{450}\text{ positive control} - Ab_{450}\text{ negative control}} \times 100{,}000$$

3.0 RESULTS.

3.1 Quantification and identification of organisms.

From 20 patients, 135 isolates of staphylococci were obtained which consisted of 3 *S.aureus* and 48 coagulase negative staphylococci (CNS) isolates from psoriatic skin and 43 *S.aureus* and 41 CNS from eczematous skin (Appendix 2). The term isolate is used as defined by Tenover *et al.*, (1995): a pure culture of bacteria obtained by the subculture of a single colony from a primary isolation plate.

3.1.1 Psoriasis.

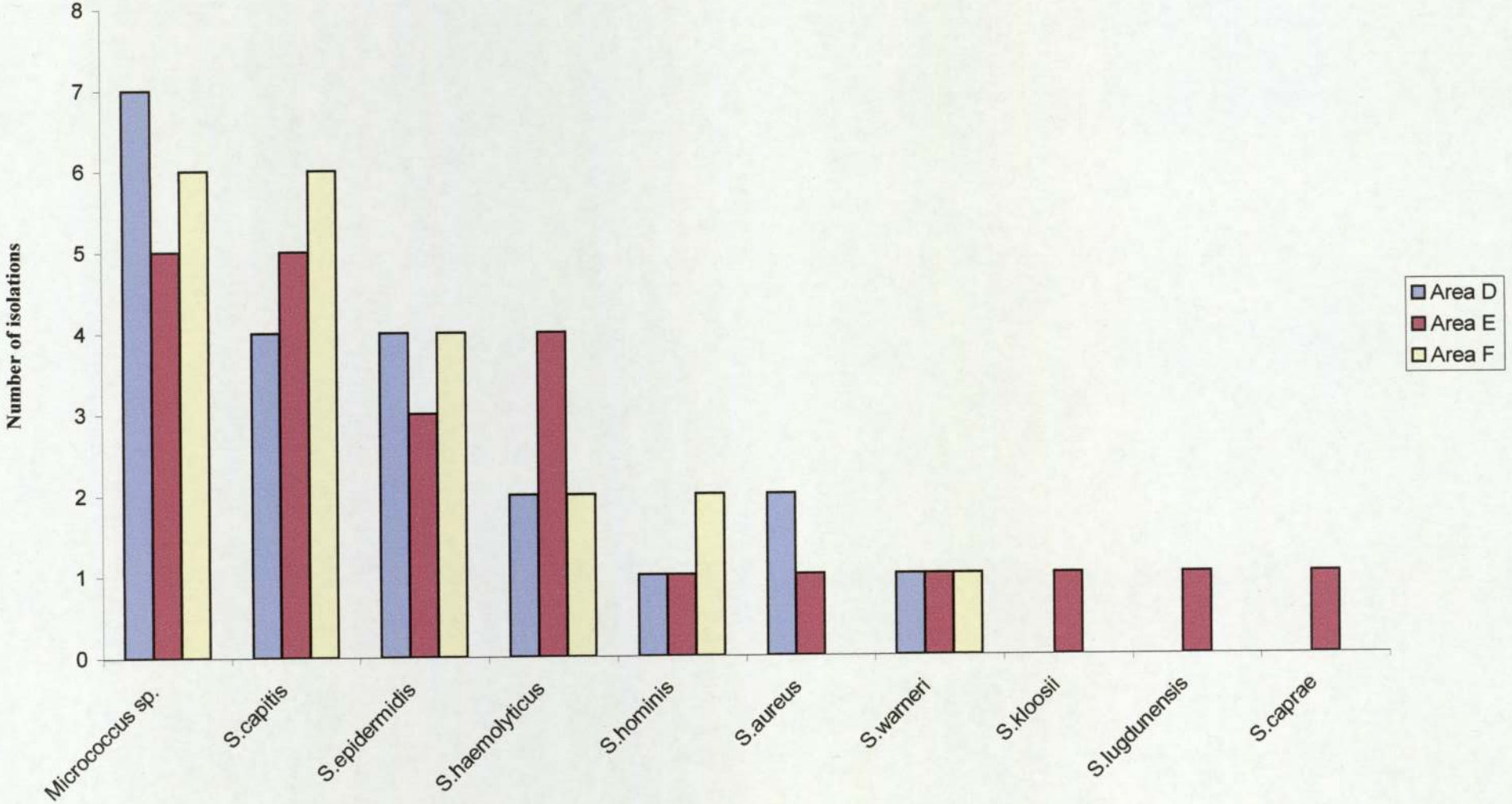
Table 4 shows the number of colony forming units (cfu) per 2 cm^2 obtained for each isolate, from each area of psoriatic skin and their identification. With reference to table 4, 3 isolates of *S.aureus* were obtained, with CNS being the predominant organisms. Figure 10 illustrates the most commonly isolated organisms from the three areas sampled. With reference to this it can be seen that the most predominant organism was *Micrococcus sp.*, followed by *S.capitis, S.epidermidis, S.haemolyticus, S.hominis, S.aureus, S.warneri, S.kloosii, S.lugdunensis* and *S.caprae.*

Several species of CNS were present in any one area, and when counts were compared, one area did not appear to be more colonised than another, the exception being the *S.aureus* of patient P6 area E.

Table 4. Quantification and types of organisms obtained from psoriatic skin.

PATIENT	SKIN COUNT (CFU FROM A 2CM² AREA)		
	D INFLAMMED EDGE OF PLAQUE	E CENTRE OF PLAQUE	F UNINVOLVED SKIN
P1	No growth	*S.haemolyticus* 40	*S.hominis* 220
P2 (11.98)	*S.capitis* 100	*S.haemolyticus* 140	*S.capitis* 280
P2 (12.99) SCALP	***S.aureus* 700** *Micrococcus sp.*20 *S.capitis* 20	*Micrococcus sp.*170 *S.lugdunensis* 20	*S.epidermidis* 20 *S.capitis* 20 *Micrococcus sp.20*
SHOULDER BLADES	*Micrococcus sp.*200	Coliform 700 *Micrococcus sp.*600	Coliform 270 *S.epidermidis* 200 *Micrococcus sp.*100
CALF	*Micrococcus sp.*140 *S.haemolyticus* 60	*S.haemolyticus* 100 *S.capitis* 60 *Micrococcus sp.*40	*S.capitis* 100 *S.haemolyticus* 20 *Micrococcus sp.*20
P3	*S.haemolyticus* 60 *S.epidermidis* 40	*S.haemolyticus* 360 *S.epidermidis* 200	*S.haemolyticus* 280 *S.capitis* 300
P4	No growth	*S.epidermidis* 20 *S.capitis* 340	*S.epidermidis* 20
P5	*S.capitis* 20 *S.epidermidis* 600	*S.capitis* 420 *S.caprae* 140	Coliform 20
P6	***S.aureus* 720**	***S.aureus* 11 520**	No growth
P7	*S.epidermidis* 40	*S.kloosii* 20	No growth
P8	*Micrococcus sp.*40	*S.capitis* 20	*S.hominis* 20
P9 (11.98)	No growth	No growth	*Corynebacterium sp.* 140
P9 (12.99) HEAD	*S.capitis* 140 *S.epidermidis* 400 *Enterococcus sp.*80 *Micrococcus sp.*20	*S.epidermidis* 20 *S.capitis* 40 *S.hominis* 180	*S.capitis* 40 *Micrococcus sp.*100
RIGHT ELBOW	*Micrococcus sp.*100 *S.warneri* 40	*Micrococcus sp.*80	*Micrococcus sp.*440 *S.warneri* 100 *S.epidermidis* 60
LEFT ELBOW	*Micrococcus sp.*180 *S.hominis* 40	*Micrococcus sp.*100 *S.warneri* 20	*Micrococcus sp.*180 *S.capitis* 20 *S.warneri* 20

Figure 10. Bar chart to illustrate the incidence of organism isolation per site in psoriasis.



3.1.2 Eczema.

Table 5 shows the number of cfu obtained for each isolate, from each of the three areas of eczematous skin sampled, along with their identification. With reference to this table and in contrast to table 4, it can be seen that *S.aureus* was the most commonly and the most predominantly isolated organism in the exudative area (A) 17/19, the dry area (B) 14/19 and the uninvolved skin (C) 11/19. The exudative area had the highest counts in all but one patient. Figure 11 highlights this and in addition shows the distribution of the CNS in these areas. With reference to figure 11 it can be seen that the most commonly isolated CNS from all three areas was *S.capitis,* followed by *S.epidermidis, Micrococcus sp., S.warneri, S.haemolyticus, S.cohnii, S.lugdunensis* and *S.kloosii.*

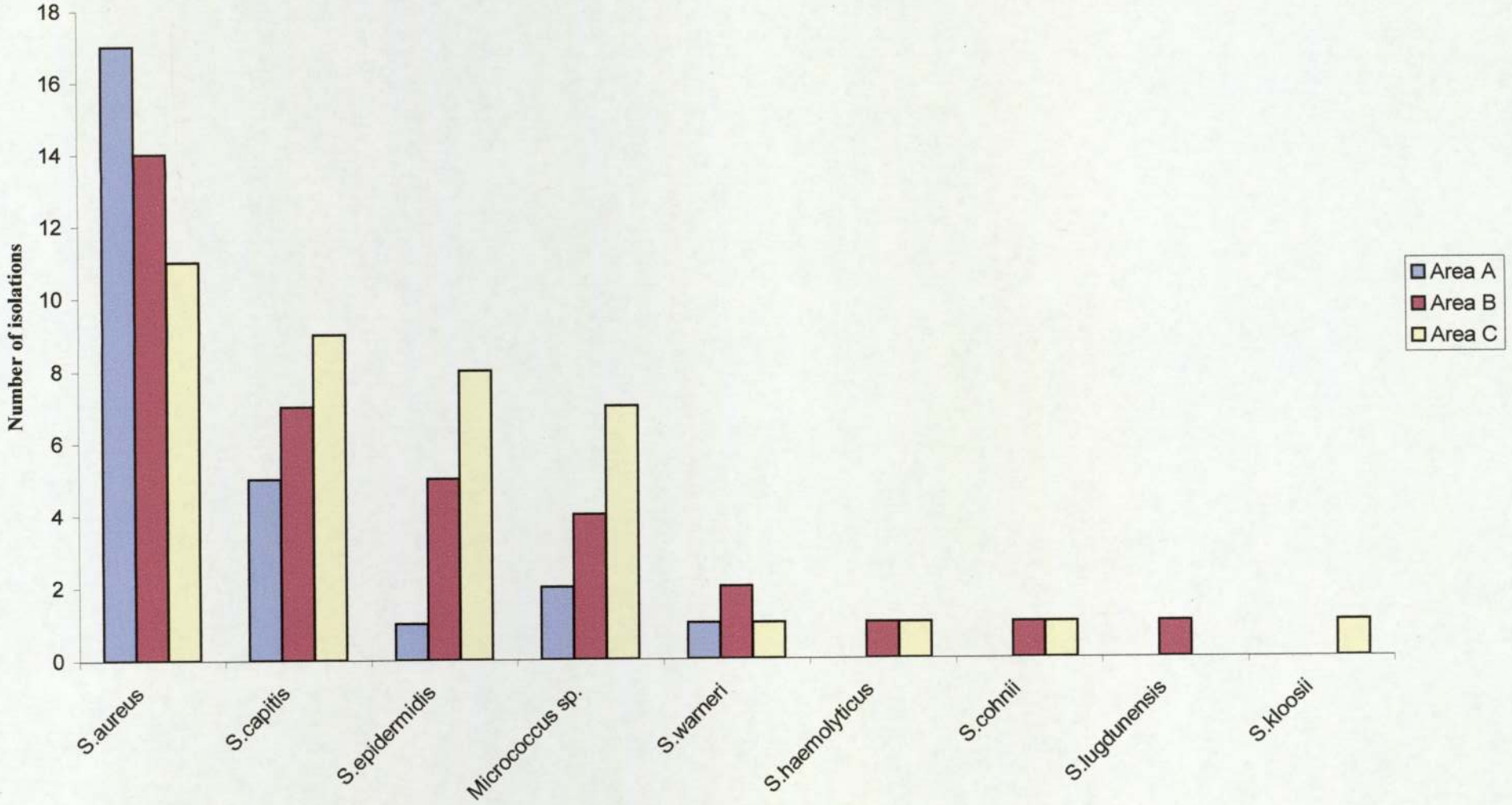
Table 5. Quantification and types of organisms obtained from eczematous skin.

PATIENT	SKIN COUNT (CFU FROM A 2 CM^2 AREA)		
	A EXUDATIVE	B DRY	C UNINVOLVED
E1	***S.aureus* 21 900**	***S.aureus* 3 540** *S.capitis* 160	*S.kloosii* 580 *S.warneri* 160
E2	***S.aureus* 500**	***S.aureus* 12 880**	No growth
E3 (11.98)	***S.aureus* 4 360** *S.capitis* 980	*Bacillus sp.* 20	***S.aureus* 140** *Micrococcus sp.* 340
E3 (12.99) Elbow crease	***S.aureus* 17 600** *S.warneri* 300	***S.aureus* 1 900** *S.epidermidis* 6 600	***S.aureus* 100** *S.capitis* 600
Knee	***S.aureus* 100 000**	***S.aureus* 15 000** *S.capitis* 400	***S.aureus* 120** *Micrococcus sp.* 400 *S.capitis* 40
Top of thigh	***S.aureus* 120 000**	***S.aureus* 6 900** *S.capitis* 400	*S.capitis* 200 *Micrococcus sp.* 140
E4	***S.aureus* 10 240**	*S.capitis* 360 *S.epidermidis* 140	*S.epidermidis* 14 080 *S.capitis* 820
E5	***S.aureus* 200** *S.epidermidis* 220	***S.aureus* 4 000**	***S.aureus* 6 320** *S.epidermidis* 1 680
E6 (11.98)	***S.aureus* 21 920**	***S.aureus* 5 600** *S.capitis* 16 960	*S.haemolyticus* 160 *S.capitis* 60
E6 (12.99) Calf	***S.aureus* 19 600** *S.capitis* 400	***S.aureus* 460** *S.cohnii* 240 *Micrococcus sp.* 180	***S.aureus* 1 480** *Micrococcus sp.* 80
Wrist	*Micrococcus sp.* 320	*Micrococcus sp.* 520 *S.epidermidis* 60 *S.warneri* 40	*Micrococcus sp.* 240 *S.cohnii* 140 *S.epidermidis* 120
Shoulder blades	***S.aureus* 6 800** *S.capitis* 1000 *Micrococcus sp.* 200	*Micrococcus sp.* 640 *S.epidermidis* 40	*Micrococcus sp.*800 ***S.aureus* 60**
E7	***S.aureus* 8 234**	***S.aureus* 3 600** *S.lugdunensis* 500	***S.aureus* 6 600** *S.capitis* 1 860

Table 5 continued.

PATIENT	SKIN COUNT (CFU FROM A 2CM² AREA)		
	A EXUDATIVE	B DRY	C UNINVOLVED
E8 (11.98)	***S.aureus* 2 680** *S.capitis* 120	***S.aureus* 460** *S.haemolyticus*180 *S.capitis* 140	*S.epidermidis* 20
E8 (12.99) Back	***S.aureus* 20 000**	***S.aureus* 4 900**	***S.aureus* 180** *S.capitis* 60
Elbow	***S.aureus* 32 800**	***S.aureus* 700** *S.epidermidis* 100	***S.aureus* 320** *S.epidermidis* 80 *S.capitis* 60
Calf	***S.aureus* 36 400** *Corynebacterium sp.* 1000	*Micrococcus sp.* 1 400 *S.warneri* 600	*Micrococcus sp.*1 800 *S.epidermidis* 150 *S.capitis* 40
E9	***S.aureus* 6 240** *S.capitis* 120	***S.aureus* 1 360** *S.capitis* 200	***S.aureus* 940** *S.epidermidis* 480
E10	No growth	***S.aureus* 120**	***S.aureus* 60** *S.epidermidis* 100

Figure 11. Bar chart to illustrate the incidence of organism isolation per site in eczema.



3.2 PFGE.

PFGE was used to analyse the genomic DNA fragments of the staphylococcus isolates produced by digestion with the rare-cutting restriction enzyme *Sma1* ($^{5'}$CCC/GGG $^{3'}$). The staphylococcal genome has a low GC content of 35-37% and cleavage with this RE generates between 15-20 DNA fragments (Iandalo, Bannantine and Stewart, 1996). Analysis of the DNA fragment profiles then allowed the relatedness of the isolates obtained, to be evaluated (Tenover *et al.*, 1995).

3.2.1 Psoriasis.

Speciated isolates of CNS obtained from the skin of eczema and psoriasis patients, were compared by the calculation of the Dice correlation coefficient and clustered by UPGMA (unweighted pair group method of arithmetic averages), to enable the construction of a dendrogram, figure 12. This approach identified 38 different strains of CNS for eczematous and psoriatic skin and demonstrated considerable heterogeneity amongst them. *S.capitis* and *S.epidermidis* were the predominant strains for both conditions, though many genotypes of each species were identified by PFGE. No common strain appeared to be associated with psoriasis or eczema. Figure 13 shows examples of the diversity of the chromosomal DNA restriction patterns obtained for some of the CNS isolates.

Statistical analysis of the bands of the CNS isolates acquired from the repeat swabs and the original isolates of patient P2 by ANOVA, gave a p value of 0.05. This indicated a significant difference between the isolates and hence, again demonstrated the genetic diversity of the CNS from one site to another and from one time period to another.

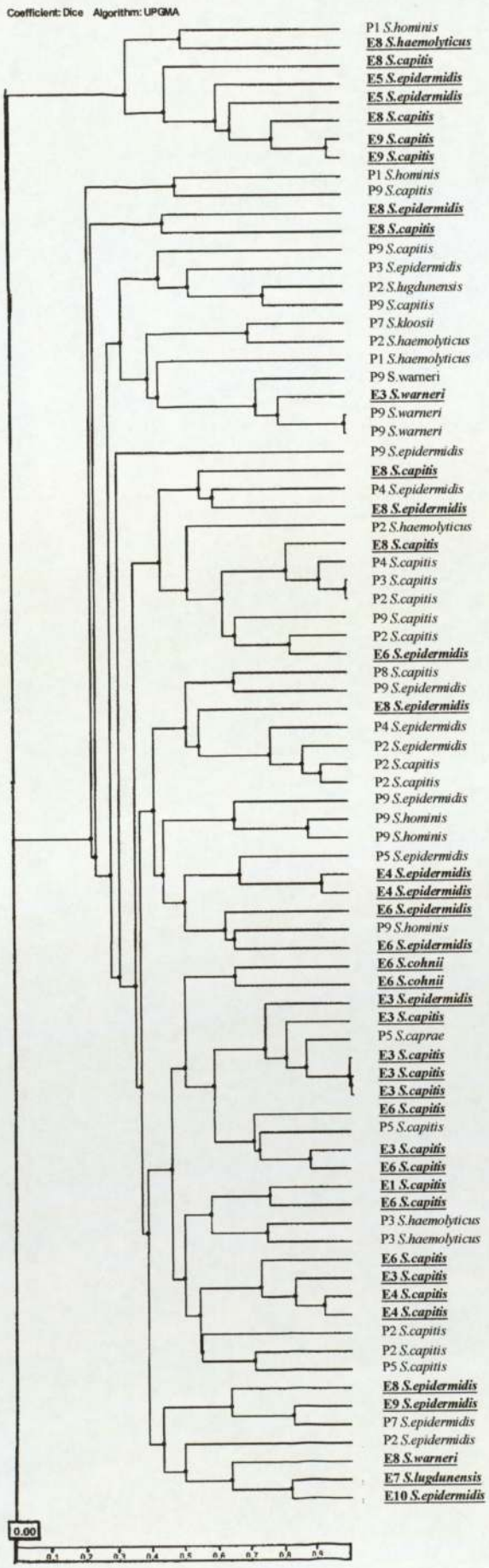


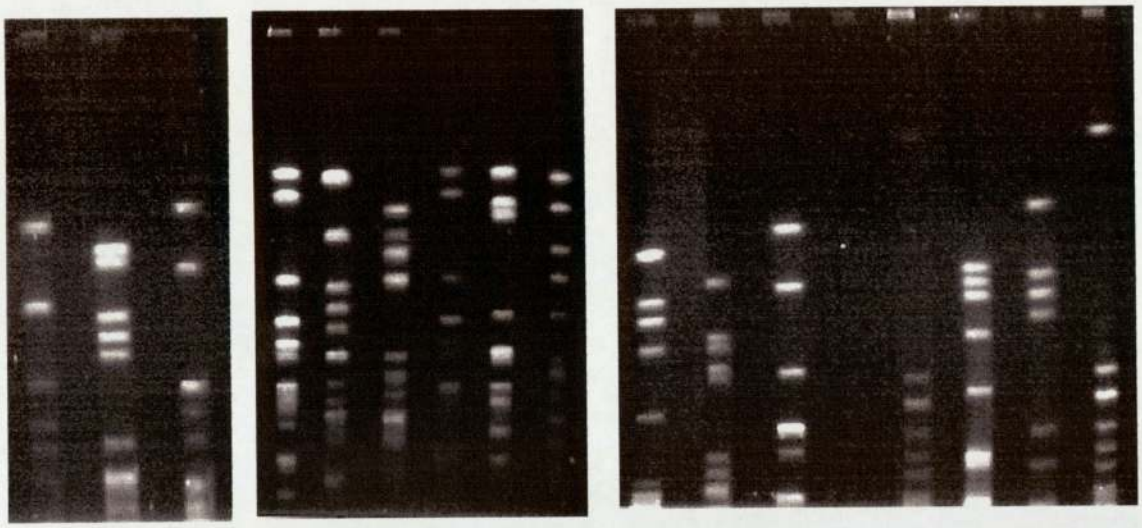
Figure 12. Dendrogram of CNS isolates from eczematous and psoriatic skin.

KEY:

E = eczema
P = psoriasis

Figure 13. Chromosomal DNA restriction patterns of CNS by PFGE:

lanes **A,B,C** = *S.lugdunensis, S.epidermidis* and *S.capitis* from patient P2; lane **D** = *S.capitis* from patient P3; lane **E** = *S.lugdunensis* from patient E7; lanes **F,G,H** = *S.epidermidis, S.capitis* and *S.caprae* from patient P5; lane **I** = *S.epidermidis* (NCTC 11047); lanes **J,K,L** = *S.warneri, S.epidermidis* and *S.capitis* from patient E8; lanes **M,N,O,P** = *S.capitis ,S.epidermidis, S.epidermidis* and *S.capitis* from patient P9.



Of the 3 *S.aureus* isolates from psoriatic skin, the two isolates from patient P6 were indistinguishable from each other but differed to the strain from patient P2 by four bands. The genotypes of these strains were also different to those obtained from eczematous skin.

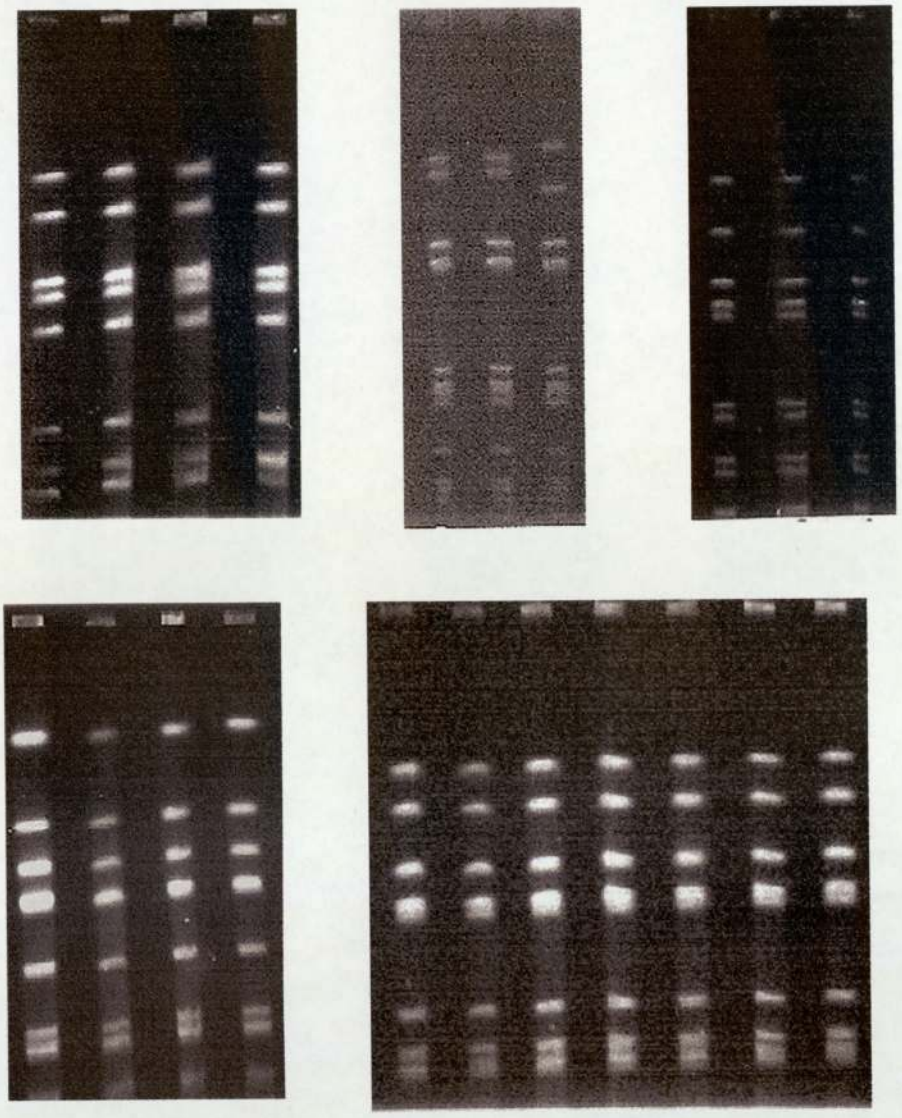
3.2.2 Eczema.

Of the 43 isolates of *S.aureus* obtained, 13 different strains were identified by PFGE. A strain is defined by Tenover *et al.,* (1995), as an isolate or group of isolates that can be distinguished from other isolates of the same genus and species by phenotypic characteristics or genotypic characteristics or both.

Multiple isolates of *S.aureus* were obtained for each patient and in 7 of the 10 patients sampled, only one strain was found per patient, for example four isolates of *S.aureus* from one patient, were all indistinguishable from each other. The restriction pattern of isolates had the same number of bands as each other and the corresponding bands, had the same apparent size. Figure 14 shows examples of the DNA restriction patterns of multiple *S.aureus* isolates from five different patients.

Table 6 shows a comparison of the fragment profiles obtained for all of the *S.aureus* strains obtained from eczematous skin, according to the criteria of Tenover *et al.,* (1995). Three of the ten patients had two different strains. The strains from patients E1, (a) and (b), differ by 1 band as did those from patient E10 (a) and (b), whereas the strains from E3 (a) and (b) differ by 7 bands. Table 6 also highlights the fact that between different patients some of the strains were closely related, but they were all different to each other, indicating that there was no common strain found on all patients.

Figure 14. Chromosomal DNA restriction patterns of multiple isolates of *S.aureus* obtained from five patients.



From this illustration it can be seen that each individual patient has their own strain of *S.aureus.*

Table 6. A comparison of band differences obtained from the PFGE of *S.aureus* strains from eczematous skin.

Patient	E1A	E1B	E2	E3A	E3B	E4	E5	E6	E7	E8	E9	E10A	E10B
E1A	X												
E1B	**1**	X											
E2	8	7	X										
E3A	8	7	**2**	X									
E3B	10	8	7	7	X								
E4	10	10	4	7	5	X							
E5	6	9	7	8	**3**	6	X						
E6	11	11	8	9	5	7	5	X					
E7	11	10	9	9	9	6	7	6	X				
E8	10	10	9	8	4	7	5	**3**	4	X			
E9	10	12	8	7	4	6	10	**3**	7	4	X		
E10A	10	9	9	8	6	7	7	5	8	5	6	X	
E10B	10	10	9	8	6	7	7	6	8	5	5	**1**	X

Comparisons were made according to the criteria of Tenover *et al.*, (1995):
2-3 bands difference = closely related
4-6 bands difference = possibly related
> 7 bands difference = not related

Three of the patients, E3, E6 and E8 were reswabbed in three different areas of the body these were: E3: elbow, knee, thigh, E6: calf, wrist, shoulder blades, E8: back, elbow, calf. The PFGE patterns for the *S.aureus* isolates from these areas were compared to those obtained from the original swabs by statistical analysis using ANOVA or one-way analysis of variance. The *p* value obtained for patients E3 and E6 was > 0.9999 and for E8 0.9998 indicating that there was no significant difference between the molecular weights of the bands for each strain, on the two occasions tested. This indicates that the same *S.aureus* could be found all over the patients body and was a constant entity. In contrast, the CNS isolates obtained from these repeats, were identified as different species and genotypes to the original.

Where multiple *S.aureus* isolates from a patient were identified as the same strain, only one of these strains was tested for the production of virulence factors.

Due to the diversity of the CNS in both conditions, attention was drawn to the *S.aureus* strains from eczematous skin to determine whether they possessed a factor common to all strains, despite differences in their genotypes.

3.3 Antibiograms and Biotyping.

Many different antibiograms and biotypes were obtained for *S.aureus* and CNS, even for those that had indistinguishable PFGE patterns. For example: for eczema the agreement of antibiograms and API profiles with PFGE results was 31% and 37% respectively. For psoriasis, the agreement of antibiograms and API profiles with PFGE results was 50%.

Of the *S.aureus* isolates from the eczema patients, none were MRSA's although for patients: **E3** 8/10, **E6** 3/7, **E8** 4/9, **E9** 1/3 of the isolates were resistant to fusidic acid,

one of the major antibiotics used to treat infected eczema. As two different genotypes for E3 were identified by PFGE, the fact that two of the isolates were sensitive seems reasonable, as the presence/absence of a band (gene) may code for this, but for the other patients, as their multiple isolates were identified as indistinguishable by PFGE, one would have expected them to have the same antibiograms.

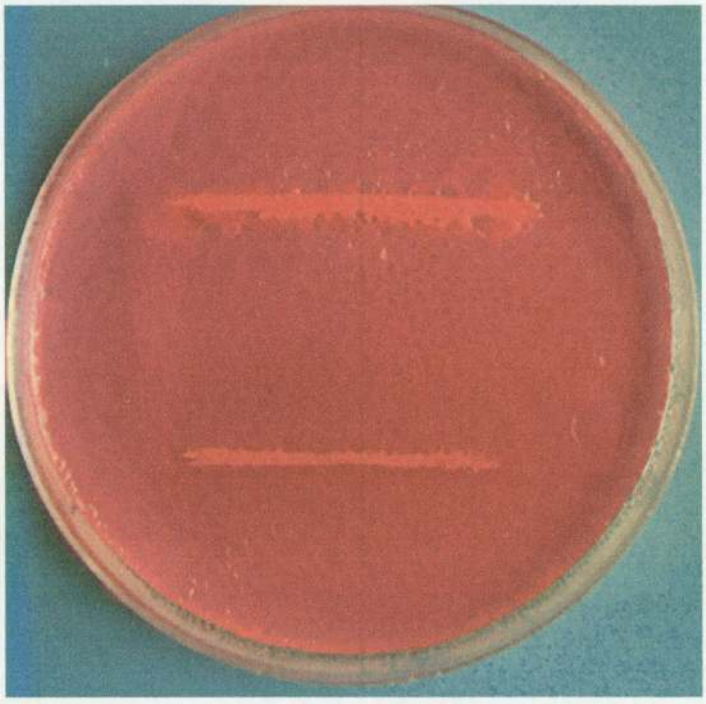
3.4 Potential virulence factors of *S.aureus* isolates.

3.4.1 PLATE ASSAYS.

3.4.1.1 Lipase activity.

The appearance of colonies producing lipase were bright pink in colour whereas the negative colonies were white/pale pink, as shown in figure 15. The plates were also viewed under ultraviolet light (350 nm) as the hydrolysis of the olive oil in the agar, by lipase activity, in the presence of rhodamine B, produced a flourescent product. The molecular mechanism underlying the formation of flourescent products generated from olive oil hydrolysis by lipase is unknown but is thought to be due to a complex formation between the cationic rhodamine B and the uranyl-fatty acid ion (Kouker and Jaegar, 1987).

Figure 15. Detection of lipase production in staphylococci.



3.4.1.2 Esterase.

Colonies that produced esterase hydrolysed the Tween 80 resulting in the precipitation of calcium chloride from the agar around the bacterial colonies, as can be seen in figure 16.

Figure 16. Detection of esterase production in staphylococci.

3.4.1.3 Extracellular polysaccharide slime layer.

A characteristic dry black appearance was produced by slime producing colonies illustrated in figure 17, in contrast to a moist red appearance by non-slime producers as shown in figure 18.

Figure 17. Slime-positive staphylococci on congo red agar.

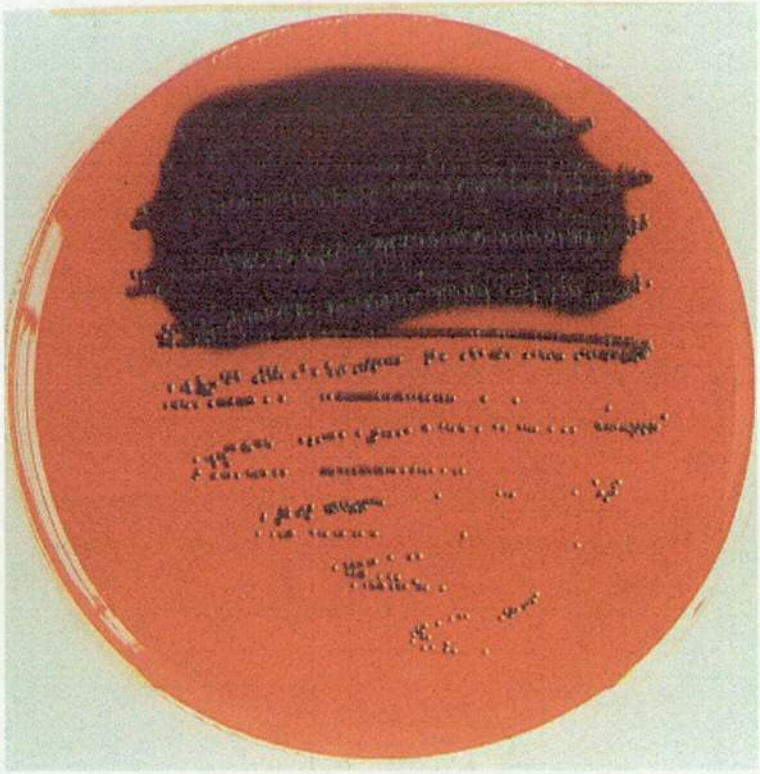
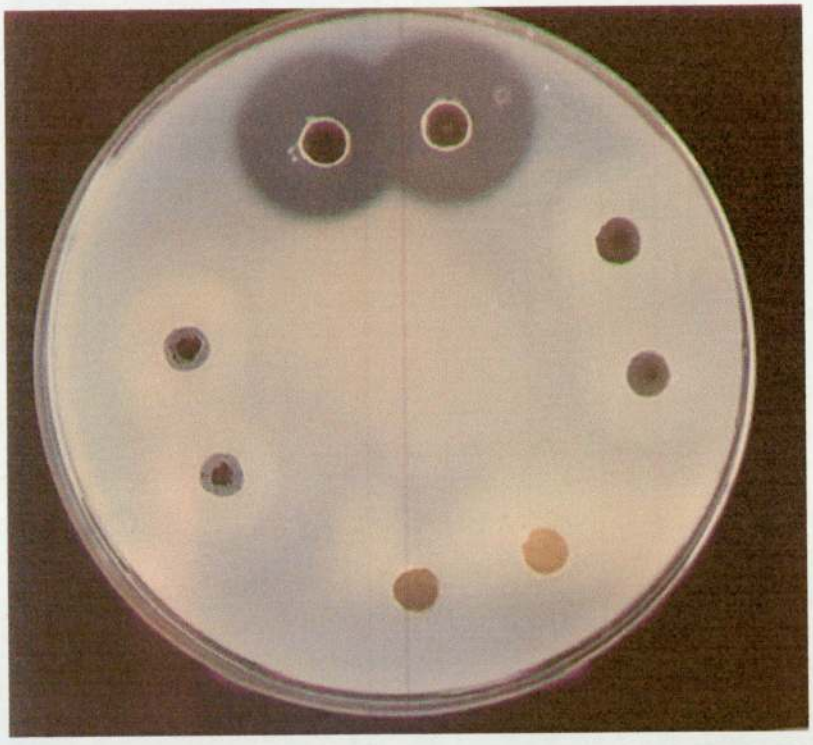


Figure 18. Slime-negative staphylococci on congo red agar

3.4.1.4 Non-specific protease.

Non-specific protease activity of the culture supernatant was indicated by a zone of clearing around the well, of the skim milk agar, as can be seen in figure 19.

Figure 19. Non-specific protease activity on skim milk agar.



3.4.1.5 Elastase.

Elastase activity was indicated by a zone of clearing around the bacterial colonies due to the degradation of elastin in the agar, illustrated in figure 20.

Figure 20. Elastase production of staphylococci.

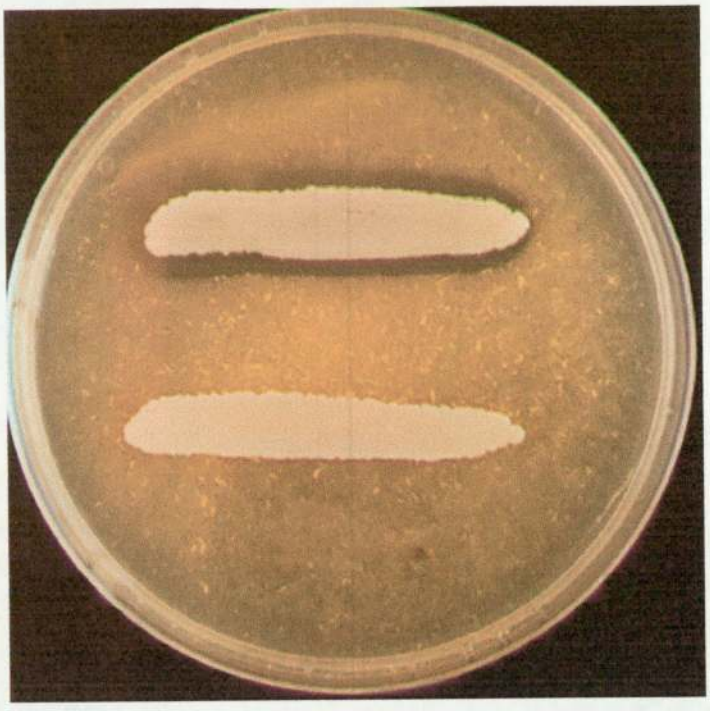


Table 7 shows the results obtained from the plate assays. With reference to this it can be seen that the only positive result was esterase.

Table 7. Virulence factors of *S.aureus* strains from eczematous skin.

PATIENT	Extracellular Polysaccharide Slime layer	Lipase	Non specific protease	Elastase	Esterase
E1	-	-	-	-	+
E2	-	-	-	-	+
E3	-	-	-	-	+
E4	-	-	-	-	+
E5	-	-	-	-	+
E6	-	-	-	-	+
E7	-	-	-	-	+
E8	-	-	-	-	+
E9	-	-	-	-	+
E10	-	-	-	-	+

3.4.2 Toxin production.

Presence of enterotoxins/TSST-1 produced by *S.aureus* caused the agglutination of sensitised latex particles and subsequent formation of a lattice structure, which when settled formed a diffuse layer on the base of the well. Absence of toxin resulted in a tight button of the latex particles. Table 8 shows the results obtained, from which it can be seen that all of the strains were negative for TSST-1, two were positive for enterotoxin A and one for enterotoxin D.

Table 8. Toxin production of *S.aureus* strains from eczematous skin.

Patient	*TSST-1*	*Enterotoxin A*	*Enterotoxin B*	*Enterotoxin C*	*Enterotoxin D*
E1	-	+	-	-	-
E2	-	-	-	-	-
E3	-	-	-	-	-
E4	-	-	-	-	-
E5	-	-	-	-	-
E6	-	-	-	-	+
E7	-	+	-	-	-
E8	-	-	-	-	-
E9	-	-	-	-	-
E10	-	-	-	-	-

3.5 Serology.

Serological tests for anti-streptolysin O (ASOT), anti-staphylolysin (AST) and IgG titres to lipid S (Elliott *et al.*, 2000, Worthington *et al.*, in press) were performed on the serum of these patients to see if the organisms on the skin surface, produced an immune response, through exposure via the skin lesions.

3.5.1 ASOT and AST.

Anti-streptolysin O titres (ASOT) of up to 200 IU/ml are considered to be normal, higher than this suggests streptococcal infection (Behring,Germany). With regards to anti-staphylolysin titres (AST), up to 2 IU/ml is normal and above this suggests staphylococcal infection (Behring, Germany).

- **ECZEMA** – with reference to table 9, it can be seen that 4/10 patients had raised ASOT's and 3/10 patients had raised AST's.
- **PSORIASIS** – with reference to table 10, and in contrast to table 9, all of the ASOT and AST's were normal.
- **CONTROLS** – with reference to table 11, it can be seen that all of the ASOT and AST's were normal.

3.5.2 Lipid S serology.

Previous studies by Worthington *et al.*, (in press), have suggested that a titre above zero is interpreted as being positive. Hence there should be no detectable titre in a healthy person.

- **ECZEMA** – 8 /10 patients had positive IgG titres see table 9, in contrast to the ASOT and AST results. 5 of these patients had a second or in one case a third serum sample tested, 6-12 months after the original, to produce in 4/5 patients a higher IgG titre.

- **PSORIASIS** – 4/10 patients had positive IgG titres as seen in table 10, in contrast to the negative titres obtained from the ASOT and AST assays. 2 of the 10 patients had a second serum sample tested for IgG titres, 12 months later to give negative results.
- **CONTROLS** – 10/10 patients did not have any detectable IgG titre see table 11.

A scatterplot to show the range of IgG titres obtained and to compare visually the results for eczema, psoriasis and normal controls is shown in figure 21.

Table 9. ASOT, AST and IgG titres to lipid S obtained from the serum of patients with atopic eczema.

PATIENT	ASOT (IU/ml)	AST (IU/ml)	Lipid S IgG TITRE Samples obtained November 1998	REPEAT Lipid S IgG TITRE Dates in brackets Denote when samples were obtained.
E1	< 200	< 2	26 636	24 303 (May 1999)
E2	400	4	451	6 998 (July 1999)
E3	200	< 2	11 813	20 836 (Dec 1999)
E4	400	2	9 706	
E5	< 200	< 2	2 106	
E6	200	6	3 310	19 337 (July 1999) 14 321 (Dec 1999)
E7	400	< 2	20 240	
E8	400	6	10 233	14 382 (Dec 1999)
E9	< 200	< 2	Not detected	
E10	< 200	< 2	Not detected	

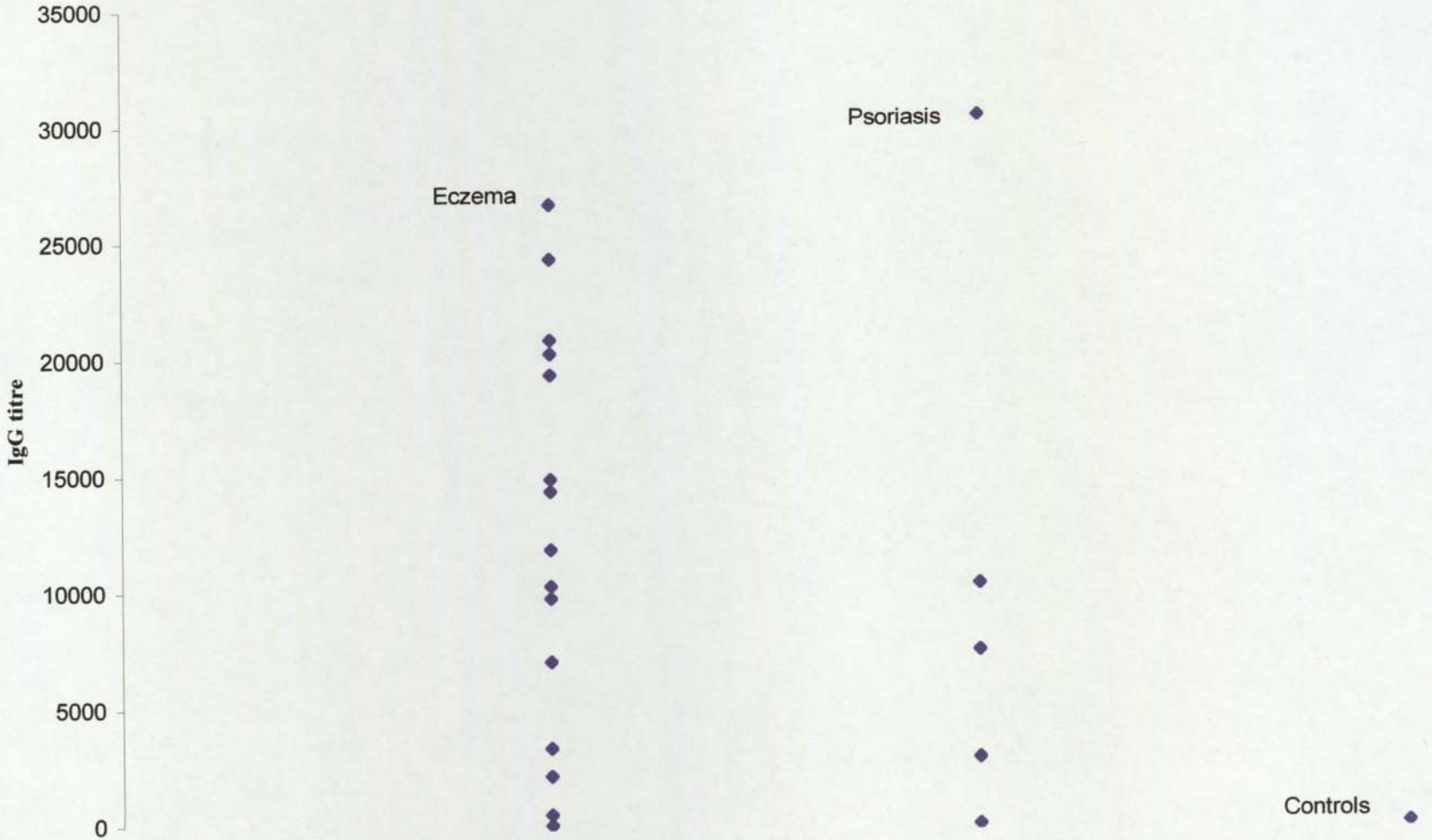
Table 10. ASOT, AST and IgG titres to lipid S obtained from the serum of patients with chronic plaque psoriasis.

PATIENT	ASOT (IU/ml)	AST (IU/ml)	Lipid S IgG TITRE Samples obtained Nov 1998	REPEAT Lipid S IgG TITRE Samples obtained Dec 1999
P1	< 200	< 2	10 308	
P2	< 200	< 2	2 859	Not detected
P3	< 200	< 2	Not detected	
P4	< 200	< 2	Not detected	
P5	< 200	< 2	Not detected	
P6	< 200	< 2	Not detected	
P7	< 200	2	30 398	
P8	200	< 2	Not detected	
P9	< 200	< 2	Not detected	Not detected
P10	< 200	< 2	7 449	

Table 11. ASOT, AST and IgG titres to lipid S from the serum of control patients.

PATIENT	ASOT (IU/ml)	AST (IU/ml)	Lipid S IgG TITRE
C1	< 200	< 2	Not detected
C2	< 200	< 2	Not detected
C3	< 200	< 2	Not detected
C4	200	< 2	Not detected
C5	200	< 2	Not detected
C6	200	< 2	Not detected
C7	< 200	< 2	Not detected
C8	< 200	2	Not detected
C9	< 200	< 2	Not detected
C10	< 200	< 2	Not detected

Figure 21. Scatterplot of IgG titres from the serum of patients with atopic eczema, chronic plaque psoriasis and normal controls.



3.5.2.1 Statistical analysis of the lipid S serology.

Various statistical tests were used to compare the titres obtained for eczema and psoriasis:

- **Fisher's Exact test** which gave a two sided P value of 0.1698
- **Mann-Whitney test** which gave a two tailed P value of 0.1903
- **Unpaired t test** which gave a two tailed P value of 0.4371

In each case the difference between the titres for eczema and those for psoriasis was considered not significant, despite a higher number of positives from the eczema patients. In both cases the titres were higher than in the controls.

4.0 DISCUSSION.

4.1 Normal skin.

The first line of defence against infection by pathogenic microorganisms is the skin. The skin surface lipids and the water from sweat keep the intact horny layer of the epidermis emulsified, soft and strong (Kagami *et al.*, 1985, Ring *et al.*, 1992).

Normal skin flora consists of three major groups of Gram-positive bacteria; the coryneforms, (*Brevibacterium, Corynebacterium, Dermabacter* and *Propionibacterium*), the micrococci and the staphylococci. In comparison, the Gram-negative bacilli constitute only a minor component of the microflora as the skin is a comparatively dry habitat, which does not encourage these organisms to multiply. Approximately twelve species of staphylococci are commonly found on human skin and are collectively known as coagulase negative staphylococci (CNS). Of these *S.epidermidis, S.hominis, S.haemolyticus* and *S.warneri* are the most abundant, however, they are rarely associated with skin disease (Fleurette *et al.*, 1987). CNS are of clinical importance when a catheter or other form of plastic prosthesis is inserted through the skin, due to their ability to adhere to plastic and subsequently cause infection. For example, over 40% of peritonitis, experienced by patients receiving continuous ambulatory peritoneal dialysis, is caused by CNS, which are often recently acquired members of skin flora (Peters, 1988).

S.epidermidis and *Propionibacterium acnes* hydrolyse the triglycerides of the lipid layer, into glycerol and free fatty acids which decreases the pH of the skin surface. The relationship of the resident flora and the lipids inhibits other pathogenic bacterial replication. Hence the barrier function of the skin depends on the intact epidermis, which in turn depends on the skin surface lipids and the resident flora (Noble,1998).

The only coagulase positive staphylococcus found in humans is *Staphylococcus aureus*, colonisation of normal skin with this organism is rare (2-25%) (Abeck *et al.*,1998). Common carrier sites include the nose, the perineum, the axillae and the toe-webs

4.2 Psoriatic skin.

From the results obtained in this study, CNS were the predominant organisms found on psoriatic skin. Ten species of CNS were identified, with *S.capitis, S.epidermidis, S.haemolyticus* and *S.hominis* being the most commonly isolated, almost identical to the flora of normal skin as mentioned above. In some cases several species of CNS were isolated for each area of plaque sampled, suggesting that no one species was associated with the inflammed edge, centre of the plaque or indeed the uninvolved skin. *S.aureus* was isolated in only two psoriatic patients, this is consistent with previous research where *S.aureus* in psoriatic plaques was found to rarely exceed 10^3 organisms per cm^2, if isolated at all (Marples *et al.*,1973).

Though psoriasis is associated with Group A streptococci, none were isolated in this investigation. The potential inhibitory action of Triton-X-100 may account for failure to isolate them (Aly *et al.*,1972).

4.3 Eczematous skin.

Staphylococcus aureus was found, to be not only the most predominant organism in the lesions, but also in the uninvolved skin. This was consistent with previous findings (Leyden *et al.*,1974, Aly *et al.*,1977, Dahl,1983, Kagami *et al.*,1985, David *et al.*,1986, White *et al.*,1986, Ring *et al.*,1992, Abeck *et al.*,1998, McFadden, 1999).

The skin of patients with atopic eczema differs from that of the normal population in that the composition of lipids in the epidermis is different. This affects the skin's function, resulting in diminished antibacterial activity and the characteristic excessive dry, flaky and cracked skin associated with eczema (Abeck *et al.*, 1998).

In normal skin transdermal water loss is reduced by lipid sheets that are made up largely of ceramides, the major water holding molecules in the extracellular space of the horny layer. This function seems to be defective in atopic skin. There are two suggestions as to why this is:

(1) It is a result of abnormal metabolism of essential fatty acids, particularly linoleic and linolenic (Munn,1999).

(2) It is a result of ceramidase secretion from the *S.aureus* isolates colonising the lesional and non-lesional skin of eczematous skin, which breaksdown ceramide into sphingosine and fatty acid (Ohnishi *et al.*, 1999).

Increased colonisation by *S.aureus* in these patients may be due to the enhanced adherence of the organism to skin, as *S.aureus* has been found to exhibit a greater degree of adherence to keratinocytes of patients with atopic eczema as compared with keratinocytes collected from those with psoriatic lesions, other skin diseases or in fact normal controls (Ring *et al.*, 1992). Fibronectin, has been characterised as one of the receptors involved in adhesion of *S.aureus* to host cells (Ring *et al.*, 1992).

4.4 Pulsed-field gel electrophoresis (PFGE).

In contrast to conventional techniques such as biotyping and antibiograms, PFGE allows a high level of discrimination and reproduceability due to the analysis of the entire bacterial chromosome. The method analyses genotypic characteristics as opposed

to the potentially variable phenotypic characteristics of the organism, where the biochemical properties and antibiotic profiles can alter each time an isolate is subcultured. The staphylococcal genome has a low GC content (35-37%) and cleavage with the restriction enzyme *Sma 1,* which cuts at GC rich sites, produces profiles of up to 18 bands which allows comparisons to be made between strains through the analysis of macrorestriction patterns (Tenover *et al.,*1995, Lang *et al.,*1999, Williams *et al.,*1999).

Disadvantages of PFGE include the need for expensive materials and equipment to run the gel and to analyse the subsequent macrorestriction patterns obtained, the limited number of samples that can be run on a gel, the use of carcinogenic substances such as ethidium bromide, and the amount of time required for sample preparation and running of the gel, usually six days (Williams *et al.,*1999).

4.4.1 Psoriasis.

PFGE identified 38 different strains of CNS, however, despite *S.capitis* and *S.epidermidis* being the predominant strains for this condition many different genotypes of each species were identified by PFGE, illustrating the diversity of the CNS on the skin surface. This was further highlighted when repeat swabs were taken to give very different genotypes to those obtained originally, suggesting that no specific strain is associated with psoriatic skin. Instead the organisms are transient and perhaps reflect contamination from clothing and / or the skin of other individuals. Rearrangement of the CNS on the skin surface can also result from washing and other manipulations which may alter the number and spectrum of strains of CNS found at any one time (Brown *et al.,*1989). This is consistent with normal skin as determined by Brown *et al.,* (1989), where the skin surface and the underlying stratum corneum were sampled.

They found that several different strains were present in samples from the skin surface whereas one or two strains were found repeatedly in the same geographic locations in the layers of the underlying stratum corneum. They concluded that CNS on the skin surface were derived from several sources whereas CNS from the underlying layers of the stratum corneum represented true resident flora of that part of the human epidermis. This may have been the case if the underlying layers of the stratum corneum in psoriatic skin was analysed.

4.4.2 Eczema.

As with psoriasis, PFGE identified many different genotypes of CNS despite the prevalence of certain species, illustrating the diversity of these organisms on the skin surface. PFGE also identified 13 different genotypes of *S.aureus,* thus, each patient had their own unique strain, or in the case of three of the patients, two different strains per person. When some of these patients were re-swabbed over different sites of the body six to twelve months later, identical *S.aureus* strains were reisolated.

PFGE has been used for typing MRSA and *S.aureus (*Lui *et al.,*1996, Cookson *et al.,*1996, Schlievert *et al.,*1993), however, the technique has not been applied to *S.aureus* isolates from atopic eczema. Instead isolates have been studied by bacteriophage typing, whereby most strains were found to belong to phage groups I or III (Dahl,1983, Kagami *et al.,* 1985,White *et al.,*1986) with 30-40% of the strains being untypable. In order to compare the results of this study with previous findings, all of the *S.aureus* isolates were sent to Colindale PHLS for phage-typing. In contrast to PFGE, phage-typing involves the comparison of isolate sensitivity patterns to phages at a standardised concentration known as the routine test dilution (RTD). *S.aureus* isolates are considered to represent the same strain if their patterns differ by less than two

strong reactions at RTD. In contrast to the findings of Dahl, 1983, Kagami et al 1985 and White *et al.,* 1986, the results from the reference laboratory showed that none of the strains were phage groups I or III, 38% were phage group II (which are particularly associated with skin isolates), 46% were distinct strains in that they showed an unrecognised phage pattern, 3% were untypable and 3% were deemed widespread sporadic (common strain but rarely associated with cross-infection). It can thus be concluded from this study that neither PFGE or phage-typing support the existence of a predominant biotype of *S.aureus* or association with a particular group of strains in atopic eczema (Jappe *et al.,*1997). Instead each patient carries their own strain which appears to be a constant entity rather than the transient CNS strains.

4.5 Colonisation or infection?

The presence of *S.aureus* on eczematous skin is universal and colonisation cannot be a criterion for infection. There are problems with the definition of 'infected eczema', whilst the discharge of pus strongly suggests infection, other appearances are not clearly diagnostic. The presence of weeping is unreliable because, although it may be due to infection, it may also be the result of a deterioration due to an allergy or simply incessant scratching (David *et al.,*1986).

Leyden *et al.,* (1974) suggested that a density of *S.aureus* greater than 10^6 / cm^2 leads to exacerbation of the disease and is an indicator for antibacterial therapy, regardless of the clinical signs, however quantitative bacteriology is not very practical and the clinical signs are often all there is to go on. It can be difficult to identify the presence of *S.aureus* in atopic eczema but there are certain characteristics which make infection highly likely. Large increases in the carriage of *S.aureus* are associated with a

progressive deterioration in the eczema, which presents with impetiginisation and weeping, excoriated papules, lichenification and crusting (Chu *et al.*,2000).

4.6 Virulence factors of *S.aureus.*

A property of staphylococci is their capacity to change specific phenotypic features rapidly and differ among variants of the same parent strain, that is, 'phase variation'. This phase variation may in itself represent a virulence factor contributing to bacterial survival and growth under changing environmental conditions (Ziebukhr *et al.*,1997). This may account for the lack of virulence found in the *S.aureus* isolated in this study. As all *S.aureus* isolates produce esterase (Rollof *et al.*,1992) the results obtained were inconclusive.

S.aureus has been found to produce slime (Baselga *et al.*,1993), lipase (Jaegar *et al.*,1994, Gotz *et al.*,1998, and Rollof *et al.*,1992) and elastase (Park *et al.*,1991).

Rollof *et al.*,1988, found that *S.aureus* isolated from deep-seated infections generally produced higher amounts of lipase than those from superficial locations e.g impetigo and that lipase production was more pronounced in *S.aureus* belonging to phage-group I, in this study none of the strains belonged to this phage-group.

S.aureus isolated from the skin of patients with atopic eczema may have the genes that code for various virulence factors but when found on the skin these may be 'switched' off, as atopic skin appears to offer no resistance to colonisation by this organism. However, on entering the bloodstream through cracked skin, these genes may become activated as the *S.aureus* then has to overcome the body's immune system. Elastase and lipase production by the *S.aureus* for example would enable it to invade other tissues.

Ring *et al.*, 1992 have suggested that *S.aureus* probably produces disease as a result of the activity of enzymes and toxic products such as haemolysin or coagulase. Products of *S.aureus* such as teichoic acid, protein A and peptidoglycan induce the release of

histamine from mast cells and basophils. Peptidoglycan enhances the expression of CD23 and the alternative pathway of complement by promoting the formation of the alternative pathway C3 convertase. In addition, platelets from patients with atopic dermatitis generate significantly higher amounts of 12-HETE and express increased numbers of receptors (CD29 / CD49f) to extracellular matrix proteins such as fibronectin, when they are stimulated with *S.aureus.*

4.7 Toxin producing *S.aureus* and superantigens.

The current view of the role of *S.aureus* in atopic eczema is that certain strains produce toxins which can act as superantigens and non-specifically stimulate the immune response leading to the exacerbation of eczema (McFadden *et al.*,1993). Superantigens have also been shown to down regulate steroid receptors in the skin leading to reduced responsiveness of the skin to topical corticosteroids (McFadden,1999). These toxins are: toxic shock syndrome-1 (TSST-1) and the staphylococcal enterotoxins SEA, SEB, SEC and SED. TSST-1 is the cause of an acute and potentially fatal illness commonly associated with tampon use in menstruating women, whereas SEA-SED, cause staphylococcal food poisoning and are potent emetic agents. The enterotoxins have recently been shown to be superantigenic, in particular SEB and SEC, which have been implicated in non-menstrual toxic shock syndrome (TSS) (Dinges *et al.*,2000). In contrast, the coagulase negative staphylococci (CNS) have not been shown to cause TSS (Dinges *et al.*,2000).

Superantigens are proteins which produce a much larger immune response than ordinary antigens. They stimulate T-cells in association with major histocompatibility complex (MHC) class II molecules, see figure 22. Unlike classical antigens, they

stimulate T-cells in a mitogenic fashion without any need for prior antigen processing, they can achieve this at minute concentrations, much lower than classical antigens.

- **Classical antigens**

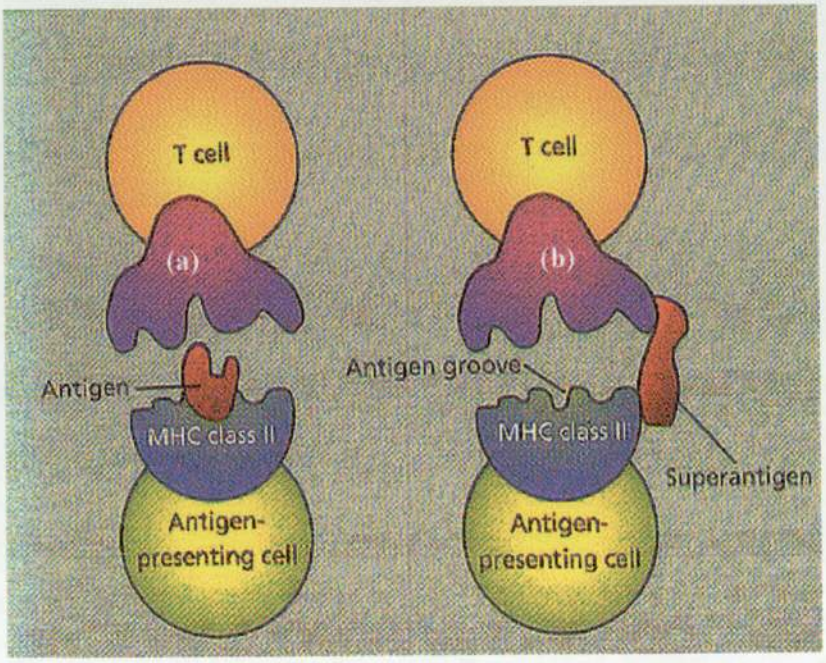
After processing, classical antigens are presented to the T-cells in the antigen groove and all five variable components of the T-cell receptor are involved in antigen recognition figure 22 (a).

- **Superantigens**

In contrast, the much larger superantigens bind to a different part of the molecule without prior antigen processing where only the Vβ variable component is involved in antigen recognition figure 22 (b).

As many as 5-10% of the T cells can recognise the superantigen, which as a result leads to massive lymphocyte stimulation and cytokine release (McFadden,1999, Schlievert,1993, Leung *et al.*,1998).

Figure 22. Antigen and superantigen binding to Major Histocompatibility Complex (MHC) and recognition by T-cell (Chu *et al.*, 2000). See text for explanation.



In atopic eczema, stimulation with superantigen leads to release of a number of proinflammatory mediators, including IL-4, IL-12, IL-13 and interferon. Predominance of IL-4 or IL-13 leads to increased synthesis of IgE antibodies, which in turn bind to mast cells, leading to the development of inflammation and eczematous lesions. Larger concentrations of superantigens induce IL-12 or interferon, in which case IgE is suppressed (Chu *et al.*,2000).

Superantigens may also release tumour necrosis factor (TNFα) from human keratinocytes. Certain lymphocytes bear a receptor known as the cutaneous lymphocyte antigen (CLA) and are preferentially taken up by the skin. Staphylococcal superantigens preferentially activate the Vβ subpopulation to which these lymphocytes belong and increase their number (McFadden,1999).

From the results obtained, none of the *S.aureus* strains produced TSST-1, two produced SEA and one SED. This is in contrast to the work of McFadden *et al.*, (1993), who showed that 24 / 37 of the *S.aureus*, produced one or more identifiable toxins. Jappe *et al.*,(1997), found that 45% of strains were capable of producing superantigens SEB, SEC and TSST-1.

All *S.aureus* isolates from atopic skin were sent to Colindale PHLS for detection of toxin genes by PCR. Table 12 shows the results obtained. All the isolates were negative for TSST-1 genes, three were positive for SEA and none for SED. In addition to these toxins they also tested for SEE, SEG, SEH, SEI, SEJ and staphylococcal exfoliative toxins A and B (eta and etb) also thought to be superantigens. Three isolates had genes for SEG, two SEH, three SEI and one eta. It is interesting to note that of the three patients who had two different genotypes of *S.aureus:* two of them, patients E3 and E10, each had one strain of the two isolated, that had toxin genes. That is E3a which

was negative, whereas E3b had the toxin gene for SEA, and E10a had the toxin genes for SEG and SEI whereas E10b was negative.

Production of these toxins poses several problems including contamination of food with enterotoxin producing *S.aureus* by eczema sufferers that are food handlers, as a result of their flaky skin.

Table 12. Toxin results obtained from Colindale PHLS.

Strain	SEA	SEB	SEC	SED	SEE	SEG	SEH	SEI	SEJ	eta	etb	TSST-1
E1a	+	-	-	-	-	-	+	-	-	-	-	-
E1b	+	-	-	-	-	-	+	-	-	-	-	-
E2	-	-	-	-	-	-	-	-	-	-	-	-
E3a	-	-	-	-	-	-	-	-	-	-	-	-
E3b	+	-	-	-	-	-	-	-	-	-	-	-
E4	-	-	-	-	-	-	-	-	-	+	-	-
E5	-	-	-	-	-	-	-	-	-	-	-	-
E6	-	-	-	-	-	+	-	+	-	-	-	-
E7	+	-	-	-	-	-	-	-	-	-	-	-
E8	-	-	-	-	-	+	-	+	-	-	-	-
E9	-	-	-	-	-	-	-	-	-	-	-	-
E10a	-	-	-	-	-	+	-	+	-	-	-	-
E10b	-	-	-	-	-	-	-	-	-	-	-	-

If infected / colonised eczematous skin is ignored, superantigens will exacerbate the eczema and down regulate the steroid receptors in the skin. The use of stronger steroids

will be of little if any use as the skin will be less responsive to these agents. As the disease becomes worse increased concentrations of topical steroids are needed to control it (McFadden, 1999).

4.8 Role of antibiotics in the management of atopic eczema.

McFadden, (1999), has suggested that antibiotics have been underused in the treatment of atopic eczema and has made the following proposals, with regards to treatment:

- Frank clinical infection should be treated with oral or topical antibiotics e.g flucloxacillin, cephalexin or fusidic acid.
- When the normal barrier function of the skin is lost due to excoriation treat with a steroid-antibiotic combination, where the antibiotic eradicates the *S.aureus* and stops the release of superantigenic toxins, and the corticosteroid reduces the effects of proinflammatory cytokines in the skin.

By combining topical glucocorticosteroids with an antibiotic, clinical efficacy may be achieved using steroids of less potency and therefore reducing the side-effects of topical steroids. Among the antibiotics used fusidic acid is the drug of choice due to its inhibition of *S.aureus* at low concentrations regardless of the patients susceptibility to methicillin or oxacillin, and its good penetration at a level similar to that of steroids, ensuring a high concentration at the site of infection.

4.8.1 Fusidic acid.

This antibiotic has been used over the last thirty-five years with a very low level of resistance, 1-2%. Resistance to this antibiotic *in vitro* is induced readily, as was probably the case in this study, but as the mutants grow slower than the sensitive bacteria, they have a lower pathogenicity and revert to full sensitivity when fusidic acid is absent. Naturally occurring resistance to fusidic acid has been known and is thought

to be associated with a permeability barrier at the cell surface reducing the entry of the antibiotic. There is no cross-resistance between fusidic acid and other antibiotics used clinically, this is probably due to the fact that fusidic acid belongs to the fusidanes a group of its own which has a structure very different from all other classes of antibiotics thus reducing the likelihood of having the same mechanism of resistance. However, to avoid development of resistance and drug induced hypersensitivity, it is also important to control the period of time that the antibiotics are used over, either topically or systemically (White *et al.*, 1985, Wilkinson,1998).

Examples of antibiotic/steroid combinations commonly used in the treatment of atopic eczema are fusidic acid / hydrocortisone and fusidic acid / betamethasone. These combinations are usually used for two weeks to eradicate the bacteria, and the steroid alone is then used (Abeck,1998, Wilkinson,1998, Javier *et al.*, 1986, Nishijima *et al.*, 1997).

4.9 Serology.

4.9.1 ASOT and AST.

Blood samples may be taken to test for anti-streptolysin O titres (ASOT) in the case of psoriatic patients and anti-staphylolysin titres for those with eczema (AST), to aid the diagnosis of infection.

- **ASOT** - Streptococci produce extracellular toxins and enzymes during growth (metabolites), some of which can cross cell membranes and enter the blood. As the concentration of the metabolite in the blood is low, antibodies formed in response to these antigens can be detected qualitatively and quantitatively. Streptolysin O is an extracellular metabolite of group A,C and G streptococci. Detection of antibodies to streptolysin O suggests recent streptococcal infection. Presence of

elevated titres helps in the diagnosis of acute rheumatic fever and post-streptococcal glomerulonephritis, streptococcal skin infections however, often result in low titres, despite their association with glomerulonephritis (Behring, Germany). This would suggest that for the purpose of this study and infact dermatology, the ASOT is of little clinical value. Also, no reference point exists for the measured value with a single determination and conclusions can only be drawn by repeating the test after 1-2 weeks and comparing the results. As patients dislike having their blood taken, it is felt that returning to the clinic for a second blood test 1-2 weeks later wouldn't be feasible. Four of the eczema patients had titres suggestive of streptococcal infection but none in psoriasis.

- **AST** – the antistaphylolysin titre is of clinical and diagnostic significance for typical staphylococcal diseases and for infections with staphylococcal involvement, for example furuncle, acne, osteomyelitis and staphylococcal pneumonia (Behring, Germany). Three of the ten eczema patients had raised titres suggestive of staphylococcal infection and none of the psoriasis patients had detectable titres. It is suggested that a single determination is of little significance and that serial titrations at bi-weekly intervals 4-6 weeks following infection yield more relevant information, again this is not very practical.

4.9.2 Lipid S ELISA.

In patients suspected of having an infection due to staphylococci, detection of antibodies to this organism is problematic and no single test is satisfactory.

In this study a novel ELISA described by Elliott *et al.*, (2000) and Worthington *et al.*, (in press), was used. This is an indirect ELISA which detects serum IgG reacting with a short chain lipoteichoic acid antigen (lipid S) from coagulase negative staphylococci

(CNS). The test has been of value in detecting patients with sepsis due to Gram-positive cocci (Elliott *et al.*, 2000, Lambert *et al.*, 2000). This assay can be used whether the patient has had or is receiving antibiotics.

From the present study, eight of the ten eczema patients and four of the ten psoriasis patients had elevated titres to lipid S. However, it has not been proven that these titres result from the presence of organisms on the skin surface. All of the eczema patients had *S.aureus* on their skin and so if the elevated titres were due to this organism, one would have expected all patients to be positive. Of the two patients that had no detectable titres one had very low counts of *S.aureus*, perhaps insufficient to produce an immune response. Alternatively this patient may have been treating herself with an anti-infective agent which had not been documented. There was also no detectable titre from the psoriasis patient who had *S.aureus* on their skin, this could have been due to the thickness of the plaque and the inability of the organism to penetrate it. If the titres were due to CNS then again, as all of the patients sampled had these organisms on their skin, every serum sample should have had elevated titres. Another possible explanation of the high titres may have been the presence of cracked skin in the eczema and psoriasis patients with positive titres, as a result entry of any gram-positive organism present on the skin entering the bloodstream would cause an immune response. With regards to psoriasis, another organism, perhaps a beta-haemolytic streptococcus, an anaerobic or slow growing bacterium, could be responsible for the positive titres.

Despite there being a higher number of positives in the eczema patients, there was no significant difference between the mean IgG titres of the patients with eczema and psoriasis. In order to be able to use this test as an aid in the clinical diagnosis of exacerbation of these conditions, further work would be necessary on much larger patient groups.

5.0 CONCLUSIONS.

One of the characteristic features of psoriasis is red lesions. This is due to an increased blood supply which contains many inflammatory cells such as polymorphs and lymphocytes. The lymphocytes seem to be of particular importance as there is growing evidence that psoriasis is an immunological disease which requires the presentation of a specific antigen by a class II positive antigen-presenting cell (APC) to CD4 T-cells in the epidermis, in order to initiate the disease, as discussed in section 1.5.1.5.

As the skin flora of psoriatic skin in this investigation consisted of so many different species and genotypes of CNS, no particular strain was associated with the condition, thus one would conclude no specific antigen for initiation and or exacerbation of the disease.

In contrast, the very nature of eczematous skin in the respect that it is dry and flaky due to a deficiency of ceramides, a result of the abnormal metabolism of essential fatty acids particularly linoleic and linolenic acid, makes atopic skin less able to repel *S.aureus,* leading to high numbers in uninvolved skin as well as lesions. Unlike CNS, *S.aureus,* plays a definite role in the exacerbation of eczema in the respect that reduction in the carriage of this organism by antibiotics, leads to an improvement in the eczema, as superantigen production and the subsequent release of mediators and cytokines that cause inflammation are prevented.

In this study, the *S.aureus* strain for each given patient was a constant entity in that it could be reisolated from different areas of the body at different times, in contrast to the CNS from both eczematous and psoriatic skin. However, no common strain of *S.aureus* was found to be associated with atopic skin and what is more, none of the isolates appeared to be particularly virulent with regards to toxin production or enzyme activity.

The results from the novel lipid S ELISA were inconclusive but with further evaluation, may be useful, particularly for atopic eczema, as an aid as to whether to treat 'colonised' eczema or not.

6.0 SUGGESTIONS FOR FURTHER WORK.

(1) A more in depth investigation into the types and numbers of organisms found in the underlying layers of the epidermis in both eczema and psoriasis patients, by means of a punch biopsy. Especially anaerobic and slow-growing organisms, rather than just staphylococci.

(2) Further investigation of the virulence of *S.aureus* associated with atopic eczema, to look for a common factor to all isolates.

(3) Further developments in the ELISA to differentiate between IgG titres to *S.aureus* and coagulase-negative staphylococci, so as to confirm involvement of either, both or none of these organisms in the disease processes of eczema and psoriasis.

(4) Repeated sampling of blood from several eczema patients, over a fixed period of time, for example, every 6 months for two years, to look for differences in IgG titres. From this it may be possible to determine what bearing this may have on the exacerbation of eczema, that is, does a high IgG titre offer some protection to these patients from recurrent infections more especially due to *S.aureus*.

APPENDIX 1. Severity scoring for eczema and psoriasis.

ECZEMA.

The rule of 9's is used to calculate body surface area:

Head = 9
Upper extremity = 9
Body anterior = 18
Body posterior = 18
Lower extremity = 18
Genital area = 1

GRADING OF SEVERITY OF ATOPIC DERMATITS

1. EXTENT

(a) Childhood and adult phase

< 9% body area..... 1
Involvement >9% < 36%..... 2
>36% involvement..... 3

(b) Infantile phase

< 18% involvement..... 1
>18% <54% involvement..... 2
>54%..... 3

2. COURSE

> 3 months remission in a year..... 1
<3 months remission in a year..... 2
Continuous course..... 3

3. INTENSITY

Mild itch, only exceptionally disturbing sleep..... 1
Itch > score of 1 < score of 3..... 2
Severe itch, usually disturbing sleep..... 3

SCORE SUMMATION

3-4 = Mild

4.5-7.5 = Moderate

8-9 = Severe

Scoring of eczema patients used in this study.

PATIENT	AGE (YEARS)	DURATION OF ECZEMA (YEARS)	SCORE	SUMMATION
E1	53	51	8	Severe
E2	21	18	2	Mild
E3	24	23	3	Mild
E4	26	25	9	Severe
E5	49	6	7	Moderate
E6	27	26	4	Mild
E7	28	25	7	Moderate
E8	38	38	5	Moderate
E9	27	26	6	Moderate
E10	50	50	4	Mild

PASI SCORING FOR PSORIASIS.

PASI = 0.1 (Eh + Ih + Dh) Ah + 0.2 (Eu + Iu + Du) Au + 0.3 (Et + It + Dt) At + 0.4

(El + Il + Dl) Al

0.1 – 0.4 correspond to the four main body areas, that is, head, upper extremities, trunk and lower extremities which correspond to 10%, 20%, 30% and 40% body surface area respectively.
h = head, **u** = upper extremities, **t** = trunk, **l** = lower extremities

A = area affected by psoriasis: 0 = no involvement
1 = <10%
2 = 10 - < 30%
3 = 30 - <50%
4 = 50 - <70%
5 = 70 - <90%
6 = 90 - 100%

E, I and **D** are used to estimate severity of psoriasis where **E** = erythema
I = induration
D = desquamation

Each are given a scale of 0 – 4. Where 0 is complete lack of involvement and 4 is the severest involvement.

SCORE SUMMATION USED IN THIS STUDY

< 5 = MILD

6-20 = MODERATE

> 20 = SEVERE

Scoring of psoriasis patients used in this study.

PATIENT	AGE (YEARS)	DURATION OF PSORIASIS (YEARS)	PASI SCORE	SUMMATION
P1	71	50	4.5	Mild
P2	63	18	21.4	Severe
P3	37	19	8.8	Moderate
P4	19	7	No score	
P5	35	5	3.3	Mild
P6	46	10	1.8	Mild
P7	32	12-13	11.4	Moderate
P8	29	10	1.9	Mild
P9	62	54	0.6	Mild
P10	Not known	5	3	Mild

APPENDIX 2. Identification and antibiograms of all staphylococcal isolates from eczematous and psoriatic skin.

PATIENT	API STAPH 32 PROFILE NUMBER	IDENTIFICATION	METH	PEN 1	CIP 5	VANC 5	GENT 10	FUS 10	ERY 5	MUP 5	MUP 200	RIF 2	TRI 1.25
E1	367336610	*S.aureus*	S	S	S	S	S	S	S	S	S	S	S
	367336610	*S.aureus*	S	S	S	S	S	S	R	S	S	S	S
	261030200	*S.capitis*	S	R	S	S	S	S	S	S	S	S	S
	66760341	*S.kloosii*	S	S	S	S	S	S	R	S	S	S	S
	363334240	*S.warneri*	S	R	S	S	S	S	R	S	S	S	S
E2	163336610	*S.aureus*	S	R	R	S	S	S	S	S	S	S	S
	363336610	*S.aureus*	S	R	R	S	S	S	S	S	S	S	S
E3	363336610	*S.aureus*	S	R	R	S	S	S	S	S	S	S	S
	363332610	*S.aureus*	S	R	R	S	S	S	S	S	S	S	S
	367336610	*S.aureus*	S	R	S	S	S	R	S	S	S	S	S
	367336610	*S.aureus*	S	R	S	S	S	R	S	S	S	S	S
	367316610	*S.aureus*	S	R	S	S	S	R	S	S	S	S	S
	367336610	*S.aureus*	S	R	S	S	S	R	S	S	S	S	S
	367336610	*S.aureus*	S	R	S	S	S	R	S	S	S	S	S
	367336610	*S.aureus*	S	R	S	S	S	R	S	S	S	S	S
	367316610	*S.aureus*	S	R	S	S	S	R	S	S	S	S	S
	367336610	*S.aureus*	S	R	S	S	S	R	S	S	S	S	S
	363332240	*S.aureus*	S	R	S	S	S	S	R	S	S	S	S
	363032200	*S.epidermidis*	S	S	S	S	S	R	S	S	S	S	S
	263032200	*S.capitis*	S	S	S	S	S	R	S	S	S	S	S
	263032200	*S.capitis*	S	S	S	S	S	R	S	S	S	S	S
	263032200	*S.capitis*	S	S	S	S	S	R	S	S	S	S	S
	367230200	*S.capitis*	S	S	S	S	S	R	S	S	S	S	S
	263020200	*S.capitis*	S	S	S	S	S	R	S	S	S	S	S
E4	263330610	*S.aureus*	S	R	S	S	S	S	S	S	S	S	S
	160030000	*S.capitis*	S	R	S	S	S	R	R	S	S	S	S
	62032200	*S.epidermidis*	S	R	S	S	S	R	R	R	R	S	S
	62032200	*S.epidermidis*	S	R	S	S	S	R	R	R	R	S	S
	162230000	*S.capitis*	S	R	S	S	S	R	R	S	S	S	S

PATIENT	API STAPH 32 PROFILE NUMBER	IDENTIFICATION	METH	PEN 1	CIP 5	VANC 5	GENT 10	FUS 10	ERY 5	MUP 5	MUP 200	RIF 2	TRI 1.25
E5	367336610	*S.aureus*	S	S	S	S	S	S	R	S	S	S	S
	362032200	*S.epidermidis*	S	S	S	S	S	R	S	S	S	S	S
	367330610	*S.aureus*	S	S	S	S	S	S	R	S	S	S	S
	367330610	*S.aureus*	S	S	S	S	S	S	R	S	S	S	S
	366032200	*S.epidermidis*	R	R	S	S	S	S	R	S	S	S	S
E6	367336610	*S.aureus*	S	R	R	S	S	R	R	S	S	S	R
	363136600	*S.aureus*	S	R	R	S	S	R	R	S	S	S	R
	367230200	*S.capitis*	S	R	S	S	S	R	R	S	S	S	S
	367200200	*S.capitis*	S	R	S	S	S	R	R	S	S	S	S
	363336610	*S.aureus*	S	S	S	S	S	R	R	S	S	S	S
	363336600	*S.aureus*	S	R	S	S	S	S	R	S	S	S	S
	363376610	*S.aureus*	S	S	S	S	S	S	R	S	S	S	S
	363376610	*S.aureus*	S	R	S	S	S	S	R	S	S	S	S
	363336610	*S.aureus*	S	R	S	S	S	S	R	S	S	S	S
	367210300	*S.capitis*	S	R	S	S	S	S	R	S	S	S	S
	63300100	*S.cohnii*	S	R	S	S	S	R	S	S	S	S	S
	167030210	*S.epidermidis*	S	R	S	S	S	R	R	S	S	S	S
	22120200	*S.warneri*	S	R	S	S	S	R	S	S	S	S	S
	366010210	*S.epidermidis*	S	R	S	S	S	R	R	S	S	S	S
	62120500	*S.cohnii*	S	R	S	S	S	R	S	S	S	S	S
	367210200	*S.capitis*	S	R	S	S	S	R	R	S	S	S	S
	267010210	*S.epidermidis*	S	S	S	S	S	R	S	S	S	S	S
E7	367336600	*S.aureus*	S	R	S	S	S	S	S	S	S	S	S
	367336610	*S.aureus*	S	R	R	S	S	S	S	S	S	S	S
	466134600	*S.lugdunensis*	S	S	S	S	S	S	S	S	S	S	S
	367336610	*S.aureus*	S	R	R	S	S	S	S	S	S	S	S
	263030200	*S.capitis*	S	S	S	S	S	S	S	S	S	S	S

PATIENT	API STAPH 32 PROFILE NUMBER	IDENTIFICATION	METH	PEN 1	CIP 5	VANC 5	GENT 10	FUS 10	ERY 5	MUP 5	MUP 200	RIF 2	TRI 1.25
E8	367336600	*S.aureus*	S	R	R	S	S	R	R	S	S	S	S
	367230200	*S.capitis*	S	R	S	S	S	R	R	S	S	S	S
	36733660	*S.aureus*	S	R	R	S	S	R	S	S	S	S	S
	226134650	*S.haemolyticus*	S	R	R	S	S	R	R	S	S	S	S
	367230200	*S.capitis*	S	R	S	S	S	R	S	S	S	S	S
	366032200	*S.epidermidis*	S	R	S	S	S	S	R	R	R	S	S
	367376610	*S.aureus*	S	R	S	S	S	S	S	S	S	S	S
	363336710	*S.aureus*	S	R	S	S	S	S	S	S	S	S	S
	367336610	*S.aureus*	S	R	S	S	S	R	S	S	S	S	S
	367336610	*S.aureus*	S	R	S	S	S	S	S	S	S	S	S
	363336700	*S.aureus*	S	R	S	S	S	S	S	S	S	S	S
	367336610	*S.aureus*	S	R	S	S	S	S	S	S	S	S	S
	367336610	*S.aureus*	S	R	S	S	S	R	S	S	S	S	S
	267230200	*S.capitis*	S	R	S	S	S	R	S	S	S	S	S
	166030010	*S.epidermidis*	S	R	S	S	S	R	R	S	S	S	S
	366032210	*S.epidermidis*	S	S	S	S	S	S	S	S	S	S	S
	367230200	*S.capitis*	S	R	S	S	S	R	R	S	S	S	S
	362330350	*S.warneri*	S	R	S	S	S	S	S	S	S	S	S
	166032210	*S.epidermidis*	S	R	S	S	S	R	S	R	R	S	S
	367230200	*S.capitis*	S	R	S	S	S	R	R	S	S	S	S
E9	367336610	*S.aureus*	S	R	R	S	S	S	S	S	S	S	S
	263230200	*S.capitis*	S	R	S	S	S	R	S	S	S	S	S
	367316710	*S.aureus*	S	R	R	S	S	S	S	S	S	S	S
	62230200	*S.capitis*	S	R	S	S	S	R	S	S	S	S	S
	367332610	*S.aureus*	S	R	R	S	S	S	S	S	S	S	S
	366032200	*S.epidermidis*	S	R	S	S	S	S	R	R	R	S	S
E10	363336610	*S.aureus*	S	R	R	S	S	S	S	S	S	S	S
	363336210	*S.aureus*	S	R	R	S	S	S	S	S	S	S	S
	166022210	*Sepidermidis*	S	R	S	S	S	S	S	S	S	S	S

PATIENT	API STAPH 32 PROFILE NUMBER	IDENTIFICATION	METH	PEN 1	CIP 5	VANC 5	GENT 10	FUS 10	ERY 5	MUP 5	MUP 200	RIF 2	TRI 1.25
P1	266234610	*S.haemolyticus*	S	S	S	S	S	R	S	S	S	S	S
	345132210	*S.hominis*	S	R	S	S	S	S	S	S	S	S	S
	66230610	*S.hominis*	S	S	S	S	S	S	S	S	S	S	R
P2	363230200	*S.capitis*	S	S	S	S	S	S	S	S	S	S	S
	226334610	*S.haemolyticus*	S	R	S	S	S	S	S	S	S	S	S
	363232200	*S.capitis*	S	S	S	S	S	S	S	S	S	S	S
	367236600	*S.aureus*	S	R	S	S	S	S	S	S	S	S	S
	361030000	*S.capitis*	S	S	S	S	S	S	S	S	S	S	S
	467134600	*S.lugdunensis*	S	S	S	S	S	S	S	S	S	S	R
	367032200	*S.epidermidis*	S	R	S	S	S	S	S	S	S	S	S
	363230200	*S.capitis*	S	S	S	S	S	S	S	S	S	S	S
	363030200	*S.epidermidis*	S	S	S	S	S	S	S	S	S	S	S
	246034600	*S.haemolyticus*	S	S	S	S	S	S	S	S	S	S	S
	326334600	*S.haemolyticus*	S	R	S	S	S	S	S	S	S	S	S
	263200200	*S.capitis*	S	R	S	S	S	S	S	S	S	S	S
	63200200	*S.capitis*	S	S	S	S	S	S	S	S	S	S	S
	262304240	*S.haemolyticus*	S	S	S	S	S	S	S	S	S	S	S
P3	226334610	*S.haemolyticus*	S	R	S	S	S	S	S	S	S	S	S
	126030200	*S.epidermidis*	S	R	S	S	S	R	S	S	S	S	S
	226334611	*S.haemolyticus*	S	R	S	S	S	S	S	S	S	S	S
	362022200	*S.epidermidis*	S	R	S	S	S	S	S	S	S	S	S
	226334611	*S.haemolyticus*	S	R	S	S	S	S	S	S	S	S	S
	263030000	*S.capitis*	S	R	S	S	S	S	S	S	S	S	S
	263220200	*S.capitis*	S	R	S	S	S	S	S	S	S	S	S
P4	162032200	*S.epidermidis*	R	R	S	S	R	R	R	R	R	S	S
	61030200	*S.capitis*	S	R	S	S	S	R	S	S	S	S	S
	162032200	*S.epidermidis*	R	R	S	S	R	R	R	R	R	S	S

PATIENT	API STAPH 32 PROFILE NUMBER	IDENTIFICATION	METH	PEN 1	CIP 5	VANC 5	GENT 10	FUS 10	ERY 5	MUP 5	MUP 200	RIF 2	TRI 1.25
P5	161030000	*S.capitis*	S	S	S	S	S	S	S	S	S	S	S
	166030300	*S.epidermidis*	S	R	S	S	S	S	S	S	S	S	S
	260030000	*S.capitis*	S	R	S	S	S	S	S	S	S	S	S
	367326000	*S.caprae*	S	S	S	S	S	S	S	S	S	S	S
P6	367336610	*S.aureus*	S	R	S	S	S	S	S	S	S	S	R
	367332610	*S.aureus*	S	R	S	S	S	S	S	S	S	S	R
P7	166010200	*S.epidermidis*	S	S	S	S	S	S	R	R	R	S	S
	66360340	*S.kloosii*	R	R	S	S	R	S	R	S	S	S	R
P8	64020200	*S.capitis*	S	S	S	S	S	S	S	S	S	S	S
	162130000	*S.hominis*	S	S	S	S	S	S	S	S	S	S	R
P9	20030000	*S.capitis*	S	R	S	S	S	S	S	S	S	S	S
	60016200	*S.capitis*	S	S	S	S	S	S	S	S	S	S	S
	122022000	*S.epidermidis*	S	S	S	S	S	S	S	S	S	S	S
	363012200	*S.epidermidis*	S	S	S	S	S	S	S	S	S	S	S
	60010200	*S.capitis*	S	R	S	S	S	S	S	S	S	S	S
	463112610	*S.hominis*	S	S	S	S	S	S	S	S	S	S	S
	61020000	*S.capitis*	S	S	S	S	S	S	S	S	S	S	S
	163330240	*S.warneri*	S	R	S	S	S	S	R	S	S	S	S
	163330240	*S.warneri*	S	R	S	S	S	S	R	S	S	S	S
	362022210	*S.epidermidis*	S	S	S	S	S	S	S	S	S	S	S
	367032710	*S.hominis*	S	S	S	S	S	S	S	S	S	S	S
	163332240	*S.warneri*	S	R	S	S	S	S	R	S	S	S	S
	61020000	*S.capitis*	S	S	S	S	S	S	S	S	S	S	S
	367032710	*S.hominis*	S	S	S	S	S	S	S	S	S	S	S
KEY:	**E**=eczema	**P**=psoriasis											
	S=sensitive	**R**=resistant											
	Meth=methicillin	**Gent**=gentamicin											
	Pen=penicillin	**Fus**=fusidic acid											
	Cip=ciprofloxacin	**Ery**=erythromycin											
	Vanc=vancomycin	**Mup**=mupirocin											
	Rif=rifampicin	**Tri**=trimethoprim											

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