THE CHARACTERIZATION, ANTIBIOTIC SCEPTIBILITY AND PLASMID PROFILES OF COAGULASE-NEGATIVE STAPHYLOCOCCI IN LAGOS.

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, UNIVERSITY OF LAGOS IN FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D) IN THE DEPARTMENT OF MEDICAL MICROBIOLOGY AND PARASITOLOGY, COLLEGE OF MEDICINE OF THE UNIVERSITY OF LAGOS, LAGOS, NIGERIA.

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CERTIFICATION

This is to certify that the thesis: "THE CHARACTERISATION ANTIBIOTIC SUSCEPTIBILITY AND PLASMID PROFILES OF COAGULASE-NEGATIVE STAPHYLOCOCCI IN LAGOS"

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is a record of original research carried out

By
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in the Department of

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DECLARATION

We hereby declare that the thesis titled **THE CHARACTERIZATION, ANTIBIOTIC SUSCEPTIBILITY AND PLASMID PROFILES OF COAGULASE- NEGATIVE STAPHYLOCOCCI IN LAGOS** is a record of original research work carried out by **Catherine Chizoba Onubogu,** in the Department of Medical Microbiology and Parasitology, College of Medicine of the University of Lagos, Nigeria.

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DEDICATION

This project is dedicated to my husband and children for their love, patience and understanding.

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ABSTRACT

A total of 745 gram-positive, catalase-positive clustering cocci were obtained from various clinical specimens which included wound, blood, urine, catheter tips, high vaginal and endocervical swabs, urethral swabs, seminal fluids, bone secretions, eye and ear swabs. These isolates were obtained over an 18-month period from the Lagos University Teaching Hospital (LUTH), Idi-Araba, and General Hospital, Ikeja. Swab samples were also obtained from skin, hands, axilla and nose of an apparently normal population and compared with isolates from clinical specimens.

Isolation, and identification of coagulase-negative staphylococci (CoNS) were carried out using conventional methods. Characterisation to species level was done using both conventional method and the API rapid Commercial kit (ID 32 STAPH). The strains were tested for slime and beta-lactamase production. Other extracellular products tested for included DNase, lipase, protease, and gelatinase activities. Susceptibility to a range of antibiotics was tested and minimum inhibitory concentrations of some of these antibiotics were determined. Some of these coagulase-negative staphylococci were screened for the presence of plasmids.

A total of 244 isolates of coagulase-negative staphylococci were obtained, of which 241 were characterised to species level while 3 were unclassified. *Staphylococcus epidermidis* (109) was the most commonly isolated species from all the specimens. Comparing the conventional methods with the rapid commercial API kit (ID 32 STAPH), there was 98.8%

specificity for the former and 95.9% specificity for the latter. A total of 40.50% were slime producers. Over one quarter(28.69%) of CoNS species showed lipase activity with greatest activity being shown by *S. haemolyticus*(54.17%). About 11.81% and 11% showed DNase and gelatinase activity respectively while 8.86% showed proteolytic activity. Beta-lactamase was detected in 69.5% of coagulase-negative staphylococci isolates.

Majority of the isolates were multidrug-resistant to commonly used antibiotics. Some of the isolates harboured plasmids with molecular weights ranging between 0.76 to 13.5 kilobase.

Various findings reported in this study suggest that, coagulase-negative staphylococci can no longer be regarded as contaminants, but play a significant role in some infections in Lagos.

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GENERAL INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Staphylococci are gram-positive, non-motile, non-sporeforming clustering cocci measuring 0.5μm in diameter which can occur singly, as short chains of 3-4 cells or as grape-like clusters of cells. They are facultatively anaerobic and produce the enzyme catalase (Kloos and Jorgensen, 1985). Most strains grow well on non-inhibitory media. They are members of the family *Micrococcaceae*. The genus *Staphylococcus* is currently composed of 33 recognized species (Kloos and Bannerman, 1994; Kloos and Bannerman, 1995; Foster *et al.*, 1997) (Table 1.1). These species include those that are indigenous to humans and animals. Species indigenous to animals and birds may be found occasionally on humans, especially when recent contact has been made (Kloos and Bannerman, 1994). Most of these staphylococci species are common inhabitants of skin and mucous membranes. The largest populations of human staphylococci are usually found in regions of the skin and mucous membranes surrounding openings of the body surfaces (Kloos, 1986; Kloos and Musselwhite, 1975).Some *Staphylococcus* species and subspecies demonstrate a marked preference for certain habitats (Kloos, 1986; Kloos and Schleifer, 1983).

Originally, of all the staphylococci species, only *Staphylococcus aureus* was considered pathogenic and was delineated from other species of staphylococci on the basis of coagulase production, mannitol fermentation and the presence of protein A on the cell wall surfaces. All other species were grouped under the species designation *Staphylococcus albus* which was later changed to *Staphylococcus epidermidis* (Kloos and Jorgensen, 1985). These criteria are used primarily by research and reference laboratories. But in many laboratories the production of the coagulase enzyme is the only test used for the

identification of *Staphylococcus aureus*. Staphylococci which do not produce coagulase are collectively referred to as coagulase-negative staphylococci (CoNS) and are frequently not identified further because they are considered non-pathogenic. Thus coagulase production continues to be widely and generally accepted as the main criterion for determining the pathogenicity of particular isolates from humans and animals.

Results of preliminary studies by Baird-Parker (1963; 1965); Mitchell, (1968); Holt, (1969) and Pelzer *et al.*, (1973) showed that coagulase-negative staphylococci represent a heterogenous group and were sub-divided into biotypes based on a few simple biochemical characters. As a group, the coagulase-negative staphylococci species are among the most frequently isolated bacteria in the clinical microbiology laboratory (Patrick, 1990; Pfaller and Herwaldt, 1988). Although historically regarded as non-pathogenic, recent studies have demonstrated that CoNS may under appropriate circumstances, produce serious human disease. Coagulase-negative staphylococci are pathogenic when alterations in the integumen allow those normal skin inhabitants to gain entry into the body. Their roles as significant pathogens following ophthalmologic (Baum, 1978), neurologic (Schoenbaum *et al.*, 1975) and cardiothoracic (Houang *et al.*, 1986) surgeries, in patients with prosthetic devices (Baddour and Christensen, 1987; Hope *et al.*, 1989) and in immunocompromised patients (Winston *et al.*, 1983) have been well documented and established. The rising incidence of bacteraemia due to coagulase-negative staphylococci in association with the use of long-term in-dwelling central venous catheters has been of great concern. One of the major problems facing the laboratory is distinguishing clinically significant pathogenic strains of coagulase-negative staphylococci from contaminant strains (Kleeman *et al.*, 1993;

Souvenir *et al.*, 1998).

HISTORICAL BACKGROUND

Classification and Taxonomy of *Staphylococcus*

Classification is simply an orderly arrangement of bacteria into groups (Brenner, 1985) and it provides a broad understanding of relationships among different organisms. Such information has great practical value. In a clinical set up, the most immediate purpose of bacterial classification is the identification of pathogens. An appropriate classification system allows scientists to choose characteristics that will allow swift and accurate categorization of newly encountered organisms. However, the classification and taxonomy of the genus *Staphylococcus* have been under investigation for many years. In most cases, it showed the need to provide simple and expedient criteria to separate staphylococci from micrococci and important classes of staphylococci from one another. This influenced the course of taxonomic studies on staphylococci and helped in the recognition of *Staphylococcus aureus* but was not helpful in the classification of other species of *Staphylococcus* (Kloos et al., 1975).

Early Classifications and Concepts.

Several workers had observed cocci in abscesses, inflammations and pus. These cocci were called different names 'micrococci' by Recklinghausen (1871), *Microsporon septicum* by Klebb (1872) and *Monads* by Hueter (1872) as quoted by Baird-Parker, (1972). But Billroth (1874) classified the cocci on the basis of their cell arrangements into '*monococcos*', '*diplococcos*', '*streptococcos*', and '*gliacoccos*'. Billroth believed that all

round and rod-shaped forms of bacteria in purulent material were stages in the life-cycle of a single organism, *Coccobacteria septicum*, whereas others considered cocci to be harmless organisms.

Ogston, a Scottish surgeon in 1880 showed for the first time that a cluster-forming coccus was the cause of certain pyogenic abscesses in man, while at the same time, Louis Pasteur reached similar conclusions in France (Pasteur, 1880). Ogston (1882) named this pyogenic organism '*Staphylococcus*' deriving the name from the Greek nouns of 'staphyle' (a bunch of grapes) and 'coccus' (a grain or berry). He used this name for the cluster-forming cocci in order to distinguish them from Billroth's chain-forming streptococci which he found to cause disease symptoms quite different from those due to staphylococci. Ogston's work played a decisive role in settling the controversy over the role, that microorganisms played in human diseases and that there were specific agents for specific diseases at a time when the concept of bacterial pleomorphism was still actively promoted.

Rosenbach (1884) isolated organisms identical to the ones described by Ogston and was probably the first to isolate and grow staphylococci in pure culture and study their characteristics in the laboratory. He observed two types of colonies - yellow and white. Strains forming yellow colonies were called *Staphylococcus pyogenes aureus* while those forming white colonies were called *Staphylococcus pyogenes albus*. These were later changed to binomials - *Staphylococcus aureus* and *Staphylococcus albus*. This caused confusion among taxonomists. A third taxon, *Staphylococcus pyogenes citreus* which was later changed to *Staphylococcus citreus* was added for strains with lemon coloured colonies

(Passet, 1885).

Further confusion arose in the classification of *Staphylococci*. Workers like Flugge in 1890 placed *Staphylococcus* in the family *Coccaceae*, which was later divided by Winslow and Rogers (1906) into two sub-families, the *Metacoccaceae* and the *Paracoccaceae*. Winslow and Winslow (1908) as quoted by Baird-Parker (1972) placed the orange staphylococci in the genus *Aurococcus* and the white in the genus *Albococcus*. Both genera with streptococci were placed in the sub-family *Paracoccaceae* while micrococci with *Sarcina* and *Rhodococcus* were placed in the *Metacoccaceae*. These sub-familial names Metacoccaceae and Paracoccaceae were later replaced with *Micrococceae Trevisan* and *Streptococceae Trevisan* (Buchanan 1911). With the re-study of the genera *Aurococcus* and Albococcus of Winslow *et al.*, (1920), they concluded that the orange and white staphylococci were not distinct and that albococci were indeed strains of *S. aureus* that had lost some of the original characteristics of the species through growth under unfavourable conditions outside the human body.

These early workers regarded staphylococci and streptococci to be parasitic organisms growing well anaerobically while micrococci and sarcinas were aerobic saprophytes. According to Baird-Parker (1972) the Committee of the Society of American Bacteriologists on Characterization and Classification of bacterial types in 1920 accepted these differences put forward by these early workers. They recommended that *Staphylococcus* becomes a genus of Streptococcaceae with the following definition - "Parasites, cells that occur in groups and short chains and very rarely in packets. Generally are stained by Gram staining and give good growth of white or orange colonies on agar.

Glucose, maltose, sucrose and often lactose are fermented with the formation of moderate amounts of acid. Gelatin is often liquified very actively".

Workers like Hucker (1924) and Rahn (1929) from their studies concluded that micrococci and staphylococci were indistinct and therefore should be classified together in the same genera. Hucker in 1948 in the sixth edition of Bergey's Manual of Determinative Bacteriology relocated the staphylococci to the genus *Micrococcus* even though they had put them in the genus *Staphylococcus* in all its first to the fifth editions.

In the mid 1950's there was renewed interest in the classification of staphylococci and micrococci. A number of workers like Van Eseltine (1955), Thatcher and Simon (1957) believed that staphylococci and micrococci should not be separated. But Evan *et al.*, (1955) were of the opinion that staphylococci should be separated from micrococci on the basis of their ability to grow anaerobically and to form acid from glucose. These findings were accepted by Breed (1957) and included in the 7th edition of Bergey's Manual of Determinative Bacteriology when he revised the classification of the members of Micrococcaceae in 1957. These were confirmed by Baird-Parker (1963).

Thus in the eighth edition of Bergey's Manual (1974) the editorial board placed the genera *Staphylococcus, Micrococcus* and *Planococcus* in the family Micrococcaceae (Table 1.2) while the genus Aerococcus formally recognised as a member of Micrococcaceae by the International Committee on Systematic Bacteriology (ICSB)-Subcommittee on the taxonomy of staphylococci and micrococci (1976) was placed in the family of Streptococcaceae.

Generic affinities of *Staphylococcus.*

The family *Micrococcaceae* comprises of four groups of gram-positive, catalase positive cocci namely the genera *Micrococcus Staphylococcus, Planococcus* and *Stomatococcus.* Grouping these genera of Gram-positive cocci together has been traditional though controversial for many years (Baird-Parker, 1972).

The genus *Stomatococcus* on the other hand, has a single species, *Stomatococcus mucilaginosus* which was described by Bergan and Kocur (1982) for organisms previously classified as *Micrococcus mucilaginosus.* A description of the genus shows that strains of the species have the morphological characteristics of the family; cocci arranged in pairs, tetrads and clusters (Ruoff, 1995). They exhibit a high guanine plus cytosine (G+C) content of 56 to 60 mol%, near to that of the members of Micrococcus (64 to 75%) and unlike that of staphylococci (30 to 39 mol%), (Bascomb and Manafi, 1998). Strains of the species show a low catalase activity and a positive benzidine activity while their responses to the production of acid from a number of carbohydrates are similar to staphylococci. The organism *Stomatococcus* is resistant to lysostaphin while lack of growth on nutrient agar containing 5% sodium chloride (NaCl) helps to differentiate it from *Micrococcus* and *Staphylococcus* species (Bergan and Kocur, 1982). It is an oral commensal organism and has been associated with occasional opportunistic infections such as bacteraemia, endocarditis due to intravenous drug use and peritonitis (Courdron *et al.*, 1987; Prag *et al.*, 1995).

The members of the genus *Planococcus* are cocci arranged in pairs or tetrads and are motile. They are able to grow on media containing as much as 12% NaCl, a characteristic that also distinguishes them from the micrococci and staphylococci (Kocur, 1986). They are positive in the catalase and benzidine tests and have a G+C content of 39 to 52 mol% (Kocur, 1986). They have not been implicated in human infections but are associated with marine environments. (Kocur, 1986). They resemble *Micrococcus* species in being strict aerobes, lacking the ability to produce bacterial lytic agents such as lysostaphin, and showing resistance to lysostaphin (Varaldo *et al.*, 1982). The members of the genus *Micrococcus* occur mostly in pairs, tetrads and irregular clusters and differ from those of *Staphylococcus* by being obligate aerobes, with a G+C content of 63 to 73 mol%, containing cytochromes a,b,c, and d, lacking teichoic acids in their cell walls, and glycine in the interpeptide bridge of their cell walls. They are resistant to furazolidone and being susceptible to bacitracin (Varaldo *et al.*,1982). Organisms that fit the current description of micrococci are commonly encountered in routine laboratories either as environmental contaminants or as commensals from normal skin and only occasionally from infections.

Previously, the genus Micrococcus contained nine species, but recent studies of fatty acid and mycolic acid patterns, peptidoglycan type, and 16S rDNA analysis of the type strains of species of *Micrococcus, Stomatococcus, Arthrobacter* etc have shown that the genus is heterogenous. Thus the isolates previously identified as *Micrococcus* species have been placed in five different genera: *Dermacoccus, Kocuria, Kytococcus, Micrococcus* and *Nesterenkonia* (Stackebrandt *et al.*, 1995). The genus *Micrococcus* now contains only two species namely *M. luteus* and *M. lylae*.

SEPARATION OF STAPHYLOCOCCI FROM MICROCOCCI

The distinction between staphylococci and micrococci is important because some species of coagulase-negative staphylococci are important opportunistic and nosocomial pathogens whereas micrococci are considered harmless saprophytes. Thus, to identify coagulase-negative staphylococci, micrococci have to be excluded first. Since micrococci are coagulase-negative, several tests have been proposed including the ability of the genus *Staphylococcus* to produce acid from glucose under anaerobic conditions (Evans *et al.*, 1955). This was criticized (Baker, 1986) because this oxidation/fermentation test, proposed by the International Association of Microbiological Societies-Subcommittee on Taxonomy of staphylococci and micrococci (1965), does not always provide clear results.

Evans and Kloos (1972) then proposed a more reliable test based on the use of semi-solid thioglycollate medium to study the anaerobic utilization of glucose in differentiating staphylococci from micrococci, but, with this test strains of *Micrococcus kristinae* can still be misclassified as staphylococci.

Several other possibilities were put forward for differentiating micrococci from staphylococci. First is the difference in the base composition of their deoxyribonucleic acid. Members of the genus *Staphylococcus* possess a low guanine plus cytosine (G + C) content of about 30 to 38 mol% in their DNA, whereas members of the genus *Micrococcus* have a high G+C content of about 66 to 73 mol%,(Kocur *et al.*, 1971).

The second difference is based on the chemical composition of the cell walls as shown by their peptidoglycan composition (Schleifer and Kandler, 1972). It is distinguished by the high amounts of glycine in staphylococci while typical micrococci do not contain glycine in the interpeptide bridges of their peptidoglycans. Even when glycine is found at all as in the case of *Micrococcus luteus* it is linked to the X-carboxyl group of glutamic acid and only one mole of glycine per mole of glutamic acid is present (Schleifer and Kandler, 1972). Also teichoic acids are present in the cell walls of staphylococci while micrococci lack these polymers (Schleifer and Kloos, 1975).

Another characteristic difference between staphylococci and micrococci is their sensitivity to lysostaphin. While staphylococci are sensitive to lysostaphin because of the peptidoglycan composition, micrococci are resistant (Schleifer and Kloos, 1975). However, some staphylococci are slightly resistant to lysostaphin due to considerable amounts of serine in the interpeptide bridges.

The presence of aliphatic hydrocarbons in the neutral lipids of micrococci and not in staphylococci also distinguishes staphylococci from micrococci (Morrison *et al.*, 1971). Many differences occur also in the *menaquinone* patterns of both staphylococci and micrococci (Jeffries *et al.*, 1969).

Studies by Kloos and collaborators (1974) on Staphylococci and Micrococci from human skin showed that 939 strains of Staphylococci tested were able to produce acid aerobically from glycerol while only 38 strains out of 650 strains of micrococci produced acid from

glycerol. They also showed that growth of most micrococci were inhibited by erythromycin at low levels (<0.2μg/ml), while all of the staphylococci were resistant to at least 0.4μg/ml. They developed a medium containing both glycerol and erythromycin for rapid separation of micrococci and staphylococci.

Subsequently, they came up with a simplified scheme for the separation of staphylococci from micrococci by advocating that a test combining the lytic action of lysostaphin and the fermentation of glycerol in the presence of erythromycin should form the best practical scheme in the routine laboratory. Thus these strains were also tested for lysozyme sensitivity. Schleifer and Kloos (1975) also pointed out that the most reliable characters for the separation of staphylococci from micrococci are the DNA base composition and the cell wall composition but since these two characters cannot be used in routine laboratories, the simple test is used.

Presently, the main characteristics used for differentiating staphylococci from micrococci include glucose fermentation, acid production from glycerol, susceptibility to lysostaphin, susceptibility to lysozyme, modified oxidase and benzidine tests, susceptibility to furazolidone and susceptibility to bacitracin (Table 1.3) Pfaller and Herwaldt, 1988).

DIFFERENT CLASSIFICATION STANDARD SCHEMES

From the minimum scheme comprising only *Staphylococcus aureus* and *Staphylococcus albus*, Baird-Parker (1963, 1965) produced a scheme suggesting six biotypes of *Staphylococcus* with some subdivisions. Further schemes were proposed by Kloos and Schleifer, (1975), Subcommittee on Nomenclature of Staphylococci and Micrococci, (1976) and extended to a 47 biotype scheme by Marples (1981).

Baird-Parker Scheme:-

What was to become the widely accepted classification for *Staphylococcus* and *Micrococcus* was proposed by Baird-Parker. (1963; 1965)(Table 1.4a). He based his classification on a few relatively simple biochemical tests. These included production of acid aerobically from arabinose, lactose, maltose and mannitol and the production of coagulase, phosphatase and acetoin.

The genus *Staphylococcus* was divided into six groups numbered SI-SVI with subgroup SVI further subdivided into three types. The genus *Micrococcus* was divided into eight subgroups MI-M8. In the scheme the primary division between *Staphylococcus* and *Micrococcus* was made on the ability to ferment glucose under anaerobic conditions. Later studies on guanine-cytosine percentage composition of the DNA showed that at least the first four micrococcal subgroups were Staphylococci while M7 and M8 represented the genus *Micrococcus*. Baird-Parker amended his scheme so that the original scheme became:

subgroup I - *S. aureus*

SII - SVI - *S. epidermidis* biotype 1-4

MI - M4 - *S. saprophyticus* biotype 1-4.

Even though the Baird-Parker schemes were based on a few simple parameters to characterize coagulase-negative staphylococci, this provided an important landmark in the knowledge of coagulase-negative staphylococci and gave room for further taxonomic research. Baird-Parker's scheme remained the method of choice until that of Kloos and Schleifer (1975).

Kloos and Schleifer Scheme:-

Kloos and Schleifer and co-workers in their comprehensive systemic studies aimed at determining natural relationships characterized nine different coagulase-negative staphylococci species from human skin (Kloos and Schleifer, 1975; Schleifer and Kloos, 1975; Kloos *et al.*, 1974). They described alternative classifications for both micrococci and staphylococci based on similar but not identical biochemical tests (Table 1.5) leading to 11 subdivisions. Most of these subdivisions were named, viz *S. aureus; S. aureus sub sp; S. simulans; S. cohnii; S. saprophyticus; S. haemolyticus; S. warneri; S. hominis; S. epidermidis, S. xylosus* and *S. capitis*. They based their classification on colony diameter, cell-aggregation, anaerobic growth in thioglycollate medium, haemolysis of bovine blood, nitrate reduction, phosphatase activity, production of acetylmethylcarbinol (acetoin) and acid from a variety of carbohydrates under aerobic conditions - monosaccharides including fructose, galactose, mannose, xylose, arabinose, ribose; disaccharides including maltose, lactose, sucrose, trehalose, turanose, while the trisaccharides included melezitose and sugar alcohols such as mannitol and xylitol.

Novobiocin and lysostaphin susceptibility were included. This scheme has also been applied to the characterization of coagulase-negative staphylococci from human infections.

Scheme based on Subcommittee on Nomenclature of Staphylococci and Micrococci:

For clinical work, a scheme (Table 1.6) was proposed by the subcommittee on the Taxonomy of Staphylococci and Micrococci (1976) as a compromise for identifying *S. aureus, S. epidermidis* and *S. saprophyticus* since they are the species most often associated with human infections. This scheme is based on only six characters including novobiocin resistance.

Expanded Scheme by Marples (1981):-

Marples and his co-workers in order to allow for strains of Staphylococci that produce acid from mannitol, phosphatase and acetoin extended the classification of Baird-Parker to include 2 additional groups(Table 1.7. They studied 300 strains by the methods of Baird-Parker (1965) and that of Kloos and Schleifer (1975) because of difficulty in interpreting results by one method. The results indicated that some of the sub-divisions were concordant while others could be accommodated by minor redefinitions of the groups though some groupings could not be made compatible as shown:

Baird-Parker Subgroup I included:

Kloos & Schleifer's *S. aureus* and S. sp (*S. intermedius*)

SII included *S. epidermidis*

M3(1) included *S. saprophyticus*

MX included *S. Cohnii*

M8 included *Micrococcus roseus*

M5 & M6 included Subgroups of *S. xylosus*

SVI(3) and M3(3) were equivalent to *S. capitis*

SVIII equivalent to *S. simulans* with minor redefinition to allow for maltose + ve from animal and acetoin - ve from humans not identifiable by Baird-Parker scheme.

Kloos and Jorgensen Schemes:-

A scheme based on colony morphology, coagulase production, oxygen requirements, haemolysis on bovine blood agar, novobiocin resistance, acetylmethylcarbinol (acetoin) production, aerobic acid production from certain carbohydrates and certain enzymes (Table 1.8a) was proposed by Kloos and Jorgensen (1985). With this scheme 20 species of staphylococcus could be differentiated. They also proposed a scheme of minimum tests for the identification of the most clinically significant species (Table 1.8b). This method like most reference methods requires large numbers of biochemical tests.

Kloos and Lambe Schemes:-

The method for identification of staphylococcus described by Kloos and Lambe (1991) (Table 1.9a) was considered by many to be the standard for final identification of *Staphylococcus*. This method like most reference methods requires large numbers of biochemical tests along with degree of experience in interpreting the reactions. With this method 27 species of staphylococci could be identified. There are at least 13 human strains

of coagulase-negative staphylococci amongst them. This scheme was based on colony morphology, coagulase production, oxygen requirements, haemolysins, resistance to certain antibiotics, various enzyme activities and aerobic acid production from certain carbohydrates (Table 1.9a). Most clinically significant species can be identified with this scheme based on several key characteristics (Table 1.9b) Kloos and Lambe (1991).

Kloos and Bannerman Schemes:-

As new species of staphylococci were identified Kloos and Bannerman (1995) proposed a scheme based on a variety of conventional phenotypic characteristics (Table 1.10a). This scheme also requires large numbers of biochemical tests. Some of the tests may not be suitable for routine use in certain clinical laboratories due to a number of specialized media. Thus most of these tests are incorporated in the commercial kit identification systems by their manufacturers. For the most clinically significant species a scheme based on several key characteristics was also proposed by Kloos and Bannerman (1995) (Table 1.10b). With this scheme 32 species of staphylococci can be identified.

EPIDEMIOLOGICAL TYPING SYSTEMS

Staphylococci are ubiquitous in nature and it is sometimes desirable to separate strains of staphylococci into types or groups for epidemiological purposes. When a species that appears to be the cause of infection is a frequent or universal member of the normal flora, simple species identification is not useful in distinguishing between infection and colonization or in tracing the source of the infecting organism. The identification of strains of coagulase-negative staphylococci (CoNS) has become important since the recognition of

the clinical significance of CoNS (Kloos and Bannerman, 1994). This is also important in distinguishing epidemiologically significant isolates from unrelated isolates of the same species especially in nosocomial outbreaks. Thus the identification of strains is necessary for monitoring the reservoir and distribution of CoNS involved in nosocomial infections and in determining the aetiologic agent.

Several different epidemiological typing methods have been applied in the study of coagulase-negative staphylococci. These techniques include colony morphology, biochemical reactions, (biotyping), antimicrobial susceptibility patterns (antibiograms), bacteriophage typing (phage typing), serological typing and molecular typing (plasmid profiles, restriction enzyme fragment patterns of plasmids and chromosomal DNA). Each of these techniques has practical limitations. The identification of strains should include at least two or more of the typing techniques for a reasonable and accurate determination (Kloos and Lambe, 1991, Pfaller and Herwaldt, 1988). Thus the information gained from the studies using useful typing methods will help in the increased understanding of the pathogenesis of coagulase-negative staphylococci. This will result in improved methods of prevention, diagnosis and therapy.

Colony Morphology:-

Colony morphology can be a very useful characteristic in the identification of species and strains especially when time is available. In this method well-isolated colonies are allowed to develop over a period of several days on a suitable agar medium at incubation temperatures of 34 to 35°C, followed by 2 days incubation at room temperature (Kloos,

1990; Kloos and Lambe, 1991). For most species more than 90% of strains can be differentiated after 72 hours (Kloos and Bannerman, 1994). Colonies of the same strain show similar features of size consistency, edge, profile, lustre and colour. This technique works best on those CoNS species that demonstrate a translucent colony type and pigments variation, for example, *S. chromogenes,* and *S. arlettae.* Certain strains may exhibit variant morphotypes and this if found would be misclassified as a different strain (Kloos and Bannerman, 1995).

Antibiogram (Antimicrobial Susceptibility Profile):-

This has been used to differentiate between strains of various organisms including clinical isolates of coagulase-negative staphy-lococci (Parisi, 1985; Birnabaum *et al.*, 1991). Antimicrobial susceptibility is frequently used by clinicians to identify clinically significant strains of *S. epidermidis* and other coagulase-negative staphylococci. This is because it is routinely performed in clinical microbiology laboratories using standardized procedures. Thus antibiograms can be used epidemiologically because the presence of a strain with a unique antibiogram can provide a marker for detecting similar strains (Pfaller and Herwaldt, 1988).

However, it has its limitations as a means of typing coagulase-negative staphylococci. The major problems with the method are multiple antibiotic resistance of nosocomial isolates of coagulase-negative staphylococci (Parisi, 1985; Christensen, *et al.*, 1983). Variation in the susceptibility testing method may result in day to day variation in the antibiogram (Parisi, 1985; Christensen, *et al.*, 1983). Instability in the expression of antibiotic resistance in

some strains of coagulase-negative staphylococci (Mickelsen *et al.*, 1985) and scope of antibiotic use within a locality varies so that results may vary from community to community (Kloos and Lambe, 1991). Christensen and co-workers (1983) analysed the different typing system and indicated that antibiogram alone could not reliably categorize two strains of coagulase-negative staphylococci as the same or different strain.

Biotyping:-

Biotyping or biochemical profile has frequently been used alone or in combination with the antibiogram as a means of distinguishing strains of coagulase-negative staphylococci (Christensen *et al.*, 1983; Mickelsen *et al.*, 1985). Several biotyping systems had been proposed for both taxonomic work and epidemiologic purposes prior to the taxonomic work of Kloos and Schleifer (1975) and development of the rapid commercial kit (Marples, 1986; Parisi, 1985). These biotyping systems were based on numerous biochemical and physiological tests of which only those of Baird-Parker (1963) and Bentley and co- workers (1968) received much attention. However, the list of characteristics used for staphylococcal strain and species identification has grown over the years (Kloos and Bannerman 1995). A combination of these biochemical characteristics known to be variable within species can aid in strain differentiation. But most tests require special media, techniques and at times instrumentation and hence these tests would be better performed in a reference laboratory. Groups have been working on a typing scheme that uses a combination of several techniques (Hebert, 1989, Herwaldt *et al.*, 1991, Geary *et al.*, 1997). They were able to show that no single method proved entirely satisfactory on all occasions.

Several manufacturers have developed rapid species and subspecies identification kits, automated systems requiring a few hours to 24 hours for completing tests (Pfaller and Herwaldt, 1988; Bascomb and Manafi, 1998). With the widespread availability of these rapid miniaturized commercial identification systems/kits many investigators have tried to use the biochemical profiles generated in the identification process as epidemiological markers (Mickelsen *et al.*, 1985, Parisi *et al.*, 1986). Investigators have also reported some limitations to the use of these systems alone in epidemiologic studies (Parisi, 1985; Parisi *et al.*, 1986). For instance, Mickelsen *et al.*, (1985) found that biotypes obtained with Staph-Ident system were not reproducible because each of the 5 isolates of *Staphylococcus epidermidis* gave a different biocode when tested on two different occassions.

Serological Typing:-

Serotyping for the purpose of identifying strains of *S. aureus* began with the work of Cowan in 1939 (Cohen, 1972) who identified strains by slide agglutination with absorbed antisera. It has been shown to be a useful epidemiological tool in the investigation of a variety of infectious diseases but has not been well developed for use with coagulase-negative staphylococci (Aasen and Oeding, 1971; Tierno and Stotzky, 1978). This is because of difficulties encountered in the preparation of specific antisera and the standardization of typing methods. Studies have shown that although, *S. aureus* and *S. epidermidis* share several group antigens but cross absorption studies have demonstrated that *S. epidermidis* has its own set of type agglutinogens (Schleifer, 1986). This information has helped in the development of typing sera and serotyping in conjunction with biotyping in a number of

studies. It has only been applied successfully in a limited number of studies (Schleifer, 1986; Tierno and Stotzky, 1978) because of difficulties in preparing specific antisera and standardization of methods. Serotyping does not appear to have much role in epidemiological studies of these organisms.

Bacteriophage Typing (Phage Typing):-

This is the most established system for epidemiological typing of Staphylococci. Since 1952, this technique has had widespread use. This led to the establishment of an international subcommittee on phage typing of staphylococci of the International Committee on Nomenclature of Bacteria (Kloos and Jorgensen, 1985). The Committee's functions were several, including standardizing the procedure for phage typing, maintaining standardized stocks of typing phages and their propagating strains. With the emergence of new strains of staphylococci that were nontypable, the bacteriophages used in the standard typing set have varied over an extended period of years. *S. aureus* is the *Staphylococcus* species most commonly typed and thus has a set of typing phages. When these typing phages isolated from *S. aureus* were applied to *S. epidermidis* this organism was rarely lysed (Parisi, 1985; Ferreiros *et al.*, 1991). Presently there are four major phage-typing sets for coagulase-negative staphylococci. These include those by Van Boven *et al.*, (1969); Pulverer *et al.*, (1973) and Dean *et al.*, (1973). These typing sets have been widely used for strain identification either alone or in combination with other methods with different degrees of success (Pereira and Melo-Cristino, 1991; Rosdahl *et al.*, 1990).

For a phage to be useful in epidemiological studies it must possess 3 characteristics (Parisi, 1985) namely:

i) It should type or lyse a large proportion of the strains.

ii) It should be reproducible; different isolates derived from the same bacterial clone should give identical reactions.

iii) It should discriminate between epidemiologically related strains from unrelated strains.

Studies carried out showed that none of the typing systems could satisfy all the parameters. Despite these problems, phage typing has been used successfully to type coagulase-negative staphylococci especially in epidemic situation (Parisi *et al.*, 1986). Christensen *et al.*, (1983) suggested that phage typing of coagulase-negative staphylococci is better reserved for epidemic rather than endemic situations. They demonstrated that phage typing alone was a poor strain discriminator in a non-epidemic situation but when combined with the antibiogram and biotyping it provided a good discrimination between strains.

Molecular typing

The molecular typing which includes plasmid pattern analysis, restriction endonuclease analysis of plasmid and chromosomal DNA, is being employed as means of identifying strains or a clonal population of staphylococci (Hawkey, 1987; Wachsmuth, 1986; Geary, 1997). Plasmid composition and restriction endonuclease analysis of specific plasmids can serve as a valuable molecular typing system especially for those strains of coagulase-negative staphylococci carrying multiple plasmids (Archer *et al.*, 1985; Parisi and Hecht,

1980; Kloos and Bannerman, 1985). A high percentage of CoNS especially *S. epidermidis* carry several different plasmids with each strain having a characteristic profile made up of its distinct plasmids (Archer *et al.*, 1984; Mickelsen *et al.*, 1985). In most staphylococcal species there is a relationship between antibiotic resistance pattern and the presence of certain plasmids carrying resistance genes (Kloos and Lambe, 1995). Thus, restriction endonuclease fragment analysis of plasmids is particularly useful in differentiating plasmids of the same size. However, some common plasmids are highly conserved and often have identical fragment patterns irrespective of the strain as in the small tetracycline plasmids and the small macrolide-lincosamide-streptogramin B (MLS) resistance plasmids (Kloos and Bannerman, 1994). Reviews have given the advantages and disadvantages of plasmid profiling (Pfaller and Herwaldt, 1988; Pfaller and Hollis, 1989). One such major disadvantage is that plasmids are sometimes unstable and may lose antibiotic resistance plasmids or cryptic plasmids. They suggested that although plasmid pattern analysis is a very powerful epidemiological tool but a combination with other typing methods will provide the most useful epidemiologic data.

Other Typing Methods:-

Other molecular typing methods which examine complex characteristics such as cellular fatty acid (CFA) analysis, multilocus enzymes electrophoretic analysis, pyrolysis-mass spectrometry, whole-cell polypeptide analysis of CoNS are being employed and reviewed for CoNS strain identification. Cellular fatty acid analysis although is relatively inexpensive, simple, quick and a large number of isolates can be tested at one time but has received little attention. This is because of the need to standardize substrates and growth

conditions in order to obtain reproducible results (Welch, 1991; Kloos and Bannerman, 1994). Multilocus enzyme electrophoresis which is based on analysis of the electrophoretic profiles of isoenzymes (Pfaller, 1992) and whole-cell polypeptide analysis of cellular proteins (Clink and Pennington, 1987) are currently being examined for strains identification of CoNS (Kloos and Bannerman, 1995). Pyrolysis-mass spectrometry in which organisms are pyrolyzed and the pyrolysates examined by mass spectrometry has given encouraging results in strain discrimination of CoNS (Freeman *et al.*, 1991). However, Kloos and Bannerman (1994) stated that it is necessary to include epidemiologically unrelated control strains so that the significance of the different spectrograms of the strains can be estimated. DNA-DNA hybridisation studies formed one of the defining criteria for the current phylogeny of CoNS species (Schleifer and Kroppenstedt, 1990). Molecular typing techniques aimed at examining the chromosomes of staphylococci have shown considerable promise in identifying strains or specific lineages. Chromosomal DNA analysis by restriction endonuclease finger printing has been used to type various pathogens including coagulase-negative staphylococci (Bialkowska-Hobrzanska *et al.*, 1990; Wilton *et al.*, 1992). Even though the technique was more discriminatory than plasmid profiling with excellent reproducibility, this techniques lacks standardization and the banding patterns are often difficult to analyze because of the large number of fragments generated by the restriction enzymes. Recent approaches to chromosomal analysis which include ribotyping and field inversion gel electrophoresis (FIGE) or pulsed-field gel electrophoresis (PFGE) have been used in the identification of the different CoNS strains (Thomas-Carter *et al.*, 1989; Izard *et al.*, 1992; Goering and Duensing, 1990; Bannerman *et al.*, 1997). These recent approaches have shown promise in

the identification of different strains of CoNS. Further studies of different CoNS species with the use of different enzymes are needed to determine how well FIFE and PFGE will discriminate among strains of CoNS.

EPIDEMIOLOGY OF COAGULASE-NEGATIVE STAPHYLOCOCCAL INFECTIONS

Infections due to coagulase-negative staphylococci have several common features and are usually nosocomial in origin (Hamory and Parisi, 1987, Jarvis and Martone, 1992, Grosserode and Wenzel, 1991). Coagulase-negative staphylococci (CoNS) are prevalent on the skin and with the frequent implantation of foreign devices into patients during hospitalization, CoNS are ideally situated to cause infections in these patients (Pfaller and Herwaldt, 1988). Colonization of foreign bodies by CoNS often leads to serious local and systemic disease. Contact spread has assumed added importance in hospitals where a large proportion of the staff and patients carry CoNS species and antibiotic resistant strains in the nose or on the skin etc (Narayani *et al.*, 1990). In hospitals the areas at highest risk for severe CoNS infections are the new-born nurseries, intensive care units, operating rooms and cancer chemotherapy wards (Valles *et al.*, 1997, Baumgart *et al.*, 1983; Winston *et al.*, 1983).

CoNS also cause infection when there are massive insults to host defences as in the outbreak of CoNS infection associated with extra-corporeal-circulation machinery described by Lathrop and co-workers (1978). Holt (1971) observed that patients with shunt infections show no white-cell reaction in liquor and in blood and the body seems to be

unaware of the massive CoNS invasion.

Several attempts have been made at the identification of the reservoirs and the site of origin of the strains of CoNS causing nosocomial infections (Jarvis and Martone, 1992, Scaberg *et al.*, 1991, Eisenberg *et al.*, 1987, Cheesbrough *et al.*, 1986). But inadequacies of epidemiologic typing systems and presence of multiple strains or several biotypes on each individual (Eisenberg et al., 1987, Parisi, 1985) have hampered some of these attempts. It is generally believed that colonization of the individual patient's skin by CoNS are the usual cause of CoNS staphylococcal bacteraemia and prosthetic device infections (Eisenberg *et al.*, 1987, Hamory and Parisi, 1987, Karchmer *et al.*, 1983). However, Wade and co-workers (1982) showed evidence to support the gastrointestinal tract as an important source for coagulase-negative staphylococcal bacteraemia. Reports have identified respiratory and genitourinary tracts among other sources (Weinstein *et al.*, 1997). Studies have also shown documented evidence for the transmission of CoNS from hospital staff to patients (Parisi, 1986, Burnie *et al.*, 1997). Epidemiological and microbiological studies have suggested that coagulase-negative staphylococcal infections of cardiac, or orthopaedic prostheses and surgeries may be initiated during surgery with the patient's flora or that of the surgeon or through the environment (Bannerman *et al.*, 1997, Clarke, 1979, Kluge *et al.*, 1974). Maderazo and co-workers (1988) in review of haematogenously acquired infection reported that 13% of the cases were caused by coagulase-negative staphylococci.

Although CoNS are generally considered to be of low virulence, the incidence of serious infections caused by these organisms has seriously increased in recent years (Pfaller and

Herwaldt, 1988, Kloos and Bannerman, 1994). The reported crude mortality rates associated with coagulase-negative staphylococcal bacteraemia are quite high ranging from 27 to 78% (Pfaller and Herwaldt, 1988). Freeman and collaborators (1987) in their study in neonatal intensive care unit (1975-1982) emphasized the need for controlled studies in which the incidence and risk factors for CoNS infections in hospitalized patients will be evaluated.

One major problem in the prevention and diagnosis of coagulase-negative staphylococcal infections in hospitalized patients is the difficulty in differentiating infection from colonization. The clinical signs and symptoms associated with both local and systemic infections are well known but are not specific and may be masked in critically ill patients. Thus, it is important to distinguish between mortality attributable to the nosocomial coagulase-negative staphylococcal bacteraemia from that due to underlying disease.

CLINICAL SIGNIFICANCE OF COAGULASE-NEGATIVE STAPHYLOCOCCI

Infections with coagulase-negative staphylococci have been reported since 1950 with increasing frequency. In 1958, Smith and co-workers noted for the first time the potential pathogenicity of CoNS in a review where he described a total of 90 cases of coagulase-negative staphylococcal septicaemia reported in the literature between 1900 and 1955. Several years later Pulverer and Halswick (1967) noted also the potential pathogencity of CoNS by reporting on 128 cases of endocarditis believed to be caused by CoNS in a paper he wrote in 1965. Quin and co-workers (1965) proposed the re-assessment of CoNS as non-pathogen since *Staphylococcus epidermidis* has been recognised in various clinical

situation. Cluff and co-workers (1968) found out that 10% of all cases of staphylococcal bacteraemia were due to *S. epidermidis*. Sapira, (1968) reported natural valve CoNS endocarditis in intravenous drug abusers. Black and co-workers (1965) noted the occurrence of nephrotic syndrome in patients with ventriculoatrial shunts that were infected with CoNS. Since this report, this association has been recorded many times (Lam *et al.*, 1969, Fokes, 1970). Dobrin and co-workers (1976) reported the biologic consequences of complement activation by complexes localised in the glomeruli of patients with chronic *S. epidermidis* bacteraemia in relation to pathogenesis of renal injury.

For many of these early studies concerning the aetiology of CoNS infections sound methodologies were not available for repeated or pure cultures determination. Hence, Kloos and Bannerman (1994) highlighted that conclusions with regards to the involvement of CoNS in infections then should be made with some caution. Due to advances in staphylococcal systematics and epidemiological typing methods the range of infections believed to be caused by CoNS especially *Staphylococcus epidermidis* was quite wide by the 1980's and 1990's. These included bacteraemia (Baumgart *et al.*, 1983, Ponce de leon *et al.*, 1986, Martin *et al.*, 1989, Spanik *et al.*, 1997); native valve endocarditis (NVE) and prosthetic valve endocarditis (Archer *et al.*, 1980; Baddour *et al.*, 1986; Richardson *et al.*, 1978); peritonitis during continuous ambulatory dialysis (Rubin *et al.*, 1980; Kraus and Spector, 1983). Other cardiac infections caused by *S. epidermidis* after cardiovascular surgery include mediastinitis (Bor *et al.*, 1983); infections of permanent pacemakers (Choo *et al.*, 1981); vascular grafts infections and intravenous catheters (Peters *et al.*, 1982; Peters and Pulverer, 1984). *S. epidermidis* has been associated with joint infections, in which

there were no predisposing factors (Morris *et al.*, 1986) and in infections of a variety of orthopaedic devices (Clarke, 1979). It accounted for almost 40% of all prosthetic joint infections (Brause, 1986) and have been implicated in pyoarthritis (Males *et al.*, 1985) and osteomyelitis (Paley *et al.*, 1986). *S. epidermidis* is a common isolate from urinary tract infections such as cystitis, urethritis, prostatitis and pyelonephritis (Leighton and Little, 1986; Wedren, 1987) and has been the primary pathogen in infections associated with cerebrospinal fluid shunt recipients (George *et al.*, 1979). Oyedeji and Babalola (1999) reported on the prevalence of *S. epidermidis* amongst CoNS isolates from neonatal conjunctivitis patients in Ile-Ife. *S. epidermidis* was also encountered in cervical adenitis (Ray-Poir and Patrick, 1993; Deighton *et al.*, 1992).

Reports showed that in children the most important infection occurs in hydrocephalic patients that have had Spitz-Holter valves implanted for the purpose of shunting spinal fluid to superior vena cava of right atrium (Callaghan *et al.*, 1961; Odio *et al.*, 1984). *Staphylococcus epidermidis* bacteraemia also occurs in neonates as a result of implanted valve (Noel and Edelson, 1984) while Patrick *et al.*, (1989) reported on persistent bacteraemia due to CoNS in low birth weight neonates without implants. Other reports on neonates include epidemic conjunctivitis in newborns caused by CoNS (Hurley, 1966). Feigin and co-workers (1973) assessed the role of *S. epidermidis* in otitis media. Several other coagulase-negative staphylococci species have been implicated in a variety of infections. These include *Staphylococcus saprophyticus* which was often regarded as a more important opportunistic pathogen than *S. epidermidis* in urinary tract infections especially in young and sexually active females causing acute cystitis or pyelonephritis in

these patients (Anderson *et al.*, 1981, Marrie *et al.*, 1982, Lee *et al.*, 1987). Hovelius and co-workers (1979) reported on the implication of *S. saprophyticus* in the aetiology of nongonococcal urethritis in men attending a venereal clinic. Other coagulase-negative staphylococci implicated and encountered also in a variety of clinical infections such as native valve endocarditis (NVE), wounds and bone infections, septicaemia, vertebral osteomyelitis, endocarditis, include *Staphylococcus haemolyticus* (Caputo *et al.*, 1987, Gruer *et al.*, 1984); *Staphylococcus warneri* (Kamath *et al.*, 1992, Karthigasu *et al.*, 1986); *Staphylococcus simulans* (Males *et al.*, 1985), *Staphylococcus lugdunesis* (Ludlam and Phillips, 1989, Shuttleworth and Colby 1992), *Staphylococcus capitis* (Lina *et al.*, 1992), *Staphylococcus schleiferi* (Freney *et al.*, 1988), *Staphylococcus hominis* (Bowman and Buch, 1984).

PATHOGENESIS OF COAGULASE-NEGATIVE STAPHYLOCOCCAL INFECTIONS

Coagulase-negative staphylococci are by now widely acknowledged to have the potential to cause human diseases under certain conditions. Initially the pathogenicity of these organisms was not well defined and their recovery from lesions with external drainage might have had little clinical significance. But the emerging role of coagulase-negative staphylococci as a major cause of foreign body infections and important nosocomial pathogens has prompted increased interest in the pathogenesis of coagulase-negative staphylococcal infections. In general, actual state of host resistance (host-factors) and virulence of the infectious agent (Microbial factor) are the factors determining the outcome of an infectious process.

Host Factors:

The host factors which often lead to serious infections with coagulase-negative staphylococci are well characterized. These factors include:

i. Breaches in natural mucocutaneous barriers due to trauma or inflammation (Kaiser, 1986, Woods *et al.*, 1986).

ii. Prior exposure to antibiotics. (Powell and Sanderson, 1987; Schwalbe *et al.*, 1987).

iii. Immunosuppression (Winston et al., 1983, Peterson *et al.*,1987).

Other high risk factors include combination of mucosal damage, granulocytopenia and concurrent colonization of the alimentary tract by coagulase-negative staphylococci as seen in patients with cancer and granulocytopenia (Hutton *et al.*, 1985, Wade *et al.*, 1982). Studies indicate that either systemic or localized opsonic deficiency may be a risk factor for coagulase-negative staphylococcal infection in neonates (Fleer *et al.*, 1985) and in continuous ambulatory peritoneal dialysis (CAPD) patients (Peterson *et al.*, 1987).

Implantation of foreign bodies:

Host defects are clearly important in the pathogenesis of coagulase-negative staphylococcal infections but another factor contributing to the increase in the number of nosocomial coagulase-negative staphylococcal infections is the presence of indwelling prosthetic devices in both compromised and non-compromised hosts. The frequent implantation of foreign materials in the form of prosthesis or devices as with indwelling intravenous catheters, cardiac valve prosthesis, prosthetic orthopaedic devices, cerebrospinal fluid

shunts, ventriculostomy drains, peritoneal dialysis catheters and transvenous cardiac pacemakers into the human body has created a range of new infection problem. Patients with such prostheses can reasonably be classified as compromised hosts. The prostheses provide unusual ecological niches with formation of an adherent biofilm with no capillary blood supply. As a result of this, the microorganisms can establish themselves virtually out of the reach of the host's cellular and humoral defences (Gristina, 1987).

The mechanism by which coagulase-negative staphylococci gain access to the surfaces of the various prosthetic devices currently is a matter of debate and intense investigation. The source of contaminating organism may be either endogenous from skin or mucosal (pulmonary or alimentary) surfaces or exogenous from the hospital environment or hospital personnel. Some studies have postulated possible routes of entry for the introduction of coagulase-negative staphylococci onto the surfaces of prosthetic devices and catheters with subsequent entry into the blood stream or other normally sterile sites. These may be:-

i. via the catheter tunnel and the exterior of the catheter (Cheesbrough *et al.*, 1986).

ii. through the catheter lumen as a result of frequent disconnections as well as the exposure of the hub to the skin of the patient and healthcare personnel performing the catheterization leading to colonisation of the hub (Cheesbrough *et al.*, 1986; Gahrn-Hansen, 1987).

iii. via surgical introduction of catheters, shunts, cardiac or orthopaedic prostheses (Burke, 1986; Karchmer *et al.*, 1983).

iv. from an endogenous source into the blood stream or normally sterile site (Burke, 1986).

Staphylococcus epidermidis is the species that most frequently colonizes such inserts. They may therefore cause meningitis or septicaemia (Schoenbaum *et al.*, 1975) relatively subacute in onset. Early-onset infections occur within several days or weeks after surgery or catheterization.

Microbial Factors

Apart from the presence of a foreign body which may facilitate infection by coagulase-negative staphylococci these organisms possess some microbial factors which help them to survive on a variety of biomaterials (mainly synthetic polymers). Studies on the pathogenesis of coagulase-negative staphylococci suggest that once these organisms overcome the normal cutaneous or mucocutaneous barriers, the process of adherence, colonization and infection follow in a sequential manner (Gristina, 1987). Specific adhesion of *S. epidermidis* RP-62A to silastic catheter surfaces can be mediated by a capsular polysaccharide-adhesin (PS/A) (Tojo *et al.*, 1988). PS/A appears to enhance the very early stages of colonization of biomaterials (Kloos and Bannerman, 1994). The ability to adhere and colonize the smooth surfaces of these prosthetic devices appears to be related to virulence. Studies have shown that once they attach to the surface of the implant a viscous extracellular polysaccharide substance or slime is produced. This slime material gives additional adhesin binding to the surface of the implant, further consolidating adhesion, aggregation and microcolony formation (Gristina, 1987; Peters, 1986). Extensive

production of slime (extracellular slime substance (ESS) by staphylococci especially S. epidermidis by Scanning electron microscopic studies shows encased multiple layers of bacteria forming a biofilm (Peters *et al.*, 1982).

This biofilm produced by the slime on the prosthetic device creates a protective micro environment (Peterson *et al.*, 1987). Slime may interfere biochemically with coagulation (Bykowska *et al.*, 1985). Recent studies have confirmed the importance of slime production for human infections. It has been shown that eradication of infection is more difficult when a slime-positive organism is involved (Kristenson *et al.*, 1986). Thus, slime production is an important factor in the colonization and infection of prosthetic devices. There is growing evidence that ESS interferes with host defense mechanisms in addition to its role in the formation of biofilm (Gray *et al.*, 1984, Gray *et al.*, 1987, Stout *et al.*, 1992).

Apart from slime production, coagulase-negative staphylococci have been shown to produce a variety of potential toxins including haemolysins, cytotoxins, deoxyribonuclease, fibrinolysin, proteinase and lipase-esterase (Gemmell, 1986; Gemmell and Schumacher-Perdreau, 1986). Strong similarities have been noted between the exoproteins produced by *S. aureus, S. epidermidis, S. haemolyticus* and *S. saprophyticus* showing that they may be important virulent factors in human infections.

Multiple antimicrobial resistance of coagulase-negative staphylococci plays a role in the pathogenesis of coagulase-negative staphylococcal nosocomial infections. Majority of the hospital strains are resistant to multiple antibiotics. Antimicrobial prescribing and usage

practices may select for multi-resistant CoNS which colonize patients and staff who then serve as a hospital reservoir for antibiotic-resistance CoNS (Archer, 1978, Christensen *et al.*, 1982a; Hamilton-Miller and Iliffe, 1985). Reports have shown that they often prove to be quite antibiotic resistant (Moller, 1988 and Aggarwal, 1991) and constitute a problem to the clinicians. However, there are few reports on the role of coagulase-negative staphylococci in infections in this environment.

OBJECTIVES OF THE STUDY

This study was designed to determine the aetiological role of various coagulase-negative staphylococci in the causation of disease in Lagos and their biological properties in order to guide medical and laboratory professionals as to the role of coagulase-negative staphylococci in clinical specimens and thus guide clinicians in the treatment of infections.

The specific objectives were:

1. To isolate, identify and characterise coagulase-negative staphylococci from various clinical specimens in our environment - Lagos.
2. To detect the possession of some pathogenic markers -enzymes, extracellular products.
3. To establish the sensitivity patterns of the species of coagulase-negative staphylococci isolated to antimicrobial agents.
4. To determine the plasmid profiles of some of the coagulase-negative staphylococci isolates.
5. To isolate, identify and characterise coagulase-negative staphylococci from normal population and to compare the species patterns with those from clinical specimens.

Table 1.1: Currently recognized *Staphylococcus* species and subspecies.

SPECIES	SUBSPECIES	NATURAL HOST(S)
S. aureus	*aureus*	Humans, mammals, birds
S. aureus	*anaerobius*	Sheep
S. epidermidis		Human (domestic mammals)
S. capitis	*capitis*	Humans
S. capitis	*urealyticus*	Humans, Some primates
S. caprae		Humans, goats
S. saccharolyticus		Humans
S. warneri		Humans, primates, domestic mammals
S. haemolyticus		Humans, primates, (domestic mammals)
S. hominis		Humans
S. lugdunensis		Humans,
S. auricularis		Humans, primates
S. cohnii	*cohnii*	Humans
S. cohnii	*ureolyticus*	Humans, primates
S. saprophyticus		Humans, mammals
S. xylosus		Humans, mammals, birds
S. arlettae		Mammals, birds
S. equorum		Horses, cattle
S. kloosii		Mammals
S. gallinarum		Poultry, birds
S. muscae		Domestic mammals (flies)
S. felis		Cats
S. simulans		Humans, mammals
S. carnosus		Meat and fish products, unknown
S. piscifermentans		Fermented fish
S. intermedius		Mammals, birds
S. delphini		Dolphins
S. schleiferi	*schleiferi*	Human infections, unknown
S. schleiferi	*coagulans*	Dogs
S. hyicus		Pigs, cattle, goats
S. chromogenes		Cattle, horses, goat
S. caseolyticus		Cattle, whales
S. lentus		Domestic mammals, dolphins
S. vitulus		Meat products, domestic mammals, whales
S. sciuri		Mammals, birds
S. pasteuri		Humans, primates
S. lustrae		Otters.

Kloos and Bannerman, 1994; Kloos and Bannerman, 1995; Foster *et al.*, 1997.

Table 1.2: Classification of the Micrococcaeceae in Bergey's Manual .

	NUMBER OF DESIGNATED SPECIES BY YEAR								
GENERA **YEAR**	**1923**	**1925**	**1930**	**1934**	**1939**	**1948**	**1957**	**1974**	**1986**
Micrococcus	23	23	32	37	44	17	16	3	9
Staphylococcus	6	5	5	6	6	-	2	3	24
Sarcina	10	10	11	11	11	6	10	-	-
Rhodococcus	5	5	6	6	-	-	-	-	-
Gaffkya	-	-	-	3	3	1	2	-	-
Planococcus	-	-	-	-	-	-	-	1	1
Stomatococcus	-	-	-	-	-	-	-	-	1

Cowan (1962); Baird-Parker (1974), Bergey's Manual, Vol.2 (1986).

Table 1.3: Differentiation tests for the separation of *Staphylococcus* from *Micrococcus*.

	TESTS	ORGANISMS	
		STAPHYLOCOCCUS	*MICROCOCCUS*
1	Glucose fermentation	+ (for most)	-(for most)
2	Acid production from glycerol	+ (for most)	-(for most)
3	Susceptibility to lysostaphin	+	-
4	Susceptibility to lysozyme	-	+
5	Modified oxidase and benzidine test	-(for most)	+
6	Susceptibility to flurazolidine	+	-
7	Susceptibility to bacitracin	-	+

Pfaller and Herwaldt (1988).

Table 1.4a: Baird – Parker, Standard Scheme: Baird-Parker, 1963, 1965.

TEST	SI	SII	SII2	SIII	SIV	SV	SVI	SY*	MI	M2	M3	MX*	M4	M5	M6	M7	M8
Coagulase	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O/F	F	F	F	F	F	F	F	F	O	O	O	O	O	O	O	O	O
Arabinose	-	-	-	-	-	-	-	-	-	-	-	-	+	V	V	-	-
Lactose	+	+	-	+	-	+	V	V	-	+	V	V	V	V	V	-	-
Maltose	+	+	+	-	V	+	V	V	V	+	V	V	V	V	V	-	-
Mannitol	+	-	-	-	-	-	+	+	-	-	+	+	+	+	+	-	-
Phosphatase	+	+	+	+	-	-	-	+	-	-	-	+	-	-	+	V	V
Acetoin (vp)	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	-	-

*Subgroups additional to those of Biard-Parker.

SVI	=	Lactose +ve
SVI (2)	=	Lactose –ve, maltose +ve
SV (3)	=	Lactose –ve, maltose -ve
M3(1)	=	Lactose +ve
M3 (2)	=	Lactose -ve maltose +ve
M3 (3)	=	Lactose –ve, maltose -ve
F	=	Fermentation
O	=	Oxidation
V	=	Variable
+	=	85-100% of strains positive

Table 1.4b: Baird-Parker Standard Scheme: Baird–Parker, 1974

	S. epidermidis				*S. saprophyticus*			
TESTS	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Acetoin (vp)	+	-	+	+	+	+	+	+
Phosphatase	d	+	-	-	-	-	-	-
Arabinose	-	-	-	-	-	-	-	+
Lactose	+	d	-	d	-	+	d	+
Maltose	+	-	d	d	d	+	+	d
Mannitol	-	-	-	+	-	-	+	+

d = Inferred Reaction (16-84% positive)

Table 1.5: Abbreviated scheme for the classification of human cutaneous staphylococci using key characters[a].

Species	Colony diameter (15 days ≥5 mm)	Anaerobic growth in thioglycolate[b]	Lysostaphin (resistant to 50μg/ml)	Coagulase	Hemolysis (bovine)	Nitrate reduction	Phosphatase	Acid (aerobically) from: β-D-(-) Fructose	D(+) Galactose	D(+) Mannose	D(+) Xylose and/or L(+) Arabinose	D(-) Ribose	Maltose	α-Lactose	Sucrose	D(+) Trehalose	D(+) Turanose	D(+) Melezitose	D-Mannitol	Xylitol	Novobiocin (resistant to 1.6μg/ml)
Staphylococcus aureus	+	+	-	+	(+)	+	+	+	+	(+)	-	+	+	(+)	+	+	(+)	-	+	-	-v[c]
***Staphylococcus* sp.**	+	(± C)	-	+	(±)	+	+	+	+	+	-	+	(-)	+	+	+	(+, ±)	-	(±, -)	-	-v
Staphylococcus simulans	(+)	+	-	-	±, -	+	(±)	+	-	(+)	-	v	±	+	+	(+)	-	-	(+)	-	-v
Staphylococcus xylosus	+	v	-	-	(-)	(+)	(+)	+	+, ±	+	+, ±	v	(+)	v	+	+	(+, ±)	-	+	(-)	+
Staphylococcus cohnii	(+)	v	-	-	(-, ±)	-	(-)	+	-	v	-	-	(±, +)	(-)	(-)	+	-	-	(+, ±)	(+, -)	+
Staphylococcus saprophyticus	+	(+, ±)	(+)	-	-	-	(-)	+	-	-	-	-	+	(+)	+	+	+	-	+	(+, ±)	+
Staphylococcus haemolyticus	(+)	(± C)	+	-	(+)	(+)	(-)	+, -	v	-	-	(-)	+	-, +	+	+	v	-	+, -	-	-v
Staphylococcus warneri	-	(+)	+	-	-, ±	(-)	(-)	+	(-)	-	-	+, -	+, ±	(-)	+	+	(-)	-	+, -	-	-v
Staphylococcus hominis	-	c	+	-	(-)	(+, ±)	(-)	+	v	-	-	-	+	v	+	+	(+)	v	-	-	-v
Staphylococcus epidermidis	-	+	+	-	-, ±	(+, ±)	+	+	(+)	(+)	-	-	+	(+)	+	-	v	(-)	-	-	-v
Staphylococcus capitis	-	v	+	-	(-)	(+)	(-)	+	-	(+)	-	-	-	-	+	-	-	-	+	-	-v

[a]A single listed symbol denotes a type character frequency of 90 to 100%; parentheses around a symbol denote a type character frequency of 70 to 89%; two symbols are listed for a character when either type is in frequency below 70%, but together equal 80 to 100%. Symbols for characters (unless otherwise noted): +, positive; ±, weak; -, negative; v, variable (+, ±, and -).

[b]Symbols for anaerobic growth: +, dense uniform; ±, gradient from dense to light down tube; ± C, gradient plus large individual colonies; c, small individual colonies to absence of visible growth; v, variable (+, ±, and ± C).

[c]Symbol: -v, negative in our study, but could conceivably be variable with exposure to novobiocin in environment.

Kloos and Schleifer, 1975.

TABLE 1.6: Scheme based on subcommittee on the Taxonomy and Nomenclature of staphylococci and micrococci 1976. Appendix I.

CHARACTER	*S. aureus*	*S. epidermidis*	*S. saprophyticus*
Coagulase	+	-	-
Sucrose (acid aerobe)	+	+	+
Trehalose (acid aerobe)	+	-	+
Mannitol (acid aerobe)	+	-	+
Phosphatase	+	+	-
Novobiocin	S	S	R

S = Susceptible
R = Resistant

Table 1.7: Expanded scheme by Marples, 1981

TEST	SI	SII	SIII	SVIC	SIV	SV	SVIh	M3	MX	M6
Coagulase	+	-	-	-	-	-	-	-	-	-
Lactose	+	v	+	V	-	+	v	v	v	v
Maltose	v	+	v	+	+	+	v	+	+	+
Mannitol	+	-	v	+	-	-	+	v	+	+
Fructose	+	+	+	V	v	v	v	+	+	+
Sucrose	+	+	+	+	+	+	v	+	-	+
Trehalose	+	-	+	-	+	+	+	+	+	+
Xylose	-	-	-	-	-	-	-	-	-	+
Phosphatase	+	v	+	-	-	-	+	-	+	v
Acetoin (VP)	v	+	-	+	+	+	+	+	+	-
Nitrate	+	+	+	+	v	+	V	-	-	+
Arginine	+	v	+	V	v	v	V	-	v	v
Urea	+	+	+	+	v	v	V	+	v	v
Lysostaphin	S	R	S	R	R	R	R	S	S	S
Haemolysis	+	+	v	v	±	±	V	-	-	-
Methicillin	S	S	S	S	S	S	S	SS	SS	?
Novobiocin	S	S	S	S	S	S	S	R	R	R

Large symbols indicate diagnostic characters; small symbols indicate incidental findings.
V = character used in defining biotypes within group.

Table 1.8a: Differentiation of *Staphylococcus* species (Kloos and Jorgensen, 1985).

Species	CHARACTERISTICS																Acid (aerobically) from												
	Colony size (large)	Colony pigment	Anaerobic growth	Aerobic growth	Coagulase	Haemolysis	Nitrate reduction	Acetoin	Cytochrome C	Phosphatase	Urease	Arginine utilization	β-Glucosidase	β-glucuronidase	β-galactosidase	Novobiocin resistance	Maltose	D(-) Trehalose	D(-) Mannitol	D(-) Xylose	Xylitol	D(-) Cellobiose	Sucrose	D(-) Turanose	D(-) Mannose	D(-)Ribose	Raffinose	α- Lactose	β-D-(-) Fructose
S. aureus	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	+	-	+	+
S. epidermidis	-	-	+	+	-	-	+	+	-	+	+	+	(d)	-	-	-	+	-	-	-	-	-	+	d	(+)	d	-	d	+
S. capitis	-	-	(+)	+	-	-	d	d	-	-	-	d	-	-	-	-	-	-	+	-	-	-	(+)	-	+	-	-	-	+
S. caprae	+	-	(+)	+	-	(d)	+	+	-	(+)	+	+	-	-	-	-	d	+	-	-	-	-	-	-	+	-	-	+	-
S. warneri	d	d	+	+	-	(d)	-	+	-	-	+	d	+	d	-	-	(+)	+	d	-	-	-	+	d	-	d	-	d	+
S. haemolyticus	+	d	(+)	+	-	(+)	d	d	-	-	-	+	d	d	-	-	+	+	d	-	-	-	+	d	-	d	-	d	d
S horminis	-	d	-	+	-	-	d	d	-	-	+	d	-	-	-	-	+	d	-	-	-	-	(+)	d	-	-	-	d	+
S. saccharolyticus	-	-	+	-	-	-	+	ND	-	d	ND	+	ND	ND	ND	-	-	-	-	-	-	-	-	ND	(+)	ND	-	-	(+)
S. auricularis	-	-	(±)	+	-	-	(d)	d	-	-	-	d	-	-	(d)	-	(+)	(+)	-	-	-	-	d	(d)	-	-	-	-	+
S. saprophyticus	+	d	(+)	+	-	-	-	+	-	-	+	-	d	-	d	+	+	+	d	-	d	-	+	+	-	-	-	d	+
S. cohnii (Human)	d	-	d	+	-	(d)	-	d	-	-	-	-	-	-	-	+	(d)	+	d	-	(d)	-	-	-	(d)	-	-	-	+
S. cohnii (Primate)	+	d	(+)	+	-	(d)	-	d	-	+	+	-	-	+	+	+	(+)	+	d	-	(d)	-	-	-	+	-	-	+	+
S. xylosus	+	d	d	+	-	-	d	d	-	d	+	-	+	d	+	+	+	+	d	+	(d)	-	+	d	+	d	-	d	+
S. simulans	+	-	+	+	-	(d)	+	-	-	(d)	+	+	-	d	+	-	-	d	+	-	-	-	+	-	d	d	-	+	+
S. carnosus	+	-	+	+	-	-	+	+	-	+	-	+	-	-	+	-	-	d	+	-	-	-	-	-	+	ND	-	d	+
S. intermedius	+	-	(+)	+	+	d	+	-		+	+	d	d	-	+	-	(±)	+	(d)		-	-	+	(d)	+	+	-	d	+
S. hyicus subsp. hyicus	+	-	+	+	d	-	+	-	-	+	d	+	d	d	-	-	-	+	-	-	-	-	+	-	+	+	-	+	+
S. hyicus subsp. Chromogenes	+	+	+	+	-	-	+	-	-	+	d	+	d	-	-	-	d	+	d	-	-	-	+	d	+	+	-	+	+
S. sciuri	+	d	(+)	+	-	-	+	-	+	+	-	-	+	-	-	+	(d)	+	+	-	-	+	+	-	(d)	+	-	(d)	+
S. lentus	-	d	(±)	+	-	-	+	-	+	+	-	-	+	-	-	+	d	+	+	(d)	-	+	+	-	(+)	+	+	d	(+)
S. caseolyticus	-	d	(±)	+	-	-	+	-	+	+	ND	ND	ND	ND	ND	-	+	d	-	-	-	ND	d	-	-	+	ND	+	+
S. gallinarum	+	d	(+)	+	-	(d)	+	-	-	(+)	+	-	+	-	-	+	+	+	+	+	d	+	+	+	+	+	+	d	+

+ = 90% or more strains; - = 90% or more strain negative; d = 11-80% of strains positive; ND = not determined. ( ) = indicate a delayed reaction.

For colony size symbol; +C for a colony diameter ≥ 6mm after incubation on P agar at 34°C to35°C for 3 days and at room temperature for additional 2days.

For anaerobic growth symbols += moderate or heavy growth down tube within 18 to24 hours; ± = heavier growth in the upper portion and weaker growth in the lower portion of tube; - = no visible growth within 48hours; ( ) =delayed growth appearing within 24-72 hours.

For haemolysis on bovine agar symbol: + = wide zone of haemolysis with 24hour to36 hours (+) delayed moderate to wide zone of haemolysis within 48 to 72 hours; - = narrow zone (≤ 1mm) or no detection of haemolysis within 72 hours.

For Novobiocin resistance: += defined as a MIC of ≥ 1.6μg/ml or a growth inhibition zone diameter of ≤ 16mm with a 5μg novobiocin disk.

Cytochrome C is determined by the modified oxidase test of Faller and Schleifer, (1981).
Urease, arginine utilisation, β- glucosidase, β-glucuronidase, β- galatosidase are characteristics that have been determined primarily by commercial rapidly – identification test system (e.g.API Staph-Ident etc.)

Table 1.8b: Minimum tests for identification of the clinically significant *Staphylococcus* species. (Kloos andJorgensen, 1985).

Character	*S. aureus*	*S.epidermidis*	*S. saprophyticus*	*S, intermedius*	*S. hyicus* Subsp. *hyicus.*
Coagulase	+	-	-	+	d
Novobiocin resistance	-	-	+	-	-
D-Mannitol	+	-	d	(d)	-
Colony pigment	+	-	d	-	-
Acetoin	+	+	+	-	-
β- galatosidase	-	-	d	+	-
Maltose	+	+	+	(±)	-
Phosphatase	+	+	-		
D-Trehalose	+	-	+		
D-xylose	-	-	-		
Sucrose	+	+	+		

Table 1.9a: Differentiation of Staphylococcus species (Kloos andLambe Jr., 1991).

Species	Characteristic[a]																																					
	Colony size (large)[b]	Colony pigment[c]	Anaerobic growth[d]	Aerobic growth[e]	Staphylocoagulase	Clumping factor[f]	Heat-stable nuclease	Hemolysins[g]	Catalase[h]	Oxidase[i]	Alkaline phosphatase	Arginine arylamidase	Pyrrolidonyl arylamise[j]	Ornithine decarboxylase	Urease[k]	β-Glucosidase[l]	β-glucuronidase[l]	β-galactosidase[l]	Arginine utilization[l]	Acetoin prodcution	Nitrate reduction	Esculin hydrolysis	Novobiocin resistance[k]	Polymyxin B Resistance[l]	D- Tebalose	D- Mannitol	D- Mannose	D- Turanose	D-Xyluse	D-Cellobiose	L-Arabinose	Maltose	α- Lactose	Sucrose	N-acetylglucosamine	Raffinose
S. aureus subsp. *aureus*	+	+	+	+	+	+	+	+	+	-	+	-	-	-	d	+	-	-	+	+	+	-	-	+	+	+	+	+	-	-	-	+	+	+	+	-
S. aureus subps. *anaaerobius*	-	-	(+)	(±)	+	-	+	+	-	-	+	ND	ND	ND	ND	-	-	-	ND	-	-	-	-	ND	-	ND	-	ND	-	-	-	+	-	+	-	-
S. epidermidis	-	-	+	+	-	-	-	(d)	+	-	+■	-	-	(d)	+	(d)	-	-	d	+	+	-	-	+	-	-	(+)	(d)	-	-	-	+	d	+	-	-
S. capitis	-	-	(+)	+	-	-	-	(d)	+	-	-	-	-	-	-	-	-	-	d	d	d	-	-	-	-	+	+	-	-	-	-	-	(+)	-	-
S. capitis subsp. *ureolyticus*	-	(d)	(+)	(±)	-	-	-	(d)	+	-	-	-	(d)	-	+	-	-	-	+	d	+	-	-	ND	-	+	+	-	-	-	-	+	(d)	+	-	-
S. caprae	d	-	(+)	+	-	-	-	(d)	+	-	(+)	-	d	-	+	-	-	-	+	+	+	-	-	-	(+)	d	+	-	-	-	-	(d)	+	-	-	-
S. saccharolyticus	-	-	+	(±)	-	-	-	-	-	-	d	-	ND	ND	ND	ND	ND	ND	+	ND	+	ND	-	ND	-	-	(+)	ND	-	-	-	-	-	-	ND	-
S. warneri	d	d	+	+	-	-	-	(d)	+	-	-	-	-	-	+	+	+	-	d	+	d	-	-	-	+	d	-	(d)	-	-	-	+	d	+	+	-
S. haemolyticus	+	d	(+)	+	-	-	-	(+)	+	-	-	-	+	-	-	d	d	-	+	+	+	-	-	-	+	d	-	(d)	-	-	-	+	d	(+)	d	-
S. horminis	-	d	-	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	+	d	d	-	-	-	d	-	-	+	-	-	-	+	+	+	+	-
S. lugdunensis	d	d	+	+	-	(+)	-	(+)	+	-	-	-	+	+	d	+	-	-	-	+	+	-	-	d	+	-	+	(d)	-	-	-	+	+	+	+	-
S. schleiferi	-	-	+	+	-	+	+	(+)	+	-	+	-	+	-	-	-	-	(+)	+	+	+	-	-	-	d	-	+	-	-	-	-	-	-	-	(+)	-
S. auricularis	-	-	(±)	(+)	-	-	-	-	+	-	-	+	d	-	-	-	-	(d)	d	-	(d)	-	-	-	(+)	-	-	(d)	-	-	-	(+)	-	d	-	-
S. saprophyticus	+	d	(+)	+	-	-	-	-	+	-	-	-	-	-	+	d	-	+	-	+	-	-	+	-	+	d	-	+	-	-	-	+	d	+	d	-
S. cohnii	d	-	d	+	-	-	-	(d)	+	-	-	-	-	-	-	-	-	-	-	d	-	-	+	-	+	d	(d)	-	-	-	-	(d)	-	-	-	-
S. cohnii subsp. *urealiticum*	+	d	(+)	+	-	-	-	(d)	+	-	+	-	d	-	+	-	+	+	-	d	-	-	+	-	+	+	+	-	-	-	-	(+)	+	-	d	-
S. xylosus	+	d	d	+	-	-	-	-	+	-	d	-	d	-	+	+	+	+	-	d	d	d	+	-	+	+	+	d	+	-	d	+	d	+	+	-
S. kloosii	d	d	-	+	-	-	-	(d)	+	-	d	-	d	-	d	d	d	d	-	d	-	d	+	-	+	+	-	-	(d)	-	d	d	(d)	(±)	-	-
S. equorum	-	-	-	(+)	-	-	-	(d)	+	-	(+)	-	-	-	+	N D	+	d	-	-	+	d	+	N D	+	+	+	d	+	(d)	+	d	d	+	d	-
S. arlettae	d	+	-	+	-	-	-	-	+	-	(+)	-	-	-	-	N D	+	d	-	-	-	-	+	N D	+	+	+	+	+	-	+	+	+	+	+	+
S. gallinarum	+	d	(+)	+	-	-	-	(d)	+	-	(+)	-	-	-	+	+	d	d	-	-	+	+	+	-	+	+	+	+	+	+	+	+	d	+	+	+
S. simulans	+	-	+	+	-	-	-	(d)	+	-	(d)	-	+	-	+	-	d	+	+	d	+	-	-	-	d	+	d	-	-	-	-	(±)	+	+	+	-
S. carnosus	+	-	+	+	-	-	-	-	+	-	+	-	+	-	-	-	-	+	+	+	+	-	-	-	d	+	+	-	-	-	-	-	d	-	ND	-
S. intermeduis	+	-	(+)	+	+	d	+	d	+	-	+	-	+	-	+	d	-	+	d	-	+	-	-	-	+	(d)	+	d	-	-	-	(±)	d	+	+	-

S. delphini	+	-	(+)	+	+	-	-	+	+	-	+	ND	ND	ND	+	ND	ND	ND	+	-	+	ND	-	ND	-	(+)	+	ND	-	ND	-	+	+	+	ND	ND
S. hyicus	+	-	+	+	d	-	+	-	+	-	+	-	-	-	d	d	+	-	+	-	+	-	-	+	+	-	+	-	-	-	-	+	+	+	-
S. chromogenes	+	+	+	+	-	-	-	-	+	-	+	-	d	-	+	d	-	-	+	-	+	-	-	+	+	d	+	d	-	-	-	d	+	+	d	-
S. caseolyticus	-	d	(±)	+	-	-	ND	-	+	+	-	ND	+	-	-	-	-	-	d	-	+	ND	-	-	d	-	-	-	-	-	-	+	+	d	ND	ND
S. sciuri	+	d	(+)	+	-	-	-	-	+	+	+	-	-	-	-	+	-	-	-	-	+	+	+	-	+	+	(d)	(±)	(d)	+	d	(d)	(d)	+	d	-
S. lentus	-	d	(±)	(+)	-	-	-	-	+	+	(±)	-	-	-	-	+	-	-	-	-	+	+	+	-	+	+	(+)	(±)	(±)	+	d	d	d	+	d	+

[a]Symbols (unless otherwise indicated): +, 90% or more strains positive; ±, 90% or more strains weakly positive; -, 90% or more strains negative; d, 11 to 89% of strains positive; ND, not determined. Parenthesis indicates a delayed reaction.

[b]Positive is defined as a colony diameter of ≥6mm after incubation on P agar at 34 to 35°C for 3 days and at room temperature (ca. 25°C) for an additional 2 days .

[c]Positive is defined as the visual detection of carotenoid pigments (e.g.(yellow, yellow-orange, or orange) during colony development at normal incubation or room temperatures. Pigments may be enhanced by the addition of milk, fat, glycerol monoacetate or soaps of P agar.

[d]In a semisolid thioglycolate medium. Symbols: +, moderate or heavy growth down the tube within 18 to 24 h; ±, heavier growth in the upper portion of the tube and weaker growth in the lower, anaerobic portion of the tube; -, no visible growth within 48h, but very weak diffuse growth or a few scattered, small colonies may be observed in the lower portion of tube by 72 to 96h. parentheses indicate delayed growth appearing within 24 to 72 h, sometimes noted as large discrete colonies in the lower portion of the tube.

[e]On P agar or bovine, sheep, or human blood agar at 34 to 37°C. *S. equorum* grows slowly at 35 to 37°C; its optimum growth temperature is 30°C. anaerobic species *S. saccharolyticus* and *S. aureus* subsp. *anaerobius* grow very slowly in the presence of air. Aerobic growth may be increased slightly by subculture in the presence of air. *S. aureus* subsp. *anaerobius* requires the addition of blood, serum, or egg yolk for growth on primary isolation medium. *S. auricularis, S lentus*, and *S. vitulus* produce just detectable colonies on P agar in 24 to 36h and these colonies remain very small (1 to 2mm in diameter).

[f]Detected in rabbit or human plasma (slide coagulase test). Human plasma is preferred for the detection of clumping factor with *S. lugdunensis* and *schleiferi.* Latex agglutination is somewhat less reliable for detection of clumping factor or fibrinogen affinity factor in *S. lugdunensis.*

[g]Hemolysis on bovine blood agar (58). Symbols: +, wide zone of hemolysis within 24 to 36h; (+) delayed moderate to wide zone of hemolysis within 48 to 72h; (d), no or delayed hemolysis; -, no or only very narrow zone (≤1 mm) of hemolysis within 72h. Some of the strains designated negative may produce a slight greening or browning of blood agar.

[h]Catalase and cytochrome synthesis cannot be induced in *S. aureus* subsp. *anaerobius* by the addition of H_2O_2 or hemin to the culture medium. Catalase can be induced in *S. saccharolyticus* by hemin supplementation. In this species, cytochromes a and b are present in small quantities.

[i]Determined by the modified oxidase test of Faller and Schleifer (1981) to detect the presence of cytochrome C.

[j]Pyrrolidonyl arylamidase, urease, β-glucosidase, β-glucuronidase, β-galactosidase, and arginine utilization are characteristics that have been determined primarily by commercial rapid-identification test systems (e.g. API Staph Trac, ATP 32 Staph, Baxter-MicroScan Pos Combo Type 5 panel and RAPID POS ID panel, Becton Dickson Minitek Gram-Positive Set, and Vitek Systems Gram Positive Identification Card).

[k]Positive is defined as an MIC of ≥ 1.6μg or a growth inhibition zone diameter of ≤ 16mm with a 5μg novobiocin disk.

[l]Positive is defined as a growth inhibition zone diameter of <10 mm with a 300-U polymyxin B disk.

[m]Alkaline phosphatase activity is negative for approximately 6 to 15% of strains of *S. epidermidis*, depending on the population sampled. A low but significant number of clinical isolates have been phosphatase negative.

Table 1.9b: Key tests for identification of most clinically significant *Staphylococcus* species (Kloos andLambe Jr., 1991).

Species	Colony pigment[b]	Staphylocoagulase	Clumping factor[b]	Heat-stable nuclease	Alkaline phosphatase	Pyrrolidonyl arylamidase[b]	Ornithine decarboxylase	Urease[b]	β-galactosidase[b]	Acetoin prodcution	Novobiocin resistance[b]	Polymyxin B Resistance[b]	D - Trehalose	D - Mannitol	D - Mannose	D - Turanose	D -Xylose	D -Cellobiose	Maltose	Sucrose
S. aureus	+	+	+	+	+	-	-	d	-	+	-	+	+	+	+	+	-	-	+	+
S. epidermidis	-	-	-	-	+	-	(d)	+	-	+	-	+	-	-	(±)	(d)	-	-	+	+
S. haemolyticus	d	-	-	-	-	+	-	-	-	+	-	-	+	d	-	(d)	-	-	+	+
S. lugdunensis	d	-	(+)	-	-	+	+	d	-	+	-	d	+	-	+	(d)	-	-	+	+
S. schleiferi subsp. schleiferi	-	-	+	+	+	+	-	-	(+)	+	-	-	d	-	+	-	-	-	-	-
S. saprophyticus	d	-	-	-	-	-	-	+	+	+	+	-	+	d	-	+	-	-	+	+
S. intermedius	-	+	d	+	+	+	-	+	+	-	-	-	+	(d)	+	d	-	-	(±)	+
S. hyicus	-	d	-	+	+	-	-	d	-	-	-	+	+	-	+	-	-	-	-	+

[a]Symbols: +, 90% or more strains positive; ±, 90% or more strains weakly positive; - 90% or more strains negative; d, 11 to 89% of strains positive. Parentheses indicate a delayed reaction.
[b]Descriptions are the same as in Table 1.9a.

Table 1.10a: Differentiation of Staphylococcus species (Kloos and Bannerman, 1995).

Species	Characteristic[a]																																									
	Colony size (large)[b]	Colony pigment[c]	Anaerobic growth[d]	Aerobic growth[e]	Staphylocoagulase	Clumping factor[f]	Heat-stable nuclease	Hemolysins[g]	Catalase[h]	Oxidase[i]	Alkaline phosphatase	Arginine arylamidase	Pyrrolidonyl arylamise[j]	Ornithine decarboxylase	Urease[k]	β-Glucosidase[l]	β-glucuronidase[m]	β-galactosidase[n]	Arginine utilization[o]	Acetoin production	Nitrate reduction	Esculin hydrolysis	Novobiocin resistance[p]	Polymyxin B Resistance[q]	D- Trehalose	D- Mannitol	D- Mannose	D- Turanose	D-Xylose	D-Cellobiose	L-Arabinose	Maltose	α- Lactose	Sucrose	N-acetylglucosamine						
S. aureus subsp. *Aureus*	+	+	+	+	+	+	+	+	+	-	+	-	-	-	d	+	-	-	+	+	+	-	-	+	+	+	+	+	-	-	-	+	+	+	+	-					
S. aureus subps. *Anaaerobius*	-	-	(+)	(±)	+	-	+	+	-	-	+	ND	ND	ND	ND	-	-	-	ND	-	-	-	-	ND	-	ND	-	ND	-	-	-	+	-	+	-	-					
S. epidermidis	-	-	+	+	-	-	-	(d)	+	-	+[s]	-	-	(d)	+	(d)	-	-	d	+	+	-	-	+	-	-	(+)	(d)	-	-	-	+	d	+	-	-					
S. capitis subsp.*capitis*	-	-	(+)	+	-	-	-	(d)	+	-	-	-	-	-	-	-	-	-	d	d	d	-	-	-	-	+	+	-	-	-	-	-	(+)	-	-					
S. capitis subsp. *Ureolyticus*	-	(d)	(+)	(±)	-	-	-	(d)	+	-	-	-	(d)	-	+	-	-	-	+	d	+	-	-	ND	-	+	+	-	-	-	-	+	(d)	+	-	-					
S. caprae	d	-	(+)	+	-	-	-	(d)	+	-	(+)	-	d	-	+	-	-	-	+	+	+	-	-	-	(+)	d	+	-	-	-	-	(d)	+	-	-	-					
S. saccharolyticus	-	-	+	(±)	-	-	-	-	-	-	d	-	ND	ND	ND	ND	ND	ND	+	ND	+	ND	-	ND	-	-	(+)	ND	-	-	-	-	-	-	ND	-					
S. warneri	d	d	+	+	-	-	-	(d)	+	-	-	-	-	-	+	+	+	-	d	+	d	-	-	-	+	d	-	(d)	-	-	-	(+)	d	+	-	-					
S. pasteuri[a]	d	d	+	+	-	-	-	(d)	+	-	-	-	-	-	+	+	+	-	d	d	d	-	-	ND	+	d	-	(d)	-	-	-	(d)	d	+	-	-					
S. haemolyticus	+	d	(+)	+	-	-	-	(+)	+	-	-	-	+	-	-	d	d	-	+	+	+	-	-	-	+	d	-	(d)	-	-	-	+	d	+	+	-					
S. hominis	-	d	-	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	d	d	d	-	-	-	d	-	-	+	-	-	-	+	d	(+)	d	-					
S. hyicus	d	d	+	+	-	(+)	-	(+)	+	-	-	-	+	+	d	+	-	-	-	+	+	-	-	d	+	-	+	(d)	-	-	-	+	+	+	+	-					
S. schleiferi subsp. *Schleiferi*	-	-	+	+	-	+	+	(+)	+	-	+	-	+	-	-	-	-	(+)	+	+	+	-	-	-	d	-	+	-	-	-	-	-	-	-	(+)	-					
S. schleferi subsp. *Coagulans*	d	-	+	+	+	-	+	(+)	+	-	+	-	ND	-	ND	ND	ND	ND	+	+	+	-	-	ND	-	d	+	-	-	-	-	-	d	d	ND	-					
S. muscae	-	-	+	+	-	-	-	(+)	+	-	+	-	ND	-	-	ND	ND	-	-	-	+	-	-	ND	+	-	-	+	+	-	-	-	-	+	ND	-					
S. auricularis	-	-	(±)	(+)	-	-	-	-	+	-	-	+	d	-	-	-	-	(d)	d	-	(d)	-	-	-	(+)	-	-	(d)	-	-	-	(+)	-	d	-	-					
S. saprophyticus	+	d	(+)	+	-	-	-	-	+	-	-	-	-	-	+	d	-	+	-	+	-	-	+	-	+	d	-	+	-	-	-	+	d	+	d	-					
S. cohnii subsp.*cohnii*	d	-	d	+	-	-	-	(d)	+	-	-	-	-	-	-	-	-	-	-	d	-	-	+	-	+	d	(d)	-	-	-	-	(d)	-	-	-	-					
S. cohnii subsp. *Urealitycum*	+	d	(+)	+	-	-	-	(d)	+	-	+	-	d	-	+	-	+	+	-	d	-	-	+	-	+	+	+	-	-	-	-	(+)	+	-	d	-					
S. xylosus	+	d	d	+	-	-	-	-	+	-	d	-	d	-	+	+	+	+	-	d	d	d	+	-	+	+	+	d	+	-	d	+	d	+	+	-					
S. kloosii	d	d	-	+	-	-	-	(d)	+	-	d	-	d	-	d	d	d	d	-	d	-	d	+	-	+	+	-	-	(d)	-	d	d	(d)	(±)	-	-					
S. equorum	-	-	-	(+)	-	-	-	(d)	+	-	(+)	-	-	-	+	ND	+	d	-	-	+	d	+	ND	+	+	+	d	+	(d)	+	d	d	+	d	-					

S. arlettae	d	+	-	+	-	-	-	-	+	-	(+)	-	-	-	-	ND	+	d	-	-	-	-	+	ND	+	+	+	+	+	-	+	+	+	+	-	+
S. gallinarum	+	d	(+)	+	-	-	-	(d)	+	-	(+)	-	-	-	+	+	d	d	-	-	+	+	+	-	+	+	+	+	+	+	+	+	d	+	+	+
S. simulans	+	-	+	+	-	-	-	(d)	+	-	(d)	-	+	-	+	-	d	+	+	d	+	-	-	-	d	+	d	-	-	-	-	(±)	+	+	+	-
S. carnosus	+	-	+	+	-	-	-	-	+	-	+	-	+	-	-	-	-	+	+	+	+	-	-	-	d	+	+	-	-	-	-	-	d	-	ND	-
S. piscifermentans	+	-	+	+	-	-	-	-	+	-	+	-	ND	ND	+	+	-	(d)	+	-	+	d	-	ND	+	d	-	-	-	-	-	d	d	d	ND	-
S. felis	+	-	+	+	-	-	-	(d)	+	-	+	ND	ND	ND	+	-	-	+	+	-	+	ND	-	ND	+	+	+	ND	-	-	-	-	+	d	+	-
S. intermedius	+	-	(+)	+	+	d	+	d	+	-	+	-	+	-	+	d	-	+	d	-	+	-	-	-	+	(d)	+	d	-	-	-	(±)	d	+	+	-
S. delphini	+	-	(+)	+	+	-	-	+	+	-	+	ND	ND	ND	+	ND	ND	ND	+	-	+	ND	-	ND	-	(+)	+	ND	-	ND	-	+	+	+	ND	ND
S. hyicus	+	-	+	+	d	-	+	-	+	-	+	-	-	-	d	d	+	-	+	-	+	-	-	+	+	-	+	-	-	-	-	-	+	+	+	-
S. chromogenes	+	+	+	+	-	-	-	-	+	-	+	-	d	-	+	d	-	-	+	-	+	-	-	+	+	d	+	d	-	-	-	d	+	+	d	-
S. caseolyticus	-	d	(±)	+	-	-	ND	-	+	+	-	ND	+	-	-	-	-	-	d	-	+	ND	-	-	d	-	-	-	-	-	-	+	+	d	ND	ND
S. sciuri	+	d	(+)	+	-	-	-	-	+	+	+	-	-	-	-	+	-	-	-	-	+	+	+	-	+	+	(d)	(±)	(d)	+	d	(d)	(d)	+	d	-
S. lentus	-	d	(±)	(+)	-	-	-	-	+	+	(±)	-	-	-	-	+	-	-	-	-	+	+	+	-	+	+	(+)	(±)	(±)	+	d	d	d	+	d	+
S. vitulus	-	+	-	(+)	-	-	-	-	+	+	-	ND	-	-	-	d	-	-	-	-	+	d	+	ND	(d)	+	-	-	d	d	-	-	-	+	-	-

[a]Symbols (unless otherwise indicated): +, 90% or more strains positive; ±, 90% or more strains weakly positive; -, 90% or more strains negative; d, 11 to 89% of strains positive; ND, not determined. Parenthesis indicates a delayed reaction.

[b]Positive is defined as a colony diameter of ≥6mm after incubation on P agar at 34 to 35°C for 3 days and at room temperature (ca. 25°C) for an additional 2 days.

[c]Positive is defined as the visual detection of carotenoid pigments (e.g.(yellow, yellow-orange, or orange) during colony development at normal incubation or room temperatures. Pigments may be enhanced by the addition of milk, fat, glycerol monoacetate or soaps of P agar.

[d]In a semisolid thioglycolate medium. Symbols: +, moderate or heavy growth down the tube within 18 to 24 h; ±, heavier growth in the upper portion of the tube and weaker growth in the lower, anaerobic portion of the tube; -, no visible growth within 48h, but very weak diffuse growth or a few scattered, small colonies may be observed in the lower portion of tube by 72 to 96h. Parentheses indicate delayed growth appearing within 24 to 72 h, sometimes noted as large discrete colonies in the lower portion of the tube.

[e]On P agar or bovine, sheep, or human blood agar at 34 to 37°C. *S. equorum* grows slowly at 35 to 37°C; its optimum growth temperature is 30°C. anaerobic species *S. saccharolyticus* and *S. aureus* subsp. *anaerobius* grow very slowly in the presence of air. Aerobic growth may be increased slightly by subculture in the presence of air. *S. aureus* subsp. *anaerobius* requires the addition of blood, serum, or egg yolk for growth on primary isolation medium. *S. auricularis, S lentus*. and *S. vitulus* produce just detectable colonies on P agar in 24 to 36h and these colonies remain very small (1 to 2mm in diameter).

[f]Detected in rabbit or human plasma (slide coagulase test). Human plasma is preferred for the detection of clumping factor with *S. lugdunensis* and *S. schleiferi*. Latex agglutination is somewhat less reliable for detection of clumping factor or fibrinogen affinity factor in *S. lugdunensis*.

[g]Hemolysis on bovine blood agar. Symbols: +, wide zone of hemolysis within 24 to 36h; (+) delayed moderate to wide zone of hemolysis within 48 to 72h; (d), no or delayed hemolysis; -, no or only very narrow zone (≤ 1 mm) of hemolysis within 72h. Some of the strains designated negative may produce a slight greening or browning of blood agar.

[b]Catalase and cytochrome synthesis cannot be induced in *S. aureus* subsp. *anaerobius* by the addition of H_2O_2 or hemin to the culture medium. Catalase can be induced in *S. saccharolyticus* by hemin supplementation. In this species, cytochromes a and b are present in small quantities.

[i]Determined by the modified oxidase test to detect the presence of cytochrome C.

[j]Determined primarily by commercial rapid-identification tests.

[k]Positive is defined as an MIC of ≥ 1.6μg or a growth inhibition zone diameter of ≤16mm with a 5μg novobiocin disk.

[l]Positive is defined as a growth inhibition zone diameter of <10 mm with a 300-U polymyxin B disk.

[m]Alkaline phosphatase activity is negative for approximately 6 to 15% of strains of *S. epidermidis*, depending on the population sampled. A low but significant number of clinical isolates have been phosphatase negative.

[n]rRNA gene restriction site polymorphism using pBA2 as a probe can distinguish this species from other staphylococcal species, including *S. warneri*.

Table 1.10b: Key tests for identification of most clinically significant *Staphylococcus* species (Kloos and Bannerman, 1995).

species	Colony pigment[b]	Staphylocoagulase	Clumping factor[b]	Heat-stable nuclease	Alkaline phosphatase	Pyrrolidonyl arylamidase[b]	Ornithine decarboxylase	Urease[b]	β-galactosidase[b]	Acetoin prodcution	Novobiocin resistance[b]	Polymyxin B Resistance[b]	D - Trehalose	D - Mannitol	D - Mannose	D - Turanose	D -Xylose	D -Cellobiose	Maltose	Sucrose
S. aureus	+	+	+	+	+	-	-	d	-	+	-	+	+	+	+	+	-	-	+	+
S. epidermidis	-	-	-	-	+	-	(d)	+	-	+	-	+	-	-	(±)	(d)	-	-	+	+
S. haemolyticus	d	-	-	-	-	+	-	-	-	+	-	-	+	d	-	(d)	-	-	+	+
S. lugdunensis	d	-	(+)	-	-	+	+	d	-	+	-	d	+	-	+	(d)	-	-	+	+
S. schleiferi subsp. schleiferi	-	-	+	+	+	+	-	-	(+)	+	-	-	d	-	+	-	-	-	-	-
S. saprophyticus	d	-	-	-	-	-	-	+	+	+	+	-	+	d	-	+	-	-	+	+
S. intermedius	-	+	d	+	+	+	-	+	+	-	-	-	+	(d)	+	d	-	-	(±)	+
S. hyicus	-	d	-	+	+	-	-	d	-	-	-	+	+	-	+	-	-	-	-	+

[a]Symbols: ±, 90% or more strains weakly positive; +, 90% or more strains positive; - 90% or more strains negative; d, 11 to 89% of strains positive. Parentheses indicate a delayed reaction.

[b]Descriptions are the same as in Table 1.10a.

CHAPTER 2

ISOLATION AND CHARACTERIZATION OF COAGULASE - NEGATIVE STAPHYLOCOCCI FROM VARIOUS CLINICAL SPECIMENS IN LAGOS.

INTRODUCTION

Coagulase-negative staphylococci (CoNS) are frequently isolated in cultures from a variety of clinical specimens. In many cases these staphylococci are "picked up" from the skin during specimen collection and are not involved in any disease process, while in others they have been shown to cause infection (Kloos and Bannerman, 1994, Pulverer, 1985). Recently, the CoNS have been studied extensively because of their pathogenicity and involvement in human and animal diseases (Buttery *et al.*, 1997, Weinstein *et al.*, 1997, Mahoudeau *et al.*, 1997, Kloos and Bannerman, 1994, Schumacher Perdreau, 1991; Doern, 1989; Pfaller and Herwaldt, 1988; Marrie *et al.*, 1982).

The emergence of coagulase-negative staphylococci as one of the major nosocomial pathogens implicated in a variety of infections has been reported (Kloos and Bannerman, 1994; Jarvis and Martone, 1992; Scaberg *et al.*, 1991). Coagulase-negative staphylococcal infections can be life-threatening in seriously ill and immuno-compromised patients, for example, patients in intensive care units, premature newborns, cancer and transplant patients. This is due to the increase in the use of transient or permanent medical devices such as intravascular catheters and prosthetic devices (Kloos and Bannerman 1994). The need to rapidly identify this group of bacteria is essential and proper characterization of the organisms is important as culture results may be misinterpreted resulting in confusion. This has been facilitated by the use of different classification schemes (Kloos and Schleifer, 1975; Kloos and Lambe, 1991; Kloos and Bannerman, 1995) and the use of rapid commercial identification systems. This micromethod could either be with manual or automated instrumentations (Kloos *et al.*, 1992). Most of these systems have been

able to identify the coagulase-negative staphylococci species to sub-species level.

Coagulase-negative staphylococcal infections are on the increase. In Nigeria, few reports on this group of organisms are available. Kolawole (1987) reported on the occurrence and possible involvement of CoNS in bacterial infections among hospital patients at Ile-Ife at a Conference in Jos. Oyedeji and Babalola (1999) reported on the involvement of CoNS in neonatal conjunctivitis at Ile-Ife while Udo and co-workers (1997) reported on the slime production, beta-lactamase production and antimicrobial resistance of sixty-eight strains of coagulase-negative staphylococci isolated from various clinical specimens at Calabar. The purpose of this work therefore was to determine the occurrence and possible involvement of CoNS in infections from various clinical specimens in Lagos. The species pattern of CoNS isolated was determined. This study also compared the conventional methods of identification of staphylococci to the commercial and rapid identification systems.

MATERIALS AND METHODS

Study Population and Bacterial Strains:

Seven hundred and forty-five isolates of gram-positive, catalase - positive clustering cocci were obtained from various clinical specimens from the Clinical Microbiology Laboratories of Lagos University Teaching Hospital (LUTH) Idi-Araba and General Hospital Ikeja (GHI) between December 1994 and May 1996. These were clinical specimens collected from in-patients and out-patients comprising of neonates, children and adults of both sexes. Specimens included high vaginal swabs (HVS), endocervical swabs, pus from drainages, swabs from infections of the skin, ear, eye, surgical sites, burns, septic wounds, bones, abscesses/boils, urethra, mid-stream urine from urinary tract infections, pyelonephritis etc., cerebrospinal fluid, seminal fluid derived from infections of the urinary tract and infertility, blood cultures in cases of blood stream infections (BSI), peritonitis, and endocarditis.

Collection of specimens:

Daily visits were made to the LUTH and GHI microbiology laboratories. All gram-positive clustering cocci were obtained from these laboratories by sub-culturing unto nutrient agar slope. Detailed information collected on each specimen / isolate included name of the patient, age, sex, source of specimen, clinical condition being queried and whether in- or out-patient. Primary isolation of the microorganism associated with the diseases was performed on the following media, Nutrient agar, MacConkey agar and Blood agar in those Laboratories (LUTH and GHI).

Clinical significance:

The clinical significance is based on the review of Kloos and Bannerman, (1994) and (1995) which stipulates (i) the isolation of a strain in pure culture from the infected site or body fluid (because most contaminated clinical specimens produce mixed cultures of different strains and/or species, although, some infections may be the consequence of more than one strain or species), (ii) the repeated isolation of the same strain or combination of strains over the course of the infection. Thus, isolation in pure culture, presence of leucocytes and significant counts in cases of urine specimens and one or two consecutive blood cultures where possible was the pre-condition for judging a given isolate to represent a probable infection.

Isolation procedure:

Processing of the isolates was carried out in the Microbiology and Biotechnology Laboratories of the Nigerian Institute for Medical Research (NIMR) Yaba. All subcultured isolates were incubated aerobically for 24 hours and at times for 48 hours at 37°C. Each of the overnight gram-positive clustering cocci was inoculated on to Nutrient agar, P agar (Naylor and Burgi, 1956), and Mannitol salt agar (Appendix 1). Each plate was subsequently incubated aerobically at 37°C for 18-24 hours and at times for 48-72 hours in the case of P agar plates. Colonial characteristics including size and pigmentation were observed daily on P agar. On the selective mannitol salt agar strains capable of growth, fermentation of mannitol were distinguished within 24 hours. Growth indicated salt tolerance and change of the colour of the medium from red to yellow indicated fermentation of mannitol. Gram's reaction, coagulase production and catalase activity were carried out.

Coagulase production:

Bound (also called clumping factor) and free coagulase activities were determined using the conventional slide test method of Cadness-Graves *et al.*, (1943) and tube test method of Gillespie, (1943). Both tests were performed as described by Cowan and Steel (1993). See appendix one for descriptions of the tests.

Catalase Activity:

Catalase activity was carried out on 18-24 hours old cultures on nutrient agar slant using standard technique (Cheesbrough, 1985). (Appendix one for description of the test).

Gram's Reaction:

Gram staining of discrete colonies of pure culture of isolates was performed using standard method as described by Cheesbrough (1985). (Appendix one). All gram-positive, catalase-positive, coagulase-negative cocci were subcultured on to sterile nutrient agar slants and stored for further biochemical tests.

DIFFERENTIATION OF ISOLATES (CoNS) FROM MICROCOCCI

This was carried out based on tests recommended by Schleifer and Kloos, (1975) using the differentiation tests for the production of acid from glycerol (1%) in the presence of 0.4µg of erythromycin per ml, susceptibility to lysostaphin (400µg/ml) and resistance to lysozyme (25µg/ml). Oxidase activity and aerobic requirement of each isolate were determined as described by Kloos *et al.*, (1974). See appendix one for descriptions of various tests.

DETERMINATION OF SPECIES BY CONVENTIONAL BIOCHEMICAL TESTS:

All gram-positive, catalase-positive, coagulase-negative staphylococci were further characterized using the method of Kloos and Schleifer (1975). This method uses 13 key characteristics including coagulase activity, haemolysis, nitrate reduction, phosphatase activity, lysostaphin susceptibility, novobiocin susceptibility, aerobic acid production from lactose, xylose, sucrose, arabinose,maltose, ribose, fructose, trehalose, xylitol and mannitol and anaerobic growth in thioglycolate medium. Other tests used to classify the isolates included acetoin production and oxidase production. The procedures for determining these characteristics were slightly modified from the original descriptions. See appendix one for descriptions of various procedures.

Haemolysis:

Differences in the haemolytic activity of species were demonstrated with bovine agar (5% sheep blood in P agar) (Kloos and Schleifer, 1975).

Nitrate Reduction:

Nitrate reduction was detected using the conventional tube test with nitrate broth after 5 days of incubation. (Cowan and Steel, 1993, Difco Manual, 1984).

Phosphatase Activity:

Phosphatase activity was determined by the splitting of phenolphthalein phosphate, in phenolphthalein phosphate agar releasing free phenolphthalein, (Collins and Lyne, 1970).

Acetoin production:

Acetoin production was detected using the rapid paper disk method of Davis and Hoyling (1973).

Novobiocin Susceptibility:

Novobiocin susceptibility was determined using 5μg novobiocin susceptibility disks (Oxoid) on inoculated Mueller Hinton agar. (Kloos and Schleifer, 1975).

Lysostaphin Susceptibility:

Lysostaphin (Sigma Chemicals) susceptibility of strains was determined on P agar plates containing (200μg) per ml of P agar. (Schleifer and Kloos, 1975).

Anaerobic growth in Thioglycolate Medium:

Tubes containing 8ml of cooled (50-52°C) thioglycollate semi solid medium (Brewer's fluid thioglycollate medium plus 0.3% agar) (Evan and Kloos, 1972) were inoculated with a 0.1ml saline suspension of an overnight culture and incubated for 5 days at 35°C

Oxidase Activity:

Oxidase activity was determined on 18-24 hours old cultures by technique of Collins and Lyne (1970) on a clean Whatman No. 1 filter paper previously dipped in oxidase reagent.

Aerobic Acid Production from Carbohydrates:

Acid production from various carbohydrates was detected under aerobic conditions using an agar plate method (Kloos and Schleifer, 1975) by inoculating a carbohydrate agar. Cultures

were incubated for 24 to 72 hours.

DETERMINATION OF SPECIES BY API STAPH-IDENT SYSTEMS (ID 32 STAPH):

Each isolate was grown on P agar and several identical colonies were picked for the preparation of an inoculum suspension having a turbidity equal to 0.5 Mcfarland standard. About fifty-five microlitre (55µl) of inoculum suspension was used to inoculate each cupule of ID 32 STAPH strip containing 32 cupules, 26 of which contained dehydrated test substrates. Each strip permits the determination of urease production arginine dihydrolase, ornithine decarboxylase, esculin hydrolysis, nitrate reduction, acetoin production, b-galactosidase, arginine arylamidase, alkaline phosphatase, pyrrolidonyl arylamidase, b-glucuronidase, novobiocin resistance, fermentation of glucose, fructose, mannose, maltose, lactose, trehalose, mannitol, raffinose ribose, cellobiose, sucrose, turanose, arabinose, N-acetyl-glucosamine.

Inoculation of the strips, incubation and reading of the strips were done according to manufacturer's instructions (see appendix Three). The results were coded into a numerical profile and the species ascertained using Analytical Profile Index register - ATB 32 STAPH.

CONTROL STRAINS: Control strains; *Staphylococcus aureus* ATCC 25923, *S. epidermidis* ATCC 14990, *Staphylococcus saprophyticus* ATCC 15305.

STATISTICAL ANALYSIS:

Data were analysed using an IBM-Compatible PC with Minitab and Fig-P Statistical packages.

RESULTS

A total of seven-hundred and forty-five (745) gram-positive clustering cocci were obtained from various clinical specimens from LUTH and GHI microbiology laboratories. Tests for Gram's reaction, production of the enzyme coagulase (free, bound or both) and catalase activity yielded 488 gram-positive, catalase-positive, coagulase-positive clustering cocci and 257 gram-positive catalase-positive, coagulase-negative clustering coccci.

SEPARATION OF THE COAGULASE-NEGATIVE STAPHYLOCOCCI FROM MICROCOCCI SPECIES.

Out of the 257 gram-positive, catalase-positive, coagulase-negative clustering cocci obtained, 11 isolates were found to be *Micrococcus* species. These were excluded from the study.

DETERMINATION OF COAGULASE-NEGATIVE SPECIES BY CONVENTIONAL BIOCHEMICAL TESTS.

A total of 246 coagulase-negative staphylococcci were characterised by the conventional biochemical methods of Kloos and Schleifer(1975). The sources of CoNS isolates are shown in Table 2.1 while the results of the biochemical tests are shown in Table 2.2. The 236 isolates were characterised to species level, while 10 isolates were unclassified (Table 2.3). The species distribution is shown as follows: *S. epidermidis* (109); *S. saprophyticus* (36); *S. capitis*(27); *S. simulans*(24); *S. haemolyticus*(19); *S. warneri* (9); *S. hominis*(5); *S. cohnii*(1); *S. xylosus* (6), Unclassified(10).

Isolates of several species were able to produce acid aerobically (see Plate 2.1) from at least five of the carbohydrates tested except for 3 isolates of *S. capitis* and 2 isolates of the unclassified group which produced acid aerobically from only three carbohydrates. Isolates of *S. xylosus* were the only isolates out of the 246 CoNS that produced acid from xylose and arabinose. None of the isolates identified as *S. epidermidis* produced acid from mannitol.

DETERMINATION OF SPECIES BY API STAPH-IDENT SYSTEMS (ID 32 STAPH).

Out of the 246 presumed CoNS isolates being characterised by the rapid commercial kit, (ID 32 STAPH) 241 isolates were characterised to species level while 2 isolates were identified as *Stomatococcus* species. In all, 3 isolates were still unclassified while the 2 *Stomatococcus* species were excluded from the study. The species distribution using API rapid kit (ID 32 STAPH) is shown as follows (Table 2.4). *S. epidermidis* 109 (44.3%); *S. saprophyticus* 36(14.6%); *S. capitis* 27 (11.0%); *S. cohnii* 1(0.4%); *S.simulans* 24 (9.8%); *S. haemolyticus* 24 (9.8%); *S. warneri* 7(2.8%); *S. xylosus* 6(2.4%); *S. hominis* 5(2.0%); *S. lugdunensis* 2(0.8%); *Unclassified* 3(1.2%); and *Stomatococcus* species 2(0.8%). Using the API Kit (ID 32 STAPH) 2 of the isolates identified previously as *S. warneri* were identified as *S. lugdunensis,* while 5 out of the 10 unclassified CoNS by the conventional biochemical tests were identified as *S. haemolyticus* . The two isolates found to be *Stomatococcus* species were from the 10 CoNS unidentified by conventional biochemical tests.

COMBINING CHARACTERISATION TO SPECIES LEVEL BY BOTH CONVENTIONAL BIOCHEMICAL METHOD OF KLOOS AND SCHLEIFER, (1975) AND RAPID API COMMERCIAL KIT-ID 32 STAPH.

A total of 244 isolates of CoNS out of 246 presumed isolates of CoNS obtained after seperation of CoNS from *Micrococci* species were obtained. In all, 241 of these CoNS were characterised to species level. The species distribution is shown in Table 2.5 and Figure 2.1. *S. epidermidis* was the most commonly isolated CoNS from all clinical specimens. It accounted for 109 (44.3%)of CoNS isolates. Thirty-six strains (14.6%) were *S. saprophyticus*, while 27(11.0%) strains were identified as *S. capitis*. Twenty-four (9.8%) strains each were identified as *S. simulans* and *S. haemolyticus* respectively. Other CoNS species, *S. hominis* 5(2.0%), *S. lugdunensis* 2(0.8%), *S. cohnii* 1(0.4%), *S. warneri* 7(2.8%) *and S. xylosus* 6(2.4%) were isolated. Three (1.2%) CoNS isolates remained unclassified. Comparision between the conventional biochemical method and the ID 32 STAPH is shown in Table 2.6.

DISCUSSION

In this study, a total of 244 isolates of CoNS were isolated and characterised. Significant species-to-species differences have emerged. *Staphylococcus epidermidis* accounted for 44.3% of all clinical isolates and the most prevalent in almost all groups of clinical speciemens. It accounted for 38.1% of wound isolates, 76.9% of blood culture isolates, 36.5% of urine isolates, 46.1% of urethra discharge isolates, 50.0% of bone secretions in cases of osteomyelitis, 75% of eye swabs in cases of conjunctivitis and 100% in tissue biopsies in cases of gastritis. In isolates from high vaginal swabs, endocervical swabs, semen and seminal fluids, *Staphylococcus epidermidis* accounted for an appreciable number of isolates while there were no isolates of *Staphylococcus epidermidis* from ear swabs in cases of otitis media and from sterile fluid in a case of congestive heart failure. This prevalence of *Staphylococcus epidermidis* is consistent with other studies on species characterization of CoNS that relate *Staphylococcus epidermidis* to be the most prevalent and major species of clinical relevance (Archer, 1984, Deighton *et al.*, 1988, Pfaller and Herwaldt, 1988, Kleeman *et al.*, 1993, Kloos and Bannerman, 1994, Udo *et al.*, 1997).

Several species of CoNS inhabit the skin (Kloos, 1986, Kloos, 1990). Studies on clinical isolates of CoNS show that 57-85% are *Staphylococcus epidermidis* (Eng *et al.*, 1982, Sewell *et al.*, 1982, Deighton *et al.*, 1988). Also *in-vitro* studies using rat model of endocarditis and *in-vivo* studies of phagocytic killing indicate the greater virulence of *S. epidermidis* when compared with other CoNS (Baddour *et al.*, 1984). It is not surprising that *Staphylococcus epidermidis* accounted for 44.3% of all the isolates and the most prevalent in this study. Higher results might also have been achieved if more specimens

had been obtained from patients who had prosthetic and indwelling devices implanted in them, and also in immunocompromised patients. The infection rate of *S. epidermidis* has been correlated with the increase in the use of prosthetic and indwelling devices such as intravascular catheters etc; the growing number of immunocompromised patients in the hospitals (Kloos and Barnnerman 1994, 1995); and the special ability to infect these devices by *S. epidermidis* (Gristina, 1987, Peters, 1986).

Staphylococcus epidermidis was not isolated from sterile fluid specimen from a congestive heart failure patient. The sample size was small and due to this no definite conclusion as to the incidence or prevalence of *S. epidermidis* in this group of patients could be made in this environment. Although, Feigin and co-workers (1973) assessed the role of *S. epidermidis* in otitis media in neonates, no *S. epidermidis* was isolated from otitis media specimens. It shows that *S. epidermidis* might not be one of the bacteria or opportunistic CoNS species involved in otitis media in this environment.

About 76.9% of all blood culture isolates yielded *S. epidermidis* species. These were isolates from patients sufferring from neonatal sepsis, neonatal tetanus, broncho pneumonia, endocarditis and pyrexia of unknown origin. The clinical relevance or significance of CoNS isolates in blood cultures becomes necessary. Major problems facing most laboratories are in differentiating true opportunistic pathogens from contaminants. Weinstein and co-workers (1997) stated that about 85% of blood culture isolates are contaminants even though some reports had lower figures. This was attributed usually to skin contamination at the time blood is taken. Many reports suggested that before any

inference as to the pathogenic significance of CoNS in cases of Blood Stream Infections(BSI) eg bacteraemia, septicaemia, etc., is drawn consecutive or multiple blood cultures from the same patients should be done (Weinstein *et al.*, 1997, Kloos and Bannerman, 1995, Kloos and Bannerman, 1994, Archer, 1985).Suggested laboratory criteria for true bacteraemia include growth within 48 hours and multiple blood cultures positive for the same organism. In contrast, increased duration of time before positivity, polymicrobial growth of skin organism, or growth during antibiotic treatment suggest contamination (Souvenir *et al.*, 1998).

In this study, 10 out of 38 isolates from blood culture specimens were from multiple specimens. The rest were single blood culture specimens. Determinig the clinical significance of all the CoNS blood culture isolates will be difficult because only 10 isolates were from multiple specimens. Based on the review of Kloos and Bannerman, 1994, and 1995, 26.3% of the blood culture isolates were true pathogens. About 60% of the *S. epidermidis* isolated from blood was from neonates including 2 neonates with low birthweights, who had various clinical infections. Since the initial descriptions in the mid 1960s of CoNS especially *S. epidermidis* as important neonatal pathogens (Buetow *et al.*, 1965, McCraken and Shinefield, 1966) many reports have shown the increase in incidence of CoNS infections in neonates especially the presence of *S. epidermidis* in multiple blood cultures in neonates with low birth weights and preterm infants who are known to be immunologically immature (Patrick *et al.*, 1989, Gongora-Rubio *et al.*, 1997). Reports attributed the most frequent source of infection with the presence of indwelling central intravascular catheters used for administering total parenteral nutrition and contaminated infusions (Goldman and Pier 1993; Valles *et al.*, 1997). In this study, most of the blood

culture isolates were from critically ill patients thus some of these isolates especially in multiple blood culture isolates are most likely to be pathogens. CoNS infections often can be life-threathening in these patients (Kloos and Bannerman, 1994). Other reports elsewhere in Nigeria have implicated *S. epidermidis* in septicaemia especially in neonatal septicaemia (Udo *et al.*, 1997 and Olusanya *et al* ., 1991). These reports are also in agreement with the findings in this study.

The range of other infections believed to be caused by *S. epidermidis* are wide by the 1980s. These include wound infections, urinary tract infections, osteomyelitis, amongst other infections (Kloos and Bannerman, 1994). Coagulase-negative staphylococci especially *S. epidermidis* has been implicated in wound infections and other pyogenic lesions (Wilson and Stuart, 1965, Pulverer, 1985). In this study, *S. epidermidis* accounted for 39.0% of wound isolates from burns, skin sepsis, multiple boils, chronic ulcer and post operation wound infections from hospitalised patients. *S. epidermidis* is widely distributed over the body surface. Since these isolates were isolated in pure cultures from some chronic and purulent draining materials it is obvious that the infections are opportunistic. The isolates from post operation wound infections might also be nosocomial in origin either introduced during surgery or during management of the surgical incisions.

Osteomyelitis due to CoNS has been documented and is frequently a postsurgical complication following insertion of orthopaedic appliances for reduction of fractures (Waldovogel *et al* ., 1970). It could result from open fractures in which the bone was in communication with the outside environment. Since proper laboratory procedures were

adopted and surgical extraction of the pus was used in some cases the *S. epidermidis* obtained thus accounted for true pathogens.

About 75% of CoNS species isolated from urinary catheter tips in this study were *S. epidermidis*. This might be as a result of the exposure of the catheter to the skin of the patients and the special ability of *S. epidermidis* to adhere to foreign body surfaces (Christensen *et al.*, 1986). CoNS infections are believed to arise by direct extension of skin flora into wounds and catheter entry sites. Depending on the ability of CoNS to adhere, colonise and avoid the immune system of the host, they may develop the lifestyle of a pathogen and produce products that will affect the host (Kloos and Bannerman, 1994).

The involvement of CoNS in neonatal conjunctivitis has been studied. Oyedeji and Babalola (1999)reported the involvement of CoNS in neonatal conjunctivitis with *S. epidermidis* accounting for 47.6% of CoNS species and 13.6% of all bacterial isolates obtained. Seventy-five percent of the isolates from conjunctivitis specimens were *S. epidermidis*. The high percentages obtained in this study is likely due to the small number of conjunctivitis specimens sampled. The small number of specimens processed might also account for the 100% recorded for *S. epidermidis* in tissue biopsy isolates from patients with gastric ulcer (gastritis). In this case, two consecutive specimen of each patients all yielded *S.epidermidis* except in one set of isolates in which different strains of *S. epidermidis* were obtained as shown by the profile index numbers of API Kit (ID 32 STAPH).

The prevalence of 36.5% recorded for *S. epidermidis* in urine isolates and 46.1% for urethra isolate is likely due to the fact that most of the urine samples were from patients with urinary tract infection, pyelonephritis, prostatis and benign prostrate hypertrophy. In urine specimens that grew CoNS, significant colony counts and leucocytes in the urine sediments were obtained. Thirty-two out of forty-one urine samples isolates were from urinary tract infections (UTI). It is well known that *S. saprophyticus* is the leading agent among CoNS causative agents in UTI especially among women. In this study, 29.3% of the CoNS strains from urine were *S. saprophyticus,* while 36.5% were *S. epidermidis.* In contrast, some other workers found more of *S. saprophyticus* than *S. epidermidis* (Marrie *et al.,* 1982, Wallmark *et al.,* 1978) in urine specimens. The present findings obtained in this study is in agreement with the findings of Narayani *et al.,* (1990); Loo *et al.,* (1984) and emphasize the prevalence of *S. epidermidis* in most clinical infections. Most of the urethra discharge specimens are from patients suffering from urethritis and UTIs. *S. epidermidis* has been implicated in UTI and prostatitis. The result obtained in this study is not surprising since the isolates were obtained in pure culture and were isolated too from high vaginal swabs (HVS) and endocervical swabs. In male secretions (seminal fluids) from patients suffering from urethritis, prostatitis and infertility, *S. epidermidis* accounted for 21.7% of CoNS isolated thus showing the prevalence and presence of *S. epidermidis* among the various clinical specimens.

Staphylococcus saprophyticus accounted for 14.8% of the overall isolates. *S. saprophyticus* has been reported as an important-opportunistic pathogen in human urinary tract infections (UTIs) especially in young sexually active females (Marrie *et al.,*1982, Wallmark *et al.,* 1978). It has also been proposed as an agent of nongonococcal urethritis in males or a

cause of other sexually transmitted disease (Hovelius *et al.*, 1979) and has also been implicated in prostatitis (Bergman *et al.*, 1989, Nickel and Costerton, 1992). In this study, the *S. saprophyticus* isolates were from urinary tract infections, pyleonephritis and urethritis; pelvic inflammatory disease (PID), infertility and others. Since CoNS are normal flora, one can not make any conclusion as to the clinical significance in cases of infertility. It accounted for 38.9% of all HVS and endocervical swabs. They were isolated in good numbers from urethra discharges, semen and prostatic fluids etc in cases of urethritis and prostatis in males. These are consistent with other studies implicating *S. saprophyticus* as an important opportunistic pathogen in human UTIs (Wallmark *et al.*, 1978,Marrie *et al.*,1982, Bhalla and Agarwal, 1986). The isolation rate of *S. saprophyticus* has to do with the type of patient population. Most of the female patients in this study were from adult population. All the isolates were obtained in pure cultures. *Staphylococcus saprophyticus* adheres better to urothelial cells than to other epithelial cells like buccal mucosal cells (Colleen *et al.*, 1979). It is rarely found in normal urethra, periurethral area (Sellin *et al.*, 1975); vagina and rectum (Paed *et al.*, 1977) in contrast to *S. epidermidis* which is present in anatomic site that contains indigenous flora. This might explain its' finding in such areas as opportunistic pathogen. Isolation of *S. saprophyticus* in wound infection is not common, even though Golledge, (1988) reported the isolation of *S. saprophyticus* from wound infection and septicaemia. In this study, *S. saprophyticus* was also isolated from wound swabs. This species is usually found in small transient population on a variety of body sites and might have been picked up from the skin.

Other species of CoNS found in the study included *S. capitis*(11.1%), *S. haemolyticus*(9.8%), *S. simulans*(9.8%), *S. warneri*(2.9%), *S. xylosus*(2.4%), *S.*

hominis(2.1%), *S. cohnii*(0.4%) and 1.3% which was unclassified. In this study, *S. capitis* which initially was not a common occurrence in many studies (Pfaller and Herwaldt, 1988), was isolated from various clinical specimens though in rather low figures except in wound infections. It accounted for 19.0% of all wound isolates after *S. epidermidis* (39.0%). This is in contrast to other reports (Varaldo *et al.*, 1984, Kleeman *et al.*, 1993, Kloos and Lambe, 1991). *S. capitis* has been reported to be found in large populations on the adult human head, especially on the scalp and forehead where sebaceous glands are numerous and well developed (Kloos, 1986, Kloos and Bannerman, 1995). The findings in this study might be due to the habitat of *S. capitis* since one isolate of this species from wound swab was from an accident victim with head injury. About 57.1% of *S. capitis* isolates were from otitis media discharge. This can be attributed to a study which reported that moderate-sized to large populations of this organism are found on the face, eyebrow and external auditory meatus (Kloos and Bannerman, 1994). The presence of *S. capitis* in blood culture isolates and UTIs is similar to that other reports found in Varaldo *et al.*, (1984), Pal and Ayyagari, (1989) and Bandres and Darouiche, (1992).

S. haemolyticus and *S. simulans* had a prevalence of 9.8% each. *S. haemolyticus* was more widely distributed than *S. simulans* in this study. *S. haemolyticus* accounted for 28.6% of otitis media isolates, 25.0% of conjunctivitis isolates, 17.4% of male secretions (seminal fluids) isolates, 16.7% of HVS and endocervical swab isolates and 14.3% of wound infections isolates. Low incidence was recorded from urine and blood isolates. This distribution of *S. haemolyticus* is not surprising since it is the second most frequently encountered species of CoNS in human infections and has been associated with various clinicl infections (Kloos and Bannerman, 1995, Low *et al.*, 1992). Although it did not

maintain that status in this study, it was probably due to the types of specimen sampled and that were available. *S. simulans* like other CoNS has been associated with some infections. This species is occasionally found on human skin (Kloos and Musselwhite, 1975) and in the urethra of healthy women (Marrie *et al.*, 1982). In this study its' isolation rate in infections is not surprising since on infrequent occasions it has been isolated from clinical specimens such as blood, urine, fluid, exudates from wounds, abscesses, lesions (Gill *et al.*, 1983, Marsik and Brake, 1982), chronic osteomyelitis and pyoarthritis (Males *et al.*, 1985), intravascular catheters (Freeman and Hjersing, 1980) and native valve endocarditis (NVE) (Jansen *et al.*, 1992).

The isolation rates being reported for *S. warneri, S. xylosus, S. hominis* and *S. cohnii* are expected since *S. epidermidis* is the most commonly isolated species of clinical importance, while data on their clinical significance are limited. The few isolates of *S. warneri* from this study were from wound infection sites, urine and bone secretions from osteomyelitis. This is in contrast to the few available data from which they were isolated mainly from blood cultures of patients suffering from endocarditis, *S. warneri* bacteraemia; vertebral osteomyelitis (Dan *et al.*, 1984, Wood *et al.*, 1989, Kamath *et al.*, 1992). Leighton and Little, (1986) isolated the organism from Urinary Tract Infections. Occurrence of *S. xylosus* in a variety of human infections has been reported with low frequencies (Kloos and Lambe, 1991). Isolation of 6(2.4%) strains of *S. xylosus* is high when compared to a lot of studies in which it was not isolated at all and at times below 0.5% of total samples(Kleeman *et al.*,1993, Narayani *et al.*, 1990). The findings here might be attributed to this species being prevalent in our environment.

S. hominis is another species of CoNS which has been reported to occur in low incidence in a variety of human infections (Bowman and Buck,1984, Kloos and Lambe,1991). This is true in this study. In the reports of studies conducted in two hospitals in USA and India respectively, *S. hominis* was among the three most common CoNS clinical isolates (Kleeman *et al.*, 1993, Narayani *et al.*, 1990). In the India study, *S. hominis* was the second isolated species both in healthy carrier and hospital patients. This showed the prevalence of *S. hominis* in those environments. The low prevalence of *S. cohnii* in this study is not surprising since it is one of the CoNS that have been reported to have had low incidence occurrence in a variety of human infections. The isolation rate of *S. cohnii* here is consistent with the study of Kleeman *et al.*,(1993). Two isolates of *S. lugdunensis* were obtained. This species is one of the relatively new species of CoNS and was originally isolated from human clinical specimens (Freney *et al.*, 1988). It was reported that this species accounted for 10% of all *Staphylococcus* species in human clinical specimens if *S. aureus* and *S. epidermidis* were excluded (Herchline and Ayers, 1991). In this study, 0.8% was obtained for *S. lugdunensis*. The reason for this percentage might be that the population of *S. lugdunensis* in human clinical specimens seems to be not very significant in Lagos. The isolation of this species was made possible by the use of rapid commercial kit API kit.

The API Kit (ID 32 STAPH) used in this study has an overall accuracy of 98.7%. When the API Kit method of characterization was compared to the conventional methods of characterization of Kloos and Schleifer (1975), there was no significant difference. But the API Kit was able to characterise 7 out of the 10 isolates that were unclassified by the conventional methods of Kloos and Schleifer (1975). Some of the isolates were also reclassified by the API Kit eg. the two isolates which were formally classified as *S. warneri*

were classified by the API Kit as *S. lugdunensis.* Also some clinical strains of *Stomatococcus* species which were misidentified as Staphylococci were re-classified. The reason for the misidentification might be because these strains showed a catalase positive reaction almost identical to those of ordinary Staphylococci strain. The API Kit was able to identify these *Stomatococcus* species which the conventional method could not do. Although, 3 isolates were still not characterised by the API Kit out of the 244 CoNS isolates, this system still provided a rapid and accurate method for identifying the various CoNS examined in this study. The 3 unclassified isolates had profile numbers that were not contained in the API profile register (ATB 32 STAPH BOOK). The main limitation to the use of this rapid commercial Kit is the availability of the kits and its cost in our environment. The accuracy in the use of some other commercial automated kits has been questioned (Bascomb and Manafi, 1998, Kloos and George, 1991, Kloos and Wolfshol, 1982). Using the conventional methods of Kloos and Schleifer (1975), most of the important prevalent human species of CoNS were identified in this study. However, with the newly described human species of CoNS for example, *S. lugdunensis* (Freney *et al.,* 1988) and borderline species, additional biochemical tests like the ones obtained in the Kloos and Bannerman (1995) scheme should be adopted. The only limitation is that some of these tests require special media and special equipments. With this scheme the newer species and subspecies of CoNS will accurately be identified where API Kit is not available.

Thus CoNS once regarded as contaminants and nonpathogens are now the subject of growing interest owing to their ability to cause infection under certain conditions, and their emergence as important nosocomial blood stream infections.

CONCLUSION

It is clear from this study that one should expect to isolate different species and strains of CoNS in our laboratories here in Lagos. *Staphylococcus epidermidis* has been shown to be the most prevalent species in various clinical specimens while *Staphylococcus saprophyticus* was shown to be an important opportunistic pathogen in urinary tract infections. *Staphylococcus capitis, S. haemolyticus* and *S. simulans* were isolated from many clinical specimens but with lower isolation rates when compared to *S. epidermidis*.

The clinical relevance and significance of CoNS continue to increase in our environment. When considering the relevance or significance of a CoNS isolate, several factors have to be considered including the source of the specimen or isolate, the relative numbers of organisms isolated, whether it is in pure or mixed culture and the clinical findings in the patient.

Organisms isolated from a closed source such as blood, subarachnoid space, joint or pleural space were considered more likely to be pathogens than organisms isolated from an open source such as wound, because of the surrounding microflora except for wounds which were fluctant or draining purulent material. Since CoNS are widely spread on the human body and can produce very large populations distinguishing the aetiologic agent(s) from contaminating flora is a serious challenge to the clinical laboratory. The solution to this problem will be facilitated by the quality of the specimen obtained from the patients and how accurately the specimens represent the infection.

Table 2.1: Sources of isolates of coagulase-negative Staphylococci in various specimens from patients with different diseases. (No. of isolates).

Clinical diagnosis	Wound	Blood	Urine	Urethra	Catheter tips	Ear	Vagina/ Cervix	Male secretions	Tissue biopsy	Bone secretions	Eye	Fluids	Total
Burns/skin sepsis	7	-	-	-	-	-	-	-	-	-	-	-	7
Multiple boils	5	-	-	-	-	-	-	-	-	-	-	-	5
Post op. Wound inf.	23	-	-	-	-	-	-	-	-	-	-	-	23
Ulcer (Leg. Penile) etc.	29	-	-	-	-	-	-	-	-	-	-	-	29
Urinary tract inf.	-	-	32	8	-	-	11	-	-	-	-	-	51
Otitis media	-	-	-	-	-	7	-	-	-	-	-	-	7
Osteomylitis	-	-	-	-	-	-	-	-	-	6	-	-	6
Urethritis	-	-	-	18	-	-	-	5	-	-	-	-	23
Prostatis/BPH	-	-	2	-		-	-	3	-	-	-	-	5
Peurperal sepsis	-	-	-	-	-	-	4	-	-	-	-	-	4
Endocarditis	-	2	-	-	-	-	-	-	-	-	-	-	2
Infertity	-	-	-	-	-	-	-	16	-	-	-	-	16
Pyleonephritis	-	-	7	-	-	-	-	-	-	-	-	-	7
Neonatal sepsis	-	23	-	-	2	-	-	-	-	-	-	-	25
Neonatal tetanus	-	2	-	-	-	-	-	-	-	-	-	-	2
Broncho Pneumonia	-	4	-	-	-	-	-	-	-	-	-	-	4
PUO	-	8	-	-	4	-	-	-	-	-	-	-	12
Thyroid septicaemia	-	-	-	-	2	-	-	-	-	-	-	-	2
Conjunctivitis	-	-	-	-	-	-	-	-	-	-	8	-	8
Pelvic infl. Dis.	-	-	-	-	-	-	3	-	-	-	-	-	3
Congestive heart Failure	-	-	-	-	-	-	-	-	-	-	-	1	1
Gastritis	-	-	-	-	-	-	-	-	4	-	-	-	4
Total	64	39	41	26	8	7	18	24	4	6	8	1	246

Key:
PUO – Pyrexia of unknown origin.
Post op. Wound inf. – post operative wound infection.
Pelvic infl. Dis. – Pelvic inflammatory disease.

Table 2.2: Biochemical characteristics of coagulase-negative Staphylococci (CoNS).

TEST	*S. capitis* (n=27)			*S. cohnii* (n=1)			*S. epidermidis* (n=109)			*S. haemolyticus* (n=19)			*S. hominis* (n=5)			*S. saprophyticus* (n=36)			*S. simulans* (n=24)			*S. warneri* (n=9)			*S. xylosus* (n=6)			*Unclassified* (n=10)		
	[a]+	±	-	+	±	-	+	±	-	+	±	-	+	±	-	+	±	-	+	±	-	+	±	-	+	±	-	+	±	-
Sucrose	22	1	4	0	0	1	109	0	0	19	0	0	5	0	0	36	0	0	24	0	0	8	1	0	0	0	6	5	1	4
Arabinose	0	0	27	0	0	1	0	0	109	0	0	19	0	0	5	0	0	36	0	0	24	0	0	9	4	2	0	0	0	10
Ribose	4	0	23	0	0	1	8	2	99	4	3	12	0	0	5	7	2	27	4	0	20	7	1	1	5	1	0	0	5	5
Trehalose	1	1	25	1	0	0	0	0	109	17	0	2	5	0	0	25	3	8	21	0	3	9	0	0	2	0	4	0	4	6
Mannitol	21	0	6	1	0	0	0	0	109	14	0	5	0	0	5	36	0	0	19	1	4	6	0	3	1	0	5	1	0	9
Fructose	26	0	1	1	0	0	109	0	0	19	0	0	5	0	0	33	1	2	24	0	0	9	0	0	0	1	5	0	2	8
Maltose	9	2	16	1	0	0	108	1	0	18	1	0	5	0	0	32	2	2	4	8	12	9	0	0	2	0	4	0	6	4
Xylitol	0	0	27	0	0	1	0	0	109	0	0	19	0	0	5	28	8	0	0	0	24	0	0	9	0	0	6	0	0	10
Lactose	0	3	24	1	0	0	97	4	8	13	3	3	4	0	1	23	2	11	22	2	0	2	0	7	0	0	6	3	2	5
Xylose	0	0	27	0	0	1	0	0	109	0	0	19	0	0	5	0	0	36	0	0	24	0	0	9	6	0	0	0	0	10
Nitrate reduction	21	0	6	1	0	0	102	0	7	18	0	1	5	0	0	5	0	31	22	0	2	4	0	5	5	0	1	6	0	4
Phosphatase activity	10	0	17	0	0	1	100	0	9	4	0	15	2	0	3	3	0	33	4	0	20	4	0	5	1	0	5	4	0	6
Acetoin production	4	15	8	0	1	0	95	13	1	8	8	3	1	1	3	25	11	0	2	2	20	6	3	0	1	2	4	2	3	5
[b]Anaerobic growth	19	8	0	0	1	0	100	9	0	5	11	3	0	1	4	20	16	0	22	2	0	8	1	0	4	2	0	4	0	6
Novobiocin resistance	0	0	17	1	0	0	0	9	100	0	0	19	0	5	0	36	0	0	0	0	24	0	0	5	5	0	1	0	0	10
[c]Haemolysis on sheep blood	0	8	19	0	1	15	30	64	17	2	0	0	1	4	3	0	33	0	0	15	9	0	4	5	0	0	6	5	0	5

KEY n= number of isolates tested

[a]= + positive: ± weak: -negative.

[b] = For anaerobic growth in thioglycollate medium

+ =Uniformly dense growth down the tube

± =Gradient of growth from dense to light down the tube

- No growth

[c] = For haemolysis + strong with wide zone

± =Trace

- =Negative.

Table 2.3: Frequency and distribution of coagulase-negative staphylococci isolated from clinical specimens by conventional method. No. (%) of isolates.

Species	Wound	Blood	Urine	Urethra	Catheter tips	Ear	Vagina/ Cervix	Male secretions	Bone secretions	Eye	Tissue biopsy	Sterile fluids	Total
S. capitis	12(18.8)	4(10.2)	2(4.9)	2(7.7)	1(12.5)	4(57.1)	-	2(8.3)	-	-	-	-	27(11.0)
S. cohnii	-	-	1(2.4)	-	-	-	-	-	-	-	-	-	1(0.4)
S. epidermidis	24(37.5)	30(76.9)	15(36.5)	12(46.2)	6(75.0)	-	4(22.2)	5(20.8)	3(50.0)	6(75.0)	4(100)	-	109(44.3)
S. haemolyticus	7(10.9)	1(2.6)	2(4.9)	-	-	2(28.6)	3(16.7)	2(8.3)	-	2(25.0)	-	-	19(7.7)
S. hominis	1(1.6)	1(2.6)	2(4.9)	-	1(12.5)	-	-	-	-	-	-	-	5(2.0)
S. saprophyticus	3(4.7)	-	12(29.3)	9(34.6)	-	-	7(38.9)	5(20.8)	-	-	-	-	36(14.6)
S. simulans	7(10.9)	1(2.6)	3(7.3)	2(7.7)	-	-	4(22.2)	6(25.0)	-	-	-	1(100)	24(9.8)
S. warneri	5(7.8)	-	2(4.9)	-	-	-	-	-	2(33.3)	-	-	-	9(3.7)
S. xylosus	2(3.1)	-	2(4.9)	1(3.8)	-	1(14.3)	-	-	-	-	-	-	6(2.4)
Unidentified	3(4.7)	2(5.1)	-	--	-	-	-	4(16.7)	1(16.7)	-	-	-	10(4.1)
Total	64(100)	39(100)	41(100)	26(100)	8(100)	7(100)	18(100)	24(100)	6(100)	8(100)	4(100)	1(100)	246(100)

Table 2.4: Frequency and distribution of coagulase-negative staphylococci isolated from clinical specimens by (API kit (ID 32 Staph) No. (%) of isolates.

Species	Wound	Blood	Urine	Urethra	Catheter tips	Ear	Vagina/ Cervix	Male secretions	Bone secretions	Eye	Tissue biopsy	Sterile fluids	Total
S. capitis	12(18.8)	4(10.2)	2(4.9)	2(7.7)	1(12.5)	4(57.1)	-	2(8.3)	-	-	-	-	27(11.0)
S. cohnii	-	-	1(2.4)	-	-	-	-	-	-	-	-	-	1(0.4)
S. epidermidis	24(37.5)	30(76.9)	15(36.5)	12(46.2)	6(75.0)	-	4(22.2)	5(20.8)	3(50.0)	6(75.0)	4(100)	-	109(44.3)
S. haemolyticus	9(14.0)	2(5.1)	2(4.9)	-	-	2(28.6)	3(16.7)	4(16.7)	-	2(25.0)	-	-	24(9.8)
S. hominis	1(1.6)	1(2.6)	2(4.9)	-	1(12.5)	-	-	-	-	-	-	-	5(2.0)
S. lugdunesis	2(3.2)	-	-	-	-	-	-	-	-	-	-	-	2(0.8)
S. saprophyticus	3(4.6)	-	12(29.3)	9(34.6)	-	-	7(38.9)	5(20.8)	-	-	-	-	36(14.6)
S. simulans	7(10.9)	1(2.6)	3(7.3)	2(7.7)	-	-	4(22.2)	6(25.0)	-	-	-	1(100)	24(9.8)
S. warneri	3(4.6)	-	2(4.9)	-	-	-	-	-	2(33.3)	-	-	-	7(2.8)
S. xylosus	2(3.2)	-	2(4.9)	1(3.8)	-	1(14.3)	-	-	-	-	-	-	6(2.4)
Unidentified	-	1(2.6)	-	-	-	-	-	1(4.16)	1(16.7)	-	-	-	3.(1.2)
Stomatococcus	1(1.6)	-	-	-	-	-	-	1(4.16)	-	-	-	-	2(0.8)
Total	64(100)	39(100)	41(100)	26(100)	8(100)	7(100)	18(100)	24(100)	6(100)	8(100)	4(100)	1(100)	246(100)

Table 2.5: Frequency and distribution of coagulase-negative staphylococci isolated from clinical specimens No. (%) of isolates.

Species	Wound	Blood	Urine	Urethra	Catheter tips	Ear	Vagina/ Cervix	Male secretions	Bone secretions	Eye	Tissue biopsy	Sterile fluids	Total
S. capitis	12(19.0)	4(10.2)	2(4.9)	2(7.7)	1(12.5)	4(57.1)	-	2(8.7)	-	-	-	-	27(11.0)
S. cohnii	-	-	1(2.4)	-	-	-	-	-	-	-	-	-	1(0.4)
S. epidermidis	24(38.1)	30(76.9)	15(36.5)	12(46.2)	6(75.0)	-	4(22.2)	5(21.7)	3(50.0)	6(75.0)	4(100)	-	109(44.3)
S. haemolyticus	9(14.0)	2(5.1)	2(4.9)	-	-	2(28.6)	3(16.7)	4(17.4)	-	2(25.0)	-	-	24(9.8)
S. hominis	1(1.6)	1(2.6)	2(4.9)	-	1(12.5)	-	-	-	-	-	-	-	5(2.0)
S. lugdunesis	2(3.2)	-	-	-	-	-	-	-	-	-	-	-	2(0.8)
S. saprophyticus	3(4.8)	-	12(29.3)	9(34.6)	-	-	7(38.9)	5(21.7)	-	-	-	1(100)	36(14.6)
S. simulans	7(11.1)	1(2.6)	3(7.3)	2(7.7)	-	-	4(22.2)	6(26.1)	-	-	-	-	24(9.8)
S. warneri	3(4.8)	-	2(4.9)	-	-	-	-	-	2(33.3)	-	-	-	7(2.8)
S. xylosus	2(3.2)	-	2(4.9)	1(3.8)	-	1(14.3)	-	-	-	-	-	-	6(2.4)
Unidentified	-	1(2.6)	-	-	-	-	-	1(4.36)	1(16.7)	-	-	-	3.(1.2)
Total	63(100)	39(100)	41(100)	26(100)	8(100)	7(100)	18(100)	23(100)	6(100)	8(100)	4(100)	1(100)	244(100)

Table 2.6 Comparison and distribution of coagulase-negative staphylococci from clinical specimens using conventional method and ID23 STAPH kit.

SPECIES	CONVENTIONAL METHOD	ID 32STAPH KIT
S. capitis	27	27
S. cohnii	1	1
S. epidermidis	109	109
S. haemolyticus	19	24
S. hominis	5	5
S. lugdunesis	-	2
S. saprophyticus	36	36
S. simulans	24	24
S. warneri	9	7
S. xylosus	6	6
S. stomatococcus spp	-	2
Unidentified	10	3
Total	246	246

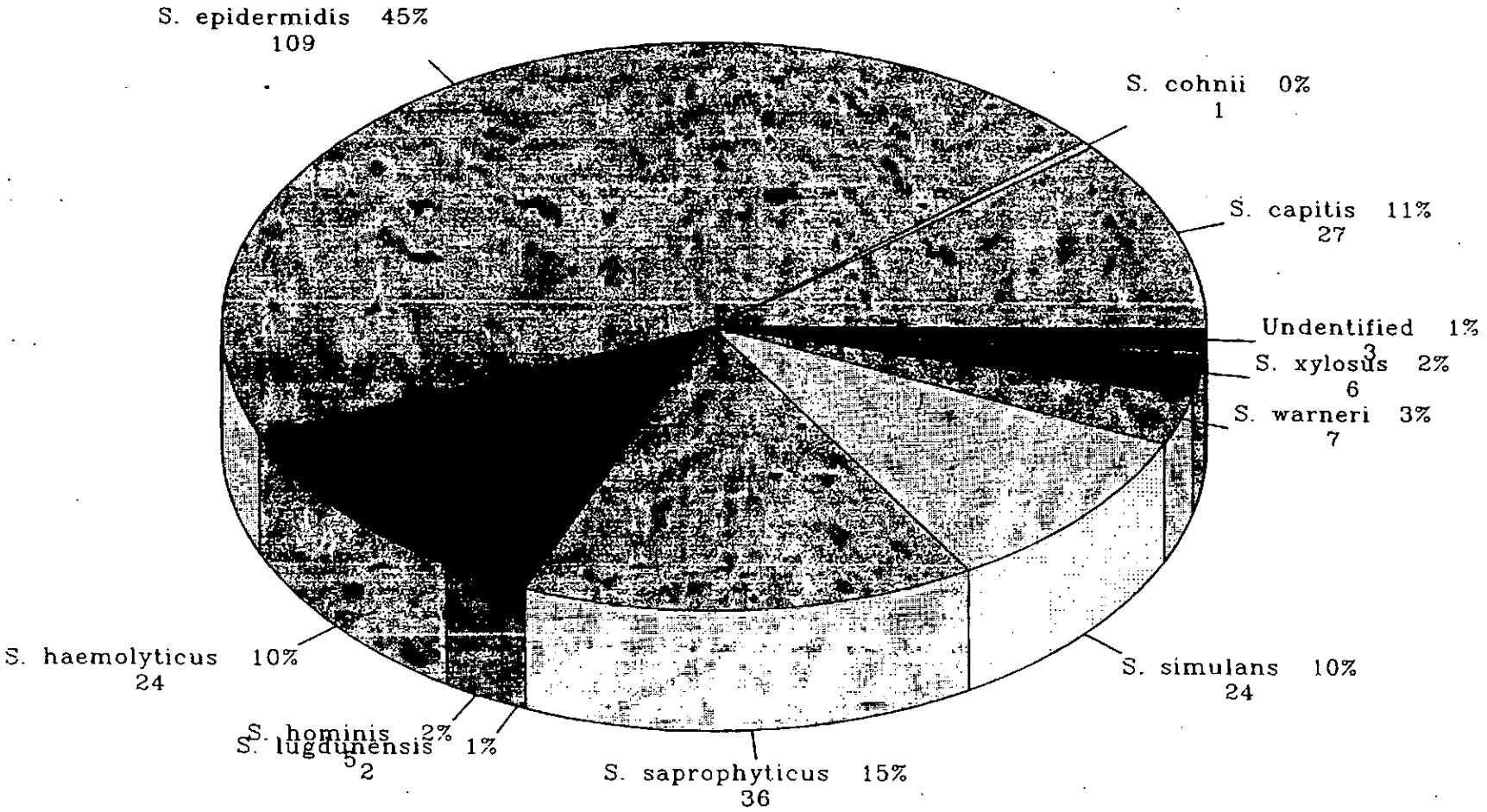
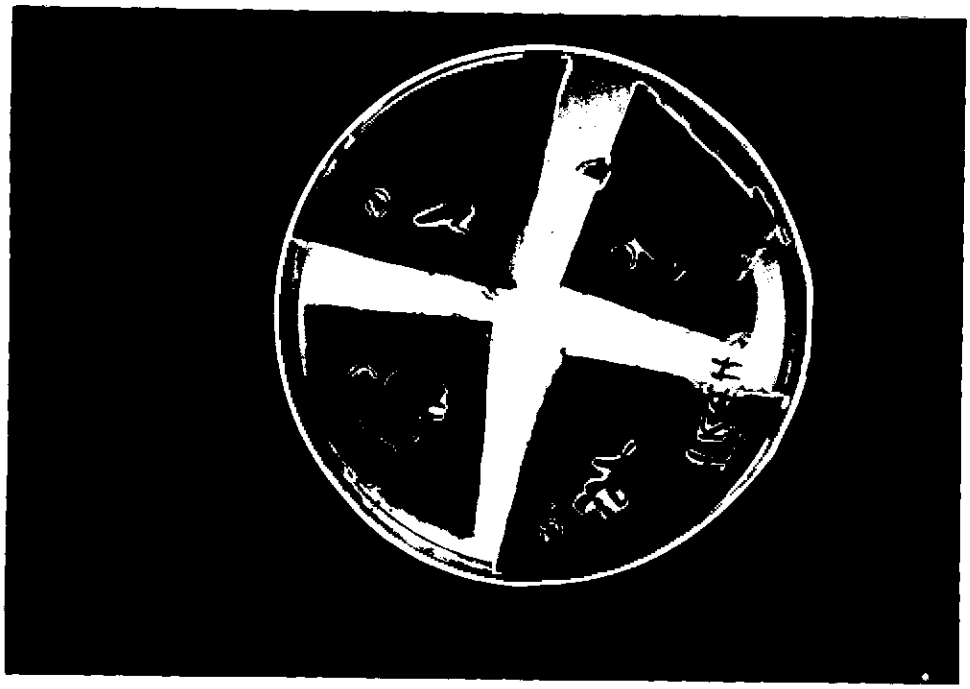
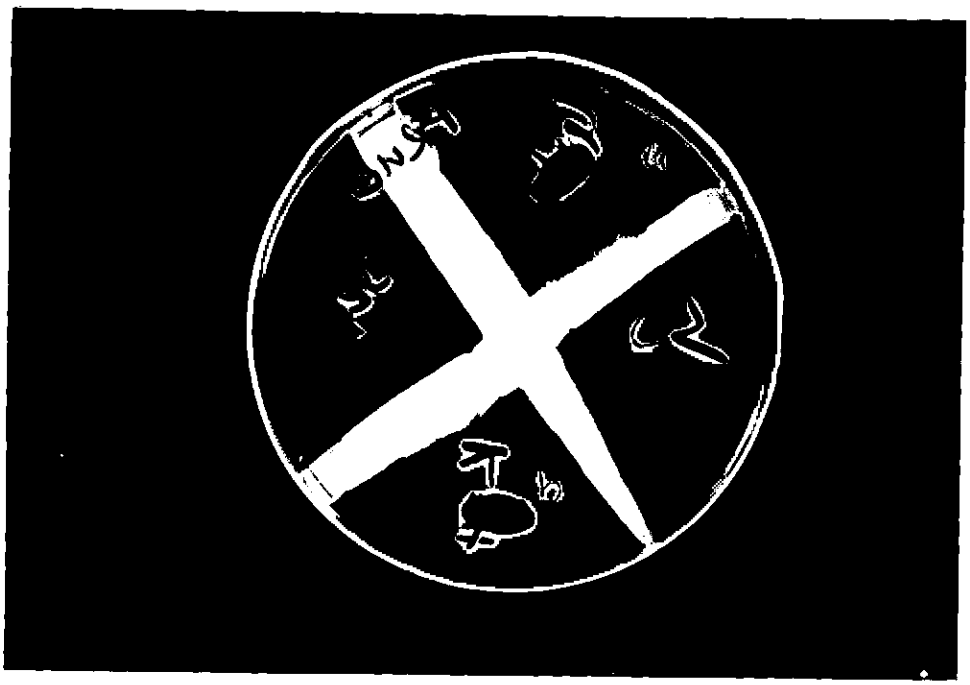


Fig. 2.1: Species distribution of coagulase-negative staphylococci isolates from clinical specimens.



A



B

Plates 2.1 Aerobic acid production from carbohydrates

A - Trehalose Carbohydrate Agar Plate.
B - Fructose Carbohydrate Agar Plate.
a = +ve (Strong acid production)
b = +ve (Moderate acid production)
c = -ve (No acid production)

CHAPTER 3

SLIME PRODUCTION AND EXTRACELLULAR PRODUCTS' ACTIVITIES OF COAGULASE-NEGATIVE STAPHYLOCOCCAL ISOLATES IN LAGOS.

INTRODUCTION

Coagulase production has been accepted in the past as the best *-in-vitro* proof of *Staphylococcus* pathogenicity. Coagulase-positive staphylococci are considered pathogenic while coagulase-negative staphylococci (CoNS) are regarded as non-pathogens or secondary contaminants when cultivated from infections (Oeding and Digranes, 1977; Parker, 1981). Today, this is not always correct because coagulase-negative staphylococci have been implicated in a variety of infections (Kloos and Bannerman, 1994; Hamory and Parisi, 1987; *Sattler et al.,* 1984; Pfaller and Herwaldt 1988; Kloos and Bannerman, 1995, Weinstein *et al.,* 1998). The balance between host resistance and the virulence of the infecting agent are the main factors determining the outcome of an infectious process. Host factors which may lead to serious infections are now well characterised. These include breaches in the natural mucocutaneous barriers (Kaiser, 1986), changes in therapeutic practice, for example prior exposure to antibiotics (Powell and Sanderson, 1987), immunosuppression (Peterson *et al.,* 1987), increased use of intravenous catheters and presence of indwelling prosthetic devices (Gristina, 1987, Burke, 1986). Apart from the presence of a foreign body which may facilitate infection by CoNS, these organisms possess microbial factors which help them to survive on a variety of biomaterials (Christensen *et al.,* 1982b). Studies have shown that once they attach to the surfaces of the implant a viscous extracellular polysaccharide substance called slime is produced (Christensen *et al.*, 1982b; Wilcox *et al.*, 1991). The biofilm produced by the slime on the devices creates a protective micro-environment (Peterson *et al.*, 1987). These staphylococci lack the enzyme

coagulase but appear to produce other virulence-factors which enable them to invade human tissues (Gemmell *et al.*, 1976; Parker, 1981)

The International Committee on the Nomenclature of bacteria in 1965 recommended that the production of both free and bound coagulase and the oxidation of mannitol both aerobically and anaerobically should be used to differentiate accurately strains of *S.aureus* from *S. epidermidis*. Other workers suggested the use of DNase or alpha haemolysin, production or the presence of protein A (Lachia and Deibel, 1969, Elek and Levy, 1950; Bernheimer, 1968; Oeding, 1967). Available evidence have shown that coagulase-negative staphylococci produce a wide variety of potential toxins and enzymes including haemolysins, cytotoxins, deoxyribonuclease (DNase), fibrinolysin, proteinase and lipase-esterase (Gemmell, 1986, Gemmell and Roberts, 1973, Gemmell and Schumacher-Perdreau, 1986). The authors noted strong similarities between the exoproteins produced by *Staphylococcus aureus* and coagulase-negative staphylococci (*S. epidermidis, S.haemolyticus and S.saprophyticus*) and suggested that these exoproteins may be important virulent factors in the infections of humans. Males *et al.*, (1975) showed differences in the frequency and toxigenicity amongst a group of *Staphylococcus epidermidis* isolates from various clinical sources. Epidemiological studies in man indicate that the ability to produce disease, as opposed to the ability to spread it, may be due to separate characteristics of the microorganism (Abramson, 1972). He cited the work of Ekstedt and Yotis (1960) which showed that intracerebral injection of coagulase or CoNS strains into mice was innocuous but that CoNS suspended in partially purified coagulase produced death in mice. This suggests that absorbed coagulase may protect the microorganism from normal bactericidal

serum factor or from phagocytosis.

The presence of DNase is often used in clinical laboratories for the presumptive characterization of *S.aureus*, either as well as or instead of coagulase for presumptive pathogenicity (Cowan and Steel, 1993). But production of DNase by strains of coagulase-negative Staphylococci has been reported by many authors (Males *et al.*, 1975; Gemmell, 1987, Vijayalakshmi *et al.*, 1980).

There is good evidence that coagulase-negative staphylococci owe at least part of their virulence for man to their ability to elaborate some of the exoproteins produced by *S.aureus* (Gemmell, 1986). Some of these exoprotein are recognised virulence factors and may play a similar role in the pathogenicity of the various coagulase-negative staphylococcal species. No study has been reported in the literature on the slime production, and some exoproteins (enzymes) production among clinical isolates of CoNS species in Lagos. The aim of this work therefore was: To provide information on slime producing ability of local strains in Lagos; To determine proteolytic, lipolytic and DNase activities among clinical isolates of these CoNS.

MATERIALS AND METHODS

Bacterial Strains:

Two hundred and thirty-seven (237) clinical isolates of coagulase-negative staphylococci (CoNS) were used for this study. The distribution of the various (CoNS) isolates was as follows: *Staphylococcus epidermidis* (105), *Staphylococcus capitis* (25), *S. cohnii* (1), *S. haemolyticus* (24), *S. hominis* (5) *S. lugdunesis* (2), *S. saprophyticus* (36), *S. simulans* (24), *S. warneri* (6), *S. xylosus* (6), Unclassified (3). These were isolated from clinical specimens obtained from the clinical microbiology laboratories of Lagos University Teaching Hospital and General Hospital, Ikeja between December 1994 and May 1996. These strains were isolated from in and out-patients suffering from infections of the urinary tract, bloodstream, eye, ear, bones, prostate, surgical site, skin etc. The specimens were processed bacteriologically using the conventional method of Kloos and Schleifer (1975) and API (ID32 STAPH) kit from Bio Merieux SA 69280 Marcy-l'Etoile France. The following reference strains *Staphylococcus aureus* ATCC 25923 obtained from Biotechnology laboratory, Nigerian Institute for Medical Research Yaba, *Staphylococcus epidermidis* ATCC 14990 and *Staphylococcus saprophyticus* ATCC 15305 obtained from Prof. W.E. Kloos, Department of Genetics, North Carolina State University USA were used as controls.

Slime Production:

Slime production was determined according to the method of Christensen *et al.*, (1982b). Briefly, a loopful of organism from a Purple agar (P-agar) or blood agar plate was inoculated into 5ml of tryptone soya broth in a test tube and incubated at 37°C for 24 hours. The content of the tube was aspirated and the tube was stained with safranin solution for 30 minutes. Slime production is indicated if a visible safranin stained film lined the wall of the tube. Slime production was recorded as weak or strong according to the density of the adherent film. *Staphylococcus epidermidis* ATCC 14990 was included as positive control.

DNase Activity:

DNase activity was detected on DNase agar (Oxoid) by the method described by Zeirdt and Golde (1970). DNase agar plates were spot inoculated wih the CoNS isolates so that growth was in plaques of about 2cm in diameter. Control organisms were also included on each plate. After overnight incubation at 35^{0}C for 18-24 hours the plate was flooded with 1N hydrochloric acid solution. Excess acid was tipped off. Clearing around the innoculum within 5 minutes of adding the acid was considered positive for DNase activity while an absence of a clearing was indicative of DNase negative strains. *S. aureus* ATTC 25923 was used as positive control and *S. epidermidis* ATCC 14990 as negative control.

Lipolytic (Lipase) and Proteolytic (Protease) Activities:

To assess lipolytic and proteolytic activities, CoNS isolates were streaked onto Baird-Parker medium (Oxoid). This medium consists of Baird-Parker agar base and 50ml egg yolk-tellurite emulsion (Oxoid SR54), which was added aseptically to the Baird-Parker agar base

after sterilization by autoclaving at 121^{0}C for 15 minutes.

The inoculated plates were examined after incubation at 35^{0}C for 3 days. On this medium, a pearly sheen was indicative of lipase activity, a zone of opacity was interpreted as phospholipase activity and clearing around single colonies was evidence of proteolysis (Deighton *et al., 1992*). *Staphylococcus aureus* ATCC 25923 was used as positive control while, *Bacillus subtilis* was the negative control.

Gelatinase Activity:

Gelatinase Activity was determined by spot inoculating a medium containing 15g gelatin (Difco), 2g of Peptone (Difco), 0.5g of yeast extract (Difco), 15g of agar and 50ml of distilled water (Shuttleworth and Colby 1992) and incubated for 48 hours at 30^{0}C. Cultures were flooded with saturated ammonium sulphate solution (Collins and lyne, 1970). Zones of clearing are formed around colonies of organisms producing gelatinase while no zones of clearing was indicative of negative result. *S. aureus* ATCC 25923 was used as positive control while *Eschericha coli* ATCC 25922 was used as negative control.

Statistical Analysis:

Data was analysed using an IBM-Compatible PC with Minitab and Fig-P Statistical Packages. Where appropriate the Chi-square (X^2) tests, analysis of variance and correlation coefficient values were used as test of significance or correlation of attributes. All statistical computations were performed to 95% confidence limits.

RESULTS

A total of 96 (40.5%)of CoNS were slime producers comprising of 40 (16.9%) strong producers and 56(23.6%) were weak producers (Table 3.1) *Staphylococcus epidermidis* accounted for 54(56.2%) of all slime producers. Out of a total of 96 CoNS slime producers, *S. epidermidis* accounted for 28 (70.0%) and 26(46.4%) of strong slime producers and weak slime producers respectively. *Staphylococcus haemolyticus* and *S.saprophyticus* accounted for 14(14.6%) and 12(12.5%) respectively of all slime producers.

Results of DNase activity, lipase activity, gelatinase activity and proteolytic activity of the various species of coagulase-negative staphylococci are contained in Table 3.2. Over one quarter of the CoNS species showed lipase activity, with greatest activities being shown by *S. haemolyticus* (54.2%), *S. lugdunensis* (50%), *S. capitis* (48.0%) and *S. simulans* (45.8%) respectively. DNase activity was detected in 11.8% of isolates while gelatinase and proteolytic activities occurred in 11.0% and 8.9% of isolates respectively.

Comparison between DNase activity with those of lipase, protease and gelatinase showed that there was no significant correlation between DNase activity with either protease or lipase activity ($p > 0.05$). A significant correlation was demonstrated between DNase activity and gelatinase activity ($r = 0.63$, $p < 0.05$) Fig. 3.1 .

DISCUSSION

About one-third of the coagulase-negative staphylococci isolates were slime producers. Slime production has been detected in most species of CoNS (Christensen *et al.*, 1983; Needham and Stempsey, 1984; Christensen *et al., 1985;* Deighton *et al.*, 1988; Pal and Ayyagari, 1989). In this study, over half of the strains of *S. epidermidis, S. cohnii, S. haemolyticus* and unclassified group produced slime or adhered to the sides of the glass test tubes compared with 33.3% of *S. saprophyticus*, 24% of *S. capitis*, 20% of *S. hominis* and 16.7% each of *S. simulans, S. warneri* and *S. xylosus*. The results obtained for *S. epidermidis* and *S. haemolyticus* is in agreement with the findings of Deighton *et al.*, (1988) while it is in contrast with their findings for other species. Deighton *et al.*, (1988) observed no slime production for *S. warneri, S. capitis, S. xylosus* and *S. cohnii* while these same species had adherence of 16.7%, 24.0%, 16.7% and 100% respectively in this study. In their study the only isolate of *S. cohnii* had no adherence while in this study the only isolate of *S. cohnii* obtained had 100% detection for slime although the adherence was weak. Since only one isolate of *S.cohnii* was obtained from either of the studies the source of the isolates becomes important. Secondly no deduction could be made because of the few strains of *S.cohnii* obtained.

The findings in this study are similar to the findings of Pal and Ayyagari (1989), who detected slime in all their isolates. Although Deighton *et al.*, (1988) and Pal and Ayyagari (1989), studies obtained their isolates from various clinical specimens, 29% of Deighton *et*

al., (1988) specimens were from catheter tips and prosthetic devices. In this study only 3.3% (Table 2.1) of the specimens were from catheter tips, whereas Pal and Ayyagari (1989) had none among the specimens. Thus, the difference in the sampling specimens or sources of the specimens might have contributed to the number, type and characteristics of the different isolates obtained in any of the studies. Christensen *et al.*, (1985) using a microtitre plate adherence method, also noted that the most strongly adherent strains were *S.epidermidis, S.haemolyticus* and *S.saprophyticus*, and only 42% of 33 *S.hominis* strains were non-adherent.

The hypothesis that slime production by some CoNS may play a part in enabling staphylococcal microcolonies to adhere to Holter shunts was first postulated by Bayston and Penny (1972). Christensen *et al.*, (1982b), investigating an outbreak of catheter-related sepsis subsequently recovered significant proportion of slime-producing strains from patients with sepsis than from contaminated blood cultures. Studies by Davenport *et al.*, (1986); Ishak *et al.*, (1985) supported the view that extracellular slime production is an important colonizing and virulence factor in CoNS infections associated with prosthetic device but Needham and Stempsey, (1984); West *et al.*, (1986) were unable to confirm these findings in their studies. Deighton *et al.*, (1988) from their observations suggested that although most CoNS, given a suitable conditions, are capable of producing slime, they only do so under environmental conditions in which extracellular polysaccharide enhances their virulence or their ability to colonize surfaces. Slime production assessed by qualitative or quantitative adherence assays has been associated with the characteristics of the strains associated with infection given a suitable growth medium. In this study, this association can

only be shown fully when other phenotyping systems for example antibiogram, plasmid analysis etc. are employed in the identification of CoNS. Thus, this will help in distinguishing between infective and non-infective isolates due to contamination or to transient skin flora bacteraemia in a patient.

Available evidence also suggests that at least part of the human virulence of CoNS can be attributed to their ability to elaborate some of the exoproteins for example proteases, urease, lipolytic enzymes, various haemolysins, DNase, gelatinase (Gemmell and Roberts, 1973, Gemmell, 1986, Gemmell, 1987). In this study all the CoNS species showed at least one of the activities except for *Staphylococcus cohnii* species which showed no activity while 68(28.7%) of CoNS showed lipase activity. Much of the lipase activity was shown by *S. capitis, S. haemolyticus, S. simulans* and *S. lugduensis*. Staphylococci produce several lipid-hydrolyzing enzymes, which collectively have been called lipases. The lipolytic activities of these enzymes have been reported as properties possessed by pyogenic staphylococci and some Clostridia (Davis, 1954). The biochemical mechanism of lipases which entail the release of free fatty acids (free oleic acid from lipoprotein a susbstrate in human plasma and the subsequent uncoupling of oxidative phosphorylation in mammalian cells is suggested as a basic concept in pathologic processes associated with staphylococcal infections. Gemmell and Roberts (1973) recognised the marked differences in the ability of various CoNS to hydrolyse various lipids and esters. They noted that in terms of lipolytic and *esterolytic* activity of CoNS that there was a much higher evidence of these enzymes and with a wider spectrum of activity in the group of CoNS strains from blood, abscesses and wounds than in the strains from the urinary tract. These bear closer resemblance to a similiar enzyme

elaborated by coagulase-positive *S. aureus* strains (Brunner *et al*; 1981).

DNase activity was shown in 28(11.8%) of the isolates. This is in agreement with the report of Gemmell, (1987) who noted that the relative incidence of this enzyme among coagulase-negative staphylococci has varied in various reports from 8-63%, but in contrast to the corresponding enzyme from *S. aureus*. Menzies (1977) reported the difference between DNase produced by coagulase-positive staphylococci (CoPS) and CoNS. He noted that CoPS strains produced heat stable DNase while the DNase produced by CoNS were heat liable. Gemmell *et al.*,(1981) by isoelectric focussing showed that DNase of CoNS were made up of three separate proteins with similar biological activity but distinct isoelectric points. The DNase cleaves the 5^1-phosphodiester bond to give a nucleoside 3^1-phosphate which then digests extracellular nucleic acid.

Coagulase-negative staphylococci have also been examined for their ability to elaborate proteolytic enzymes (Gemmell and Roberts, 1973, Gemmell, 1986, ShuttleWorth and Colby, 1992). These proteolytic enzymes represent a heterogenous group of enzyme systems using the following substrates gelatin, casein, milk agar, coagulated egg whites etc, (Abramson, 1972). Depending on the substrate, the enzymes hydrolyzing some of these substrates have been called caseinase, gelatinase, protease etc. In this study, protease (8.9%) and gelatinase (11.0%) activities were detected in most of the species except for strains of *S. cohnii, S. lugdunensis* and unclassified group. There was no gelatinase activity among the strains of *S. warneri* while none of the *S. hominis* strains showed any protease activity. In comparing DNase activity with those of lipase and protease no apparently significant

correlation was demonstrable with any of these enzymes ($p>0.05$). However, comparison between DNase activity and that of gelatinase showed significant correlation ($r=0.63$, $p<0.05$)(Figure 3.1). This might be attributed to the fact that DNase being produced is apparently not affecting the gene coding for gelatinase rather is apparently enhancing the enzyme activity by probably digesting certain genes whose gene products may impede gelatinase production.

Although the production of coagulase is characteristic of vast majority of staphylococci causing disease, epidemiological studies makes it clear that there are differences in virulence among coagulase-positive as well as coagulase-negative strains of staphylococci. Studies in mice indicate that the ability to produce disease as opposed to the ability to spread it may be due to separate characteristics of the microorganism (Eksedt and Yotis, 1960, Karas and Kapral, 1962). Thus the involvement of coagulase-negative staphylococci in various infections in many parts of the world is now well documented (Kloos and Bannerman, 1994, 1995; Pulverer, 1985, Udo *et al.*, 1997; Olusanya *et al.*, 1991; Kawamura *et al.*, 1998; Weinstein *et al.*,; 1998).

CONCLUSION

There is evidence from this study that some of the coagulase-negative staphylococci in our own environment elaborate some of the exoproteins produced by *S. aureus*. Since some of these are recognised virulence factors, they may play similar role in the pathogenicity of various coagulase-negative species in our environment. Slime production was also detected from strains of CoNS in our environment and this slime has been shown to be an important factor in colonization and infection of implanted foreign bodies and might play a role too in the establishment of infection on the surfaces of implanted foreign bodies in our environment.

Table 3.1: Slime production of clinical isolates of coagulase-negative *Staphylococci* strains.

Species	No. of strains tested	No. (%) of isolates		
		Strong slime positive	Weak slime positive	Total slime positive
Staphylococcus capitis	25	0	6(24.0)	6(24.0)
Staphylococcus cohnii	1	0	1(100.0)	1(100)
Staphylococcus epidermidis	105	28(26.7)	26(24.8)	54(51.4)
Staphylococcus haemolyticus	24	4(16.7)	10(41.7)	14(58.3)
Staphylococcus hominis	5	0	1(20.0)	1(20.0)
Staphylococcu lugdunensis	2	0	0	0(0)
Staphylococcus saprophyticus	36	5(13.9)	7(19.4)	12(33.3)
Staphylococcus simulans	24	1(4.2)	3(12.5)	4(16.7)
Staphylococcus warneri	6	0	1(16.7)	1(16.7)
Staphylococcus xylosus	6	0	1(16.7)	1(16.7)
Unclassified.	3	2(66.7)	0	2(66.7)
Total	**237**	**40(16.9%)**	**56(23.6%)**	**96(40.5)**

Table 3.2: Some extracellular products and enzymes of coagulase-negative staphylococci.

Species	No. of strains tested	No. (%) of isolates. Dnase activity	Lipase Activity	Proteolytic activity	Gelatinase activity
Staphylococcus capitis	25	2(8.0)	12(48.0)	2(8.0)	3(12.0)
Staphylococcus cohnii	1	0(0)	0(0)	0(0)	0(0)
Staphylococcus epidermidis	105	14(13.3)	18(17.2)	9(8.6)	8(7.6)
Staphylococcus haemolyticus	24	5(20.8)	13(54.3)	3(12.5)	2(8.3)
Staphylococcus hominis	5	1(20.0)	0(0)	0(0)	1(20.0)
Staphylococcu lugdunensis	2	0(0)	1(50.0)	0(0)	0(0)
Staphylococcus saprophyticus	36	2(5.6)	9(25.0)	2(5.6)	7(19.4)
Staphylococcus simulans	24	2(8.3)	11(45.8)	2(8.3)	4(16.7)
Staphylococcus warneri	6	0	2(33.3)	2(33.3)	0(0)
Staphylococcus xylosus	6	2(33.3)	2(33.3)	1(16.7)	1(16.7)
Unclassified.	3	0(0)	0(0)	0(0)	0(0)
Total	**237**	**28(11.8%)**	**68(28.7)**	**21(8.9)**	**26(11.0)**

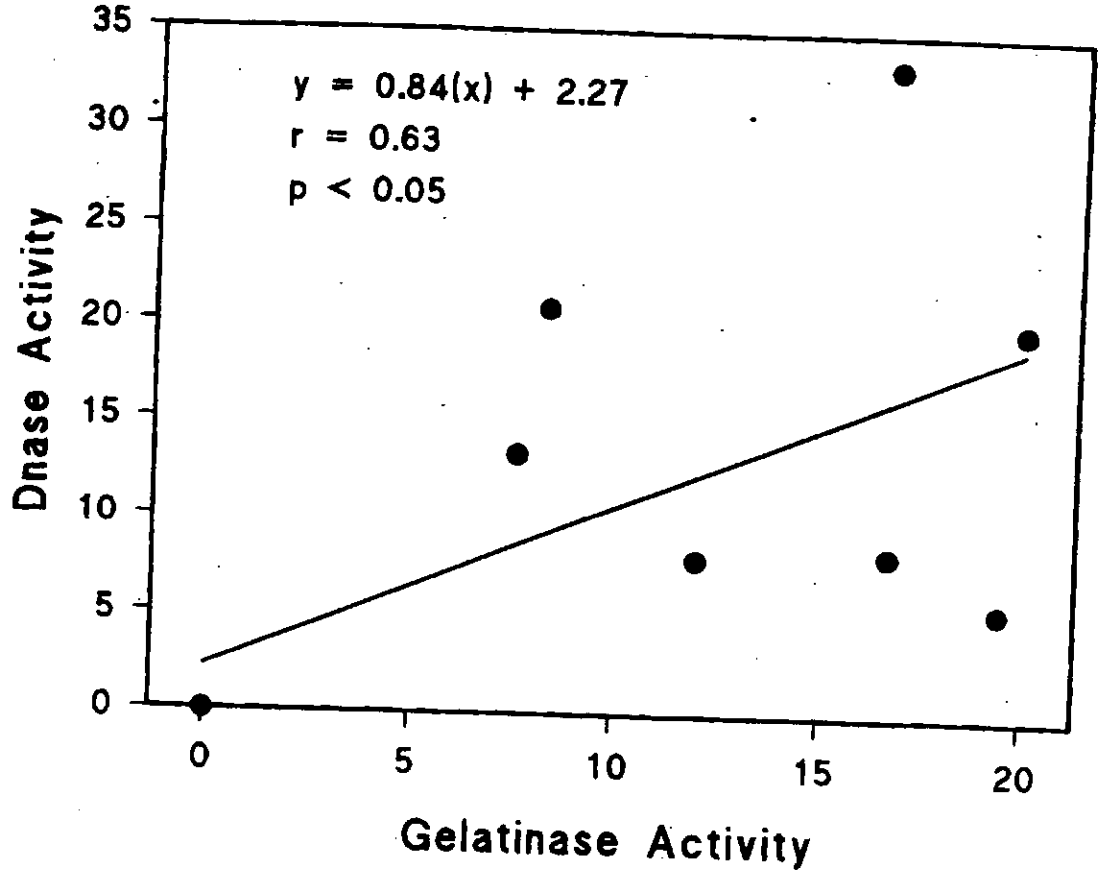


Figure 3.1. Regression line between DNAse activity and gelatinase activity of coagulase-negative staphylococci.

CHAPTER 4

ANTIMICROBIAL SUSCEPTIBILITY OF COAGULASE-NEGATIVE STAPHYLOCOCCAL ISOLATES TO SOME COMMONLY USED ANTIMICROBIAL AGENTS IN LAGOS.

INTRODUCTION

Antimicrobial resistance is increasing worldwide and common pathogenic bacteria have become increasingly resistant to widely used antimicrobials including those specified in the World Health Organisations' Model list of Essential Drugs (Farrar, 1985; WHO, 1992). Pathogenic microorganisms are part of the natural ecosystem. Microorganisms especially bacteria have always proliferated in human and animal tissues in which they have invariably caused disease, disability, deformity and even death. In some instances, they have established carrier states without apparent harm to the host (Fiennes, 1964).

Current antibiotic-prescribing practices including pre-operative antibiotic prophylaxis, have led to the selection of antibiotic-resistant organisms (Scaberg *et al.*, 1991). Changes in therapeutic practice, especially those that led to the longer survival of patients with defective immunity and increased use of intravenous catheters, have given a new importance to coagulase-negative staphylococci (CoNS) as pathogen (Lowy and Hammer, 1983). Recent reports on surveillance data taken from the National Nosocomial Infections Surveillance System in the United States of America during the late 1980s and early 1990s indicated that coagulase-negative staphylococci are among the five most commonly reported pathogen (in the fifth place at 9 to 9.7%) compared with 10 to 11.2% for *Staphylococcus aureus* in hospitals conducting hospital-wide surveillance (Jarvis and Martone, 1992; Scaberg *et al.*, 1991). Most noticeable shifts in the aetiology of nosocomial infections have been towards the more antibiotic resistant pathogens, of which the

coagulase-negative staphylococci are a major group (Bailey *et al* ., 1990).

In developing countries and in Nigeria, inappropriate use and inadequate monitoring of antibiotic therapy particularly in high-risk patients, might have further accelerated the rate of resistance development (Wolff, 1993; Odugbemi, 1981). Wrong administration of drugs has been known to increase the risk of super-infection with certain drugs or multiple drug regimens (Louria, 1971). Antimicrobial susceptibility varies among genera and species and to an extent within species therefore, there is the need to monitor antibiotic resistance in bacteria by susceptibility testing.

The aim of this study was to obtain reliable information about the susceptibility patterns of these pathogens.

MATERIALS AND METHODS

Bacterial Isolates Tested:

A total of 233 clinical isolates of coagulase-negative staphylococci collected from the Microbiology laboratories of the Lagos University Teaching Hospital Idi-Araba and General Hospital Ikeja, between December 1994 and May 1996, were characterised by method of Kloos and Schleifer (1975). These isolates were also confirmed to be coagulase-negative staphylococci by using API-Kit (ID 32 STAPH). These isolates were obtained from various clinical specimens which included high vaginal swabs, pus, blood, wound swabs, seminal fluids, eye and ear swabs, surgical sites, wound swabs. Quality control organism used was *S. aureus* ATCC 29213.

Antimcrobial Discs:

Gram-positive AB Biodisk containing the following antibiotics: ampicillin (10mg), chloramphenicol (10mg), cloxacillin (10mg), erythromycin (5mg), gentamicin (10mg), penicillin G (1 unit), streptomycin (10mg) and single discs of ceftriaxone (30mg), norfloxacin(10mg), ofloxacin(10mg), amoxycillin/clavulanic acid (30mg), methicillin (5mg), imipenem(10mg) and pefloxacin (5mg) were used.

Antimicrobial Susceptibility Testing:

Antimicrobial susceptibility testing of the clinical isolates of coagulase-negative staphylococci isolates was carried out by the disc-agar diffusion method of Bauer *et al.*,

(1966), modified and standardized by the National Committee for Clinical Laboratory Standard. A culture of each test organism in Muellar-Hinton broth (Oxoid) containing 10^7 CFU/ml equivalent to 0.5 Mcfarland turbidity standard was streaked onto Muellar-Hinton agar (Oxoid) using a sterile non-toxic swab (Sterillin Middlesex). The antibiotic discs were then placed on the surface of the innoculated agar plates for about 15 minutes. The plates were incubated at 37^0C for 24 hours. The diameter of the growth of inhibition zone around each antibiotic disc was measured in millilitres using a ruler. Interpretation of results was according to the National Committee for Clinical Laboratory Standards(NCCLS) (1993a) interpretive guidelines. Reference strains *of Staphylococcus aureus* ATCC 29213 was included as control.

Determination of methicillin susceptibility to Coagulase-negative Staphylococci:

Disc diffusion method was carried out according to NCCLS disc diffusion standard described above and as modified by Woods *et al.*, (1984). Muellar-Hinton agar containing 4% NaCl was used. A direct innoculation was used. The direct innoculation was prepared from growth on an 18 to 24 hours blood agar plate (P-sheep blood agar) by suspending colonies in 0.85% sterile saline to yield a turbidity equal to 0.5 Mcfarland standard (approximately 10^7-10^8 CFU/ml). Methicillin (5mg) discs were used. Plates were incubated at 35°C for 24 hours. Isolates with intermediate or susceptible zones at 24 hours were reincubated for an additional 24 hours. Results were read and interpreted as described above.

Determination of Minimum Inhibitory Concentration (MIC):

The MICs of some of the antimicrobial agents to the different species of CoNS were determined by the microdilution method as described by Woods and Washington (1995) based on guidelines by the National Committee for Clinical Laboratory Standard (NCCLS) (1993b). The preparation of materials, reagents, inoculum, antibiotic dilution procedure and inoculation of test plates is shown in Appendix 2.

RESULTS

Tables 4.1 - 4.11 summarized the percentages of the different species of coagulase-negative staphylococci strains to the drugs tested with their MIC values. Penicillin G (100%) was inactive against all the different strains of CoNS while Imipenem (88.9-100%) was the most effective. Other highly effective drugs with their different susceptibility ranges for different species profiles include ceftriaxone (66.7 - 100%); ofloxacin (66.7 - 100%), pefloxacin (80.6 - 100%) and norfloxacin (50 - 100%) with MIC values ranging between 2.9 - 46.9mg/ml 0.06 - 0.50mg/ml; 0.06 - 0.50mg/ml respectively. Chloramphenicol was very effective against *S. capitis* strains (91.0%); *S. hominis* (80%) and *S. cohnii* (100%); *S. epidermidis* (60.6%); *S. saprophyticus* (61.1%); *S. haemolyticus* (66.7%) with MIC range of 1000 - 4000mg/ml. Over 50% of *S. epidermidis, S. warneri, S. hominis, S. cohnii* were susceptible to Erythromycin. Gentamicin was moderately effective against *S. epidermidis, S. simulans, S. capitis, S. warneri* and *S. xylosus* with an MIC range of 46.87-3000mg/ml. Tetracycline was active against all strains of *S. hominis* and 54.5% of *S. capitis* with MIC range of 2-64mg/ml. About 53.6% of all the coagulase-negative staphylococci strains were resistant to tetracycline while 61.4% and 56.7% of CoNS were resistant to streptomycin and cloxacillin respectively. The following antibiotics; ampicillin, and amoxycillin/clavulanic acid had MIC ranges of 750 - 3000mg/ml and 23.4 - 1500 mg/ml, respectively, while 75% of CoNS were resistant to methicillin. The resistance patterns of all the species to the different drugs tested, are shown in Table 4.12 while plates 4.1 and 4.2 show the susceptibility patterns of CoNS to the gram-positive multi-discs and single disc used.

DISCUSSION

In this study all the species of coagulase-negative staphylococci (CoNS) were resistant to penicillin while a large proportion of the species were resistant to ampicillin, streptomycin, tetracycline and cloxacillin. In comparison with some reports in the literature, CoNS strains isolated in this study proved to be above the average resistance to antimicrobial agents recorded (Gill *et al.*, 1983; Marsik and Brake, 1982). This study agrees with several other studies, which have shown a high incidence of antibiotic resistance amongst CoNS isolated from both colonized and infected patients in hospitals (Deighton *et al.*, 1988, McAllister *et al.*, 1987; Younger *et al.*, 1987). Deighton *et al.*, (1988) in their study found most *S. haemolyticus* and half of *S. epidermidis* strains resistant to 5 or more antibiotics with 87% of the total isolates resistant to penicillin G. Antibiotic-resistance profiles reported from different parts of the world and from different hospitals vary (Richardson and Marples, 1982; Varaldo *et al.*, 1984; Jarlov and Hoibi, 1998), possibly reflecting different patterns of antibiotic use. Over 50% of the total CoNS strains in this study were susceptible to gentamicin, erythromycin and chloramphenicol, while some species showed over 50% resistance to these antibiotics. Varying antibiotic resistance patterns have been reported by other authors (Varaldo *et al.*, 1984; Jarlov and Hoibi, 1998).

With the increased isolation of clinically significant CoNS, interest in their susceptibility to various antimicrobial agents and the establishment of resistance to various agents has also increased (Kloos and Bannerman, 1994). *Staphylococcus epidermidis* had a wider

antimicrobial susceptibility patterns exhibited here than the other species. It has been stated that *S. epidermidis* strains tend to be resistant to a wider spectrum of antibiotics than the other CoNS strains (Marsik and Brake, 1982; Pfaller and Herwaldt, 1988; Udo *et al.*, 1997). The results in this study are in full agreement with this statement. But several other investigators have reported that strains of *S. haemolyticus* are significantly more resistant to antimicrobial agents than other staphylococcal species (Gill *et al.*, 1983, Davies *et al.*, 1986; Del Bene *et al.*, 1986). The findings in this study did not agree with this report because *S. haemolyticus* were more resistant only to ampicillin, cloxacillin, erythromycin, gentamicin, augmentin than were strains of *S. epidermidis*.

Vancomycin a glycopeptide has been considered the drug of choice when CoNS isolates are multiply resistant to commonly used antibiotics, (Kirby, 1984, Kloos and Bannerman, 1994). Although, vancomycin susceptibility was not done in this study because of the unavailability of the disc, imipenem, one of the other beta-lactams was used. In an earlier study, imipenem disc had been used to study vancomycin resistance (Schwalbe *et al.*, 1990). This antibiotic showed 93.1% efficacy to all CoNS strains in this study.

The quinolones, pefloxacin (80.6%-100%), ofloxacin (66.7%-100%) and norfloxacin (50-100%) used in this study were highly effective to CoNS. This is in agreement with the report of Kloos and Bannerman (1994). They concluded that the quinolones were a promising group of antibiotics that have a broad spectrum of activity. However, with the increased use of this group of antibiotics especially ciprofloxacin, there have been reports of resistant CoNS (Barry *et al.*, 1992; Dryden *et al.*, 1992). Studies have also indicated that

resistance to one quinolone may predispose the isolate to become resistant to other quinolones (Thomson *et al.*, 1991; Wilton *et al.*, 1992). Another antibiotic which was also highly effective was the third generation cephalosporins-ceftriaxone (66.7% - 100%). Similar results were obtained by other workers (Jones *et al.*, 1991).

All strains obtained in this study were resistant to penicillin. This result is at variance with other studies that obtained varying frequencies (Barcs *et al.*, 1989; Marples and Richardson 1981). This may be due to geographical location. Multiple drug resistance (resistance to over eight antibiotics) were encountered in this study especially with isolates from pus and other skin sites. This pattern of resistance might be probably as a result of endogenous origin of the isolates which might have acquired their pathogenicity and drug resistance from co-existing bacteria of other genera. Several authors in Nigeria have attributed the widespread resistance observed among pathogenic bacteria to commonly prescribed antibiotics to the indiscriminate use of these drugs (Olukoya *et al.*, 1988; Odugbemi, 1981).

CONCLUSION

It has been shown that strains of coagulase-negative staphylococci were highly resistant to commonly prescribed antibiotics in Lagos. Although, some of the drugs tested in this study had good *in-vitro* activity, but cost, availability and pharmacokinetics should be considered during chemotherapy especially in seriously-ill and immunocompromised patients.

Table 4.1. Antimicrobial Susceptibility profile of *Staphylococcus epidermidis* strains.

Antimicrobial agent (μg)	No. of Strains Tested	No. (%) Susceptible	No. (%) Moderately Susceptible.	No. (%) Resistant	MIC range (μg/ml)
Ampicillin (10)	104	22(21.2)	2(1.9)	80(76.9)	1500-3000
Chloramphenicol (10)	104	63(60.6)	8(7.7)	33(31.7)	1000-2000
Cloxacillin (10)	104	40(38.5)	3(2.9)	61(58.7)	375-750
Erythromycin (5)	104	58(55.8)	4(3.83)	42(40.4)	NT
Gentamicin (10)	104	61(58.7)	3(2.9)	40(38.5)	1.25-2.5
Penicillin G (1unit)	104	0(0)	0(0)	104(100)	NT
Streptomycin (10)	104	38(36.5)	1(0.9)	65(62.5)	1500-3000
Tetracycline (10)	104	45(43.3)	5(4.8)	54(51.9)	32-64
Ceftriaxone (30)	104	85(81.7)	3(2.9)	16(15.4)	2.9-5.9
Ofloxacin (10)	104	94(90.4)	2(1.9)	8(7.7)	0.20-0.35
Amoxycillin-Clavulanic acid (30)	104	54(51.9)	0(0)	50(48.1)	46.9-93.75
Methicillin (5)	104	21(20.2)	0(0)	83(79.8)	NT
Pefloxacin (5)	104	88(84.6)	4(3.8)	12(11.5)	0.25-0.50
Imipenem (10)	104	95(91.3)	1(0.9)	8(7.7)	NT
Norfloxacin (10)	104	89(85.6)	4(3.8)	11(10.6)	NT

NT = Not Tested

Table 4.2. Antimicrobial Susceptibility profile of *Staphylococcus saprophyticus* strains.

Antimicrobial agent (μg)	No. of Strains Tested	No. (%) Susceptible	No. (%) Moderately Susceptible.	No. (%) Resistant	MIC range (μg/ml)
Ampicillin (10)	36	23(63.9)	3(8.3)	10(27.8)	750-1500
Chloramphenicol (10)	36	22(61.1)	3(8.3)	11(30.6)	1000-2000
Cloxacillin (10)	36	18(50.0)	2(5.6)	16(44.4)	375-750
Erythromycin (5)	36	23(63.9)	6(16.7)	7(19.4)	
Gentamicin (10)	36	17(47.2)	1(2.8)	18(50.0)	0.31-0.63
Penicillin G (1unit)	36	0(0)	0(0)	36(100)	NT
Streptomycin (10)	36	12(33.3)	1(2.8)	23(63.9)	1500-3000
Tetracycline (10)	36	13(36.1)	1(2.8)	22(61.1)	16-32
Ceftriaxone (30)	36	27(75.0)	3(8.3)	6(16.7)	23.4-46.9
Ofloxacin (10)	36	30(83.3)	2(5.6)	4(11.1)	0.06-0.13
Amoxycillin/Clavulanic acid (30)	36	14(38.9)	1(2.8)	21(58.3)	375-750
Methicillin (5)	36	9(25.0)	2(5.6)	25(69.4)	NT
Pefloxacin (5)	36	29(80.6)	3(8.3)	4(11.1)	0.13-0.25
Imipenem (10)	36	32(88.9)	1(2.8)	3(8.3)	NT
Norfloxacin (10)	36	25(69.4)	4(11.1)	7(19.4)	NT

NT = Not Tested

Table 4.3. Antimicrobial Susceptibility profile of *Staphylococcus haemolyticus* strains.

Antimicrobial agent (µg)	No. of Strains Tested	No. (%) Susceptible	No. (%) Moderately Susceptible.	No. (%) Resistant	MIC range (µg/ml)
Ampicillin (10)	24	3(12.5)	0(0)	21(87.5)	1500-3000
Chloramphenicol (10)	24	16(66.7)	2(8.3)	6(25.0)	1000-2000
Cloxacillin (10)	24	6(25.0)	1(4.2)	17(70.8)	375-750
Erythromycin (5)	24	10(41.7)	1(4.2)	13(54.2)	
Gentamicin (10)	24	6(25.0)	1(4.2)	17(70.8)	40-80
Penicillin G (1unit)	24	0(0)	0(0)	24(100)	NT
Streptomycin (10)	24	9(37.5)	1(4.2)	14(58.3)	1500-3000
Tetracycline (10)	24	12(50.0)	1(4.2)	11(45.8)	2-4
Ceftriaxone (30)	24	17(70.8)	1(4.2)	6(25.0)	23.4-46.9
Ofloxacin (10)	24	18(75.0)	1(4.2)	5(20.8)	0.13-0.25
Amoxycillin/Clavulanic acid (30)	24	6(25.0)	0(0)	18(75.0)	750-1500
Methicillin (5)	24	7(29.2)	0(0)	17(70.8)	NT
Pefloxacin (5)	24	22(91.7)	2(8.3)	0(0)	0.06-0.13
Imipenem (10)	24	23(95.8)	1(4.2)	0(0)	NT
Norfloxacin (10)	24	24(100)	0(0)	0(0)	NT

NT = Not Tested

Table 4.4. Antimicrobial Susceptibility profile of *Staphylococcus simulans* strains.

Antimicrobial agent (μg)	No. of Strains Tested	No. (%) Susceptible	No. (%) Moderately Susceptible.	No. (%) Resistant	MIC range (μg/ml)
Ampicillin (10)	24	4(16.7)	1(4.2)	19(79.2)	750-1500
Chloramphenicol (10)	24	9(37.5)	1(4.2)	14(58.3)	2000-4000
Cloxacillin (10)	24	6(25.0)	0(0)	18(75.0)	375-750
Erythromycin (5)	24	7(29.2)	1(4.2)	16(66.7)	NT
Gentamicin (10)	24	13(54.2)	3(12.5)	8(33.3)	10-20
Penicillin G (1unit)	24	0(0)	0.(0)	24(100)	NT
Streptomycin (10)	24	4(16.7)	0(0)	20(83.3)	1500-3000
Tetracycline (10)	24	6(25.0)	0(0)	18(75.0)	16-32
Ceftriaxone (30)	24	19(79.2)	2(8.3)	3(12.5)	2.9-5.9
Ofloxacin (10)	24	17(70.8)	2(8.3)	5(20.8)	0.08-0.15
Amoxycillin/Clavulanic acid (30)	24	7(29.2)	0(0)	17(70.8)	46.9-93.75
Methicillin (5)	24	8(33.3)	0(0)	16(66.7)	NT
Pefloxacin (5)	24	22(91.7)	0(0)	2(8.3)	0.5-1.0
Imipenem (10)	24	22(91.7)	1(4.2)	1(4.2)	NT
Norfloxacin (10)	24	21(87.5)	0(0)	3(12.5)	NT

NT = Not Tested

Table 4.5. Antimicrobial Susceptibility profile of *Staphylococcus capitis* strains.

Antimicrobial agent (μg)	No. of Strains Tested	No. (%) Susceptible	No. (%) Moderately Susceptible.	No. (%) Resistant	MIC range (μg/ml)
Ampicillin (10)	22	8(36.4)	1(4.5)	13(59.1)	750-1500
Chloramphenicol (10)	22	20(91.0)	2(9.1)	0(0)	1000-2000
Cloxacillin (10)	22	8(36.4)	2(9.1)	12(54.5)	750-1500
Erythromycin (5)	22	9(40.9)	2(9.1)	11(50.0)	NT
Gentamicin (10)	22	17(77.3)	0(0)	5(22.7)	0.63-1.25
Penicillin G (1unit)	22	0(0)	0(0)	22(100)	NT
Streptomycin (10)	22	16(72.7)	2(9.1)	4(18.2)	46.87-93.75
Tetracycline (10)	22	12(54.5)	1(4.5)	9(40.9)	2-4
Ceftriaxone (30)	22	17(77.3)	5(22.7)	0(0)	2.9-5.9
Ofloxacin (10)	22	20(91.0)	2(9.1)	0(0)	0.25-0.50
Amoxycillin/Clavulanic acid (30)	22	5(22.7)	0(0)	17(77.3)	23.4-46.9
Methicillin (5)	22	4(18.2)	1(4.5)	17(77.3)	NT
Pefloxacin (5)	22	18(81.8)	2(9.1)	2(9.1)	0.5-1.0
Imipenem (10)	22	22(100)	0(0)	0(0)	NT
Norfloxacin (10)	22	17(77.3)	2(9.1)	3(13.6)	NT

NT = Not Tested

Table 4.6. Antimicrobial Susceptibility profile of *Staphylococcus warneri* strains.

Antimicrobial agent (μg)	No. of Strains Tested	No. (%) Susceptible	No. (%) Moderately Susceptible.	No. (%) Resistant	MIC range (μg/ml)
Ampicillin (10)	6	3(50.0)	0(0)	3(50.0)	750-1500
Chloramphenicol (10)	6	4(66.7)	0(0)	2(33.3)	1000-2000
Cloxacillin (10)	6	6(100)	0(0)	0(0)	187.5-375
Erythromycin (5)	6	4(66.7)	0(0)	2(33.3)	NT
Gentamicin (10)	6	6(100)	0(0)	0(0)	0.04-0.08
Penicillin G (1unit)	6	0(0)	0(0)	6(100)	NT
Streptomycin (10)	6	1(16.7)	0(0)	5(83.3)	1500-3000
Tetracycline (10)	6	2(33.3)	0(0)	4(66.7)	8-16
Ceftriaxone (30)	6	6(100)	0(0)	0(0)	2.9-5.9
Ofloxacin (10)	6	6(100)	0(0)	0(0)	0.13-0.25
Amoxycillin/Clavulanic acid (30)	6	0(0)	2(33. 3)	4(66.7)	46.9-93.75
Methicillin (5)	6	2(33.3)	0(0)	4(66.7)	NT
Pefloxacin (5)	6	6(100)	0(0)	0(0)	0.06-0.13
Imipenem (10)	6	6(100)	0(0)	0(0)	NT
Norfloxacin (10)	6	6(100)	0(0)	0(0)	NT

NT = Not Tested

Table 4.7. Antimicrobial Susceptibility profile of *Staphylococcus xylosus* strains.

Antimicrobial agent (μg)	No. of Strains Tested	No. (%) Susceptible	No. (%) Moderately Susceptible.	No. (%) Resistant	MIC range (μg/ml)
Ampicillin (10)	6	1(16.7)	2(33.3)	3(50.0)	750-1500
Chloramphenicol (10)	6	2(33.3)	0(0)	4(66.7)	1000-2000
Cloxacillin (10)	6	2(33.3)	0(0)	4(66.7)	375-750
Erythromycin (5)	6	2(33.3)	0(0)	4(66.7)	NT
Gentamicin (10)	6	4(66.7)	0(0)	2(33.3)	0.15-0.31
Penicillin G (1unit)	6	0(0)	0(0)	6(100)	NT
Streptomycin (10)	6	0(0)	2(33.3)	4(66.7)	1500-3000
Tetracycline (10)	6	2(33.3)	0(0)	4(66.7)	16-32
Ceftriaxone (30)	6	4(66.7)	0(0)	2(33.3)	11.7-23.4
Ofloxacin (10)	6	6(100)	0(0)	0(0)	0.09-0.19
Amoxycillin/Clavulanic acid (30)	6	2(33.3)	0(0)	4(66.7)	23.4-46.9
Methicillin (5)	6	2(33.3)	0(0)	4(66.7)	NT
Pefloxacin (5)	6	6(100)	0(0)	0(0)	0.25-0.50
Imipenem (10)	6	6(100)	0(0)	0(0)	NT
Norfloxacin (10)	6	3(50.0)	3(50.0)	0(0)	NT

NT = Not Tested

Table 4.8. Antimicrobial Susceptibility profile of *Staphylococcus hominis* strains.

Antimicrobial agent (μg)	No. of Strains Tested	No. (%) Susceptible	No. (%) Moderately Susceptible.	No. (%) Resistant	MIC range (μg/ml)
Ampicillin (10)	5	1(20.0)	0(0)	4(80.0)	1500-3000
Chloramphenicol (10)	5	4(80.0)	1(20.0)	0(0)	1000-2000
Cloxacillin (10)	5	4(80.0)	1(20.0)	0(0)	375-750
Erythromycin (5)	5	5(100)	0(0)	0(0)	NT
Gentamicin (10)	5	1(20.0)	0(0)	4(80.0)	40-80
Penicillin G (1unit)	5	0(0)	0(0)	5(100)	NT
Streptomycin (10)	5	2(40.0)	0(0)	3(60.0)	1500-3000
Tetracycline (10)	5	5(100)	0(0)	0(0)	2-4
Ceftriaxone (30)	5	4(80.0)	1(20.0)	0(0)	2.9-5.9
Ofloxacin (10)	5	5(100)	0(0)	0(0)	0.06-0.13
Amoxycillin/Clavulanic acid (30)	5	2(40.0)	0(0)	3(60.0)	375-750
Methicillin (5)	5	1(20.0)	0(0)	4(80.0)	NT
Pefloxacin (5)	5	5(100)	0(0)	0(0)	0.06-0.13
Imipenem (10)	5	5(100)	0(0)	0(0)	NT
Norfloxacin (10)	5	5(100)	0(0)	0(0)	NT

NT = Not Tested

Table 4.9. Antimicrobial Susceptibility profile of *Staphylococcus lugdunensis* strains.

Antimicrobial agent (μg)	No. of Strains Tested	No. (%) Susceptible	No. (%) Moderately Susceptible.	No. (%) Resistant	MIC range (μg/ml)
Ampicillin (10)	2	1(50.0)	0(0)	1(50.0)	750-1500
Chloramphenicol (10)	2	1(50.0)	0(0)	1(50.0)	2000-4000
Cloxacillin (10)	2	2(100)	0(0)	0(0)	375-750
Erythromycin (5)	2	1(50.0)	0(0)	1(50.0)	NT
Gentamicin (10)	2	0(0)	0(0)	2(100)	40-80
Penicillin G (1unit)	2	0(0)	0(0)	2(100)	NT
Streptomycin (10)	2	1(50.0)	0(0)	1(50.0)	1500-3000
Tetracycline (10)	2	2(100)	0(0)	0(0)	2-4
Ceftriaxone (30)	2	2(100)	0(0)	0(0)	2.9-5.9
Ofloxacin (10)	2	2(100)	0(0)	0(0)	0.08-0.15
Amoxycillin/Clavulanic acid (30)	2	0(0)	0(0)	2(100)	375-750
Methicillin (5)	2	1(50.0)	0(0)	1(50.0)	NT
Pefloxacin (5)	2	2(100)	0(0)	0(0)	0.13-0.25
Imipenem (10)	2	2(100)	0(0)	0(0)	NT
Norfloxacin (10)	2	2(100)	0(0)	0(0)	NT

NT = Not Tested

Table 4.10. Antimicrobial Susceptibility profile of *Staphylococcus cohnii* strains.

Antimicrobial agent (μg)	No. of Strains Tested	No. (%) Susceptible	No. (%) Moderately Susceptible.	No. (%) Resistant	MIC range (μg/ml)
Ampicillin (10)	1	0(0)	0(0)	1(100)	1500-3000
Chloramphenicol (10)	1	1(100)	0(0)	0(0)	1000-2000
Cloxacillin (10)	1	0(0)	0(0)	1(100)	750-1500
Erythromycin (5)	1	1(100)	0(0)	0(0)	NT
Gentamicin (10)	1	0(0)	0(0)	1(100)	20-40
Penicillin G (1unit)	1	0(0)	0(0)	1(100)	NT
Streptomycin (10)	1	0(0)	0(0)	1(100)	1500-3000
Tetracycline (10)	1	1(100)	0(0)	0(0)	16-32
Ceftriaxone (30)	1	1(100)	0(0)	0(0)	2.9-5.9
Ofloxacin (10)	1	1(100)	0(0)	0(0)	013.-0.25
Amoxycillin/Clavulanic acid (30)	1	0(0)	0(0)	1(100)	46.9-93.75
Methicillin (5)	1	0(0)	0(0)	1(100)	NT
Pefloxacin (5)	1	1(100)	0(0)	0(0)	0.13-0.25
Imipenem (10)	1	1(100)	0(0)	0(0)	NT
Norfloxacin (10)	1	1(100)	0(0)	0(0)	NT

NT = Not Tested

Table 4.11. Antimicrobial Susceptibility profile of unclassified coagulase-negative Staphylococci strains.

Antimicrobial agent (μg)	No. of Strains Tested	No. (%) Susceptible	No. (%) Moderately Susceptible.	No. (%) Resistant	MIC range (μg/ml)
Ampicillin (10)	3	1(33.3)	0(0)	2(66.7)	1500-3000
Chloramphenicol (10)	3	0(0)	0(0)	3(100)	2000-4000
Cloxacillin (10)	3	0(0)	0(0)	3(100)	750-1500
Erythromycin (5)	3	0(0)	0(0)	3(100)	NT
Gentamicin (10)	3	0(0)	0(0)	3(100)	40-80
Penicillin G (1unit)	3	0(0)	0(0)	3(100)	NT
Streptomycin (10)	3	0(0)	0(0)	3(100)	1500-3000
Tetracycline (10)	3	0(0)	0(0)	3(100)	32-64
Ceftriaxone (30)	3	3(100)	0(0)	0(0)	2.9-5.9
Ofloxacin (10)	3	2(66.7)	1(33.3)	0(0)	0.20-0.35
Amoxycillin/Clavulanic acid (30)	3	0(0)	0(0)	3(100)	46.9-93.75
Methicillin (5)	3	0(0)	0(0)	3(100)	NT
Pefloxacin (5)	3	3(100)	0(0)	0(0)	0.25-0.50
Imipenem (10)	3	3(100)	0(0)	0(0)	NT
Norfloxacin (10)	3	2(66.7)	0(0)	1(33.3)	NT

NT = Not Tested

Table 4.12: Antibiotic resistance patterns of coagulase-negative Staphylococcal species.

Species	No. of Strains	NUMBER (% RESISTANCE)														
		Ampicillin	Chloramphenicol	Cloxacillin	Erythromycin	Gentamicin	Penicillin	Streptomycin	Tetracycline	Ceftriaxone	Ofloxacin	Pefloxacin	Norfloxacin	Imipenem	Amoxycillin/ Clavulanic acid	Methicillin
S. capitis	22	13(59.1)	-	12(54.5)	11(50.0)	5(22.7)	22(100)	4(18.2)	9(40.9)	-	-	2(9.1)	3(13.6)	-	17(77.3)	17(77.3)
S. cohnii	1	1(100)	-	1(100)	-	1(100)	1(100)	1(100)	-	-	-	-	-	-	1(100)	1(100)
S. epidermidis	104	84(76.9)	33(31.7)	61(58.6)	42(40.4)	40(38.5)	104(100)	65(62.5)	54(45.8)	16(15.4)	8(7.7)	12(11.5)	11(10.6)	8(7.7)	50(48.1)	83(79.8)
S. haemolyticus	24	21(87.5)	6(25.0)	17(70.8)	13(54.2)	17(70.8)	24(100)	14(58.3)	11(45.8)	6(25.0)	5(20.8)	-	-	-	18(75.0)	17(70.8)
S.. hominis	5	4(80.0)	-	-	-	4(80.0)	5(100)	3(60.0)	-	-	-	-	-	-	3(60.0)	4(80.0)
S. lugdunensis	2	1(50.0)	1(50.0)	-	1(50.0)	2(100)	2(100)	1(50.0)	-	-	-	-	-	-	2(100)	1(50.0)
S. saprophyticus	36	10(27.8)	11(30.5)	16(44.4)	7(19.4)	18(50.0)	36(100)	23(63.9)	22(61.1)	6(16.7)	4(11.1)	4(11.1)	7(19.4)	3(8.3)	21(58.3)	25(69.4)
S. simulans	24	19(79.2)	14(58.3)	18(75.0)	16(66.7)	8(33.3)	24(100)	20(83.3)	18(75.0)	3(12.5)	5(20.8)	2(8.3)	3(12.5)	1(4.2)	17(70.8)	16(66.7)
S. warneri	6	3(50.0)	2(33.3)	-	2(33.3)	-	6(100)	5(83.3)	4(66.7)	-	-	-	-	-	4(66.7)	4(66.7)
S. xylosus	6	3(50.0)	4(66.7)	4(66.7)	4(66.7)	2(33.3)	6(100)	4(66.7)	4(66.7)	2(33.3)	-	-	-	-	4(66.7)	4(66.7)
Unidentified	3	2(66.7)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	-	-	-	-	-	3(100)	3(100)
Total	233	161(69.1)	74(31.7)	132(56.7)	99(42.5)	100 (42.9)	233(100)	143(61.4)	125(53.6)	33(14.2)	22(9.4)	20(8.5)	24(10.3)	12(5.2)	140(60.1)	175(75.1)

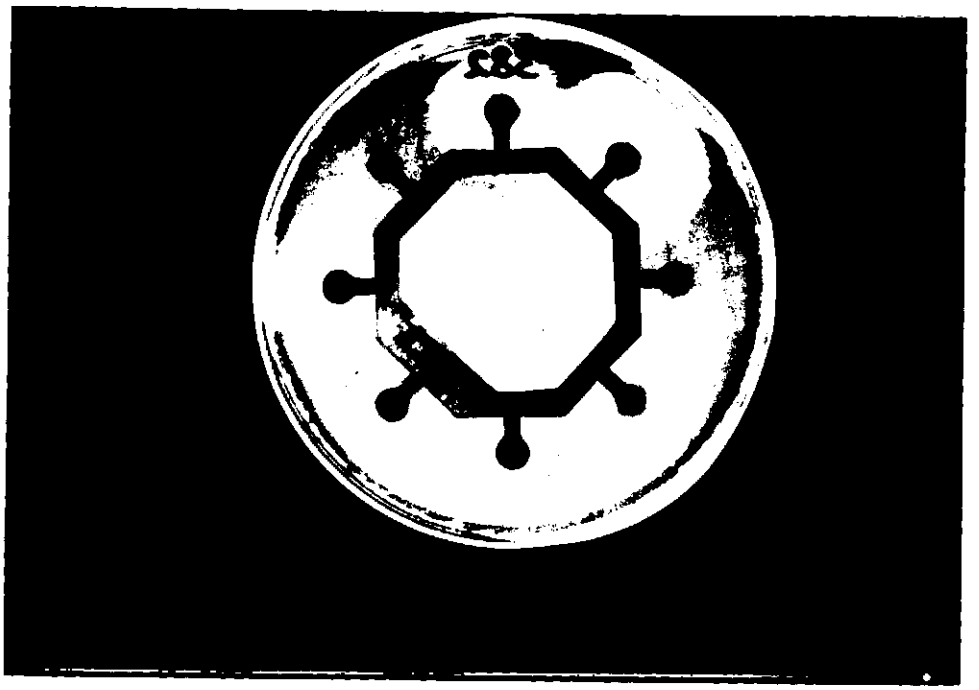


Plate 4.1a: **Antibiogram of a strain of *Staphylococcus epidermidis*.**

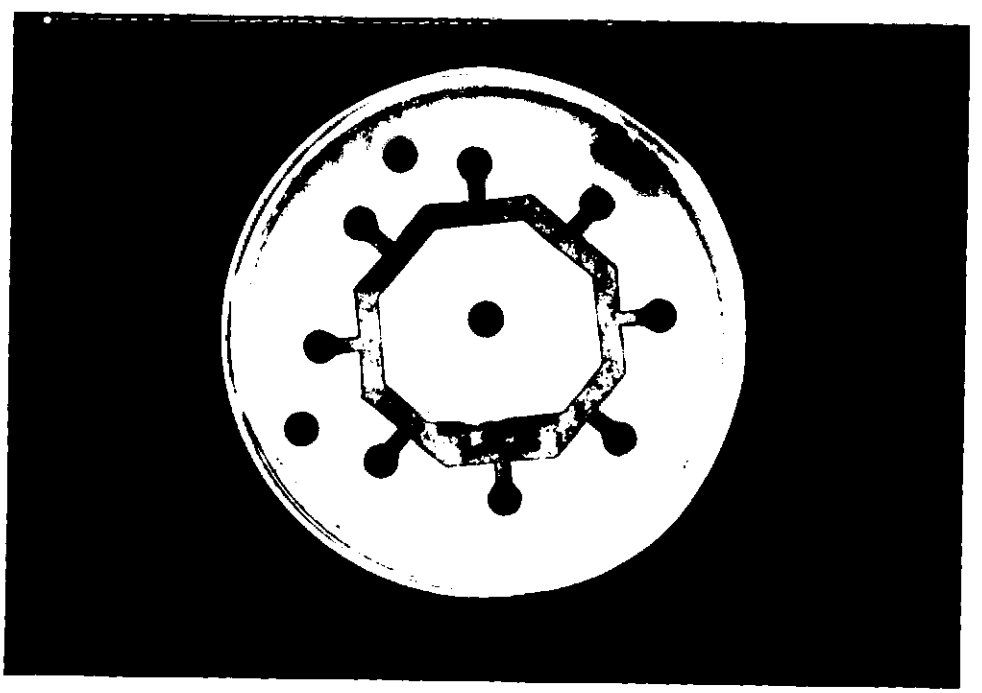


Plate 4.1b: **Multiresistance of a strain of *Staphylococcus simulans*.**

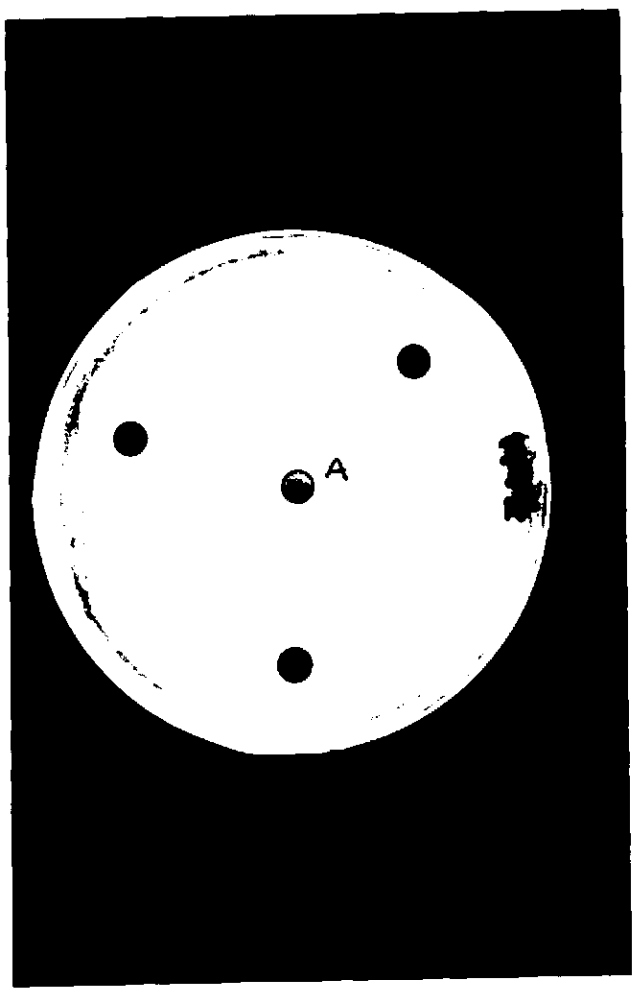


Plate 4.2: **Antibiogram of *Staphylococcus haemolyticus* and susceptibility to Imipenem antibiotic.**

A = Imipenem disc.

CHAPTER 5

BETA-LACTAMASE DETECTION AND RESISTANCE OF COAGULASE-NEGATIVE STAPHYLOCOCCI STRAINS TO SOME BETA-LACTAM ANTIBIOTICS.

INTRODUCTION

The incidence of infections caused by multi-drug resistant Gram-positive organisms is increasing despite advances in antibacterial therapy over the last 20 years. The pathogens causing these infections are frequently resistant to most currently available antibacterial agents and are therefore difficult to treat. Among these problematic Gram-positive organisms are coagulase-negative staphylococci (CoNS) which are resistant to beta-lactams, amino glycoceptides (Baquero, 1997). Bacterial strains producing beta-lactamase enzymes are growing and increasingly spreading (Fraimow and Abrutyn, 1995). The production of beta-lactamase is the predominant resistance mechanism of Gram-positive and Gram-negative bacteria to beta-lactam antibiotics (Livermore, 1991). This has led to therapeutic failure of specific beta-lactams particularly penicillin in patients associated with pathogenic bacteria producing beta-lactamase (Garau 1994, Pfaller and Herwaldt, 1988). Also the current increase of penicillin and cephalosporin resistance of nosocomial bacteria is largely caused by beta-lactamases (Swartz, 1994, French *et al.*, 1996). Production of beta-lactamases by most pathogenic bacteria poses a major problem in the usage of beta-lactam antibiotics for the treatment of diseases. Detection of beta-lactamase from pathogenic bacteria is important for optimal beta-lactam therapy.

In the gram-positive bacteria, beta-lactamases are mainly secreted into the growth medium whereas in gram-negative bacteria, they are secreted into the periplasmic space (Quintiliani and Courvelin, 1995). Thus, the extracellular nature and higher levels of beta-lactamases

produced by gram-positive bacteria make their detection relatively easier than that of gram-negative bacteria which are constitutive and often produced in smaller concentrations (Sykes, 1981). Although, the degree of extra cellularity of beta-lactamase is under genetic control, environmental conditions determine whether the extracellular enzyme will be liberated from the cell (Coles and Gross 1967). Unless the required environmental conditions are met, the beta-lactamase may remain ionically bound to the cell. Hence, several techniques have been employed for the detection of beta-lactamase production by bacteria. The most common and rapid methods include iodometric method (Rosenblatt and Neuman, 1978), acidiometric method (Tu *et al.*, 1981) and chromogenic substrates method (O' Callaghan *et al.*, 1972). These methods are known to vary in their sensitivity.

Beta-lactamase destroys penicillin by hydrolyzing the amide bond in the beta-lactam ring of 6-aminopenicillinic acid or 7- aminocephalosporanic acid and/or their N-acyl derivatives. These group of beta-lactam antimicrobial agents include the penicillin, cephalosporin, monobactams and carbapenems (figure 4.1) The major antibacterial action of all these beta-lactams is based primarily on the binding to penicillin-binding proteins (PBPs) of susceptible organisms thereby interfering with bacterial cell wall synthesis. The search for beta-lactamase stable beta-lactams began. This was rewarded in 1950's with the discovery of a penicillinase-stable compound (antistaphylococcal penicillin) methicillin (Knudsen and Rolinson, 1960). Soon after this discovery, staphylococci with intrinsic resistance to methicillin emerged in the late 1960's. Brenner and Kayser (1968) for example, reported numerous cases of nosocomial infections due to methicillin resistant staphylococci. Nosocomial infections caused by methicillin resistant coagulase-negative staphylococci still

pose a serious problem for health care institutions (Kloos and Bannerman, 1994).

Resistance to methicillin and other related beta-lactams in staphylococci especially *S. aureus* is based on the utilization of an altered penicillin binding protein (PBP) that has low affinity for the antibiotic. This altered enzyme called penicillin binding protein 2a (PBP 2a) is able to maintain cell wall integrity during growth and cell division when the native penicillin binding proteins are inactivated by beta-lactam antibiotics. *Staphylococcus aureus* contains four PBPs of which PBPs 1,2,3 are essential (Reynolds, 1988). The PBP 2a is encoded by the chromosomal gene *mecA* (Georgopapadakou, 1993) and is thought to function as a beta-lactam-resistant transpeptidase. The mecA gene is widely distributed among different species of staphylococci and is highly conserved (Ryffel *et al.*, 1990; Ubukata *et al.*, 1990). The mecA gene is found on chromosone of both coagulase-positive and coagulase-negative staphylococci. In some methicillin-resistant *Staphylococcus aureus* (MRSA) that lack the meCA gene resistance occurs by the modification of other PBPs in a beta-lactam reactivity process. Also some strains of MRSA produce plasmid-mediated penicillinase that appear to act in concert with altered PBPs in producing resistance (Jacoby and Archer, 1996).

Presently MRSA strains have spread worldwide carrying with them the mecA gene (Marples *et al.*, 1989). Penicillin-binding protein 2a or low-affinity penicillin-binding proteins presumably similar to PBP2a have been shown in a variety of coagulase-negative staphylococci (CoNS) including strains of *S. epidermidis S. haemolyticus, S. hominis, S. simulans, S. saprophyticus, S. sciuri, S. capitis, S. warneri and S. caprae* (Archer and

Pennell, 1990, Pierre *et al.*, 1990; Stratton *et al.*, 1990; Suzuki *et al.*, 1992). The authors reported that such a widespread distribution of methicillin resistance within CoNS may be due to or at least initiated by the transfer of the mecA gene among the CoNS and *S. aureus*.

Apart from methicillin, other agents synthesized to resist inactivation by beta-lactamases included second and third generation cephalosporin and meropenem (imipenem) (Moellering, 1993). Like the introduction of methicillin, newer challenges also emerged with the use of these other agents leading to hyper-production of the enzymes (Livermore 1991). Thus, newer plasmid -coded extended-broad-spectrum beta-lactamase were observed. This led to the design of beta lactamase inhibitors in order to overcome the action of beta-lactamase (Garau, 1994). Specific inhibitors of beta-lactamase include clavulanic acid, sulbactam, tazobactam, halogenated penicillinic acids and aztreonam. These inhibitors in combination with beta-lactams have shown efficacy against a wide variety of bacterial infections (Acar *et al.*, 1988). Combination of clavulanic acid/amoxycillin (augmentin), sulbactam/ampicillin (unasyn) are in use in this environment.

Beta lactamase production occurs in a large number of bacterial pathogens in this environment. Many beta-lactams are also used in the treatment of infections in this environment. The present study was therefore undertaken to determine the incidence of beta-lactamase producers among coagulase-negative staphylococci isolated in Lagos. The study was to assess the resistance pattern of these CoNS strains to some beta-lactam drugs for optimum beta-lactam therapy. In Nigeria methicillin resistance has only been reported in clinical isolates of *S. aureus* (Rotimi *et al.*, 1987; Odugbemi *et al.*, 1995; Kesah *et al.*,

1997). The frequency of methicillin resistance in CoNS is not known in Nigeria. Part of the aim of this study was to determine the incidence of methicillin resistance in CoNS infections in Lagos.

MATERIALS AND METHODS

Bacterial Strains:

Two hundred and thirty-seven (237) clinical isolates of coagulase-negative staphylococci (CoNS) were used for this study. The distribution of the various (CoNS) isolates was as follows: *Staphylococcus epidermidis* (105 isolates), *Staphylococcus capitis* (25), *S. cohnii* (1), *S. haemolyticus* (24), *S. hominis* (5), *S. lugdunesis* (2), *S. saprophyticus* (36), S. simulans (24), *S. warneri* (6), *S. xylosus* (6), Unclassified (3). These were isolated from clinical specimens obtained from the clinical microbiology laboratories of Lagos University Teaching Hospital and General Hospital, Ikeja between December 1994 and May 1996. These strains were isolated from specimens from in and out-patients suffering from infections of the urinary tract, bloodstream, eye, ear, bones, prostate, surgical site, skin etc.

Beta-Lactamase Detection:

The Beta-lactamase activity of each CoNS isolate was determined using a modified iodometric (starch paper) technique described by Odugbemi *et al.,* (1977). In this technique colonies of each CoNS isolates including positive and negative controls were collected from the surface of the culture plates and transferred to the surface of a strip of starch paper (Basildon bond) (7cm by 4cm) which was previously soaked for 10 minutes in a solution of benzyl penicillin G. (100,000 units/ml). The inoculum was spread over an area of 2-3mm and was placed at least 1.5cm apart from each other. The inocula on the starch paper contained in a Petri dish were incubated at 37°c for 30minutes, and then flooded with

Gram's iodine solution which was drained off immediately. The starch paper turned uniformly blue-black within 30 seconds with the addition of iodine. Development of a white halo which widened within 5 minutes around inoculated organism was indicative of beta-lactmase production. Beta-lactamase negative CoNS did not produce any decoloration of the surrounding area. The following organisms were included as control: *S. aureus* ATCC 29213 as positive control and *Enterococcus faecalis* ATCC 29212 as negative control.

Determination of Resistance to Beta-lactam Antibiotics.

Antimicrobial Discs:

The following antibiotics discs from Oxoid, Basingstoke Hampshire, England and AB Biodisk, Pyramidvagen, Solna Sweden were used: penicillin G(1 unit), cloxacillin (10mg), ampicillin (10mg), amoxycillin/clavulanic acid (30mg), imipenem (10mg), ceftriaxone (30mg) and methicillin (5mg).

Method:

The disc-agar diffusion method of Bauer, *et al.*, (1966) modified and standardized by the National Committee for Clinical Laboratory Standard was used as described previously in chapter 4.

Determination of methicillin resistance to Coagulase-negative Staphylococci:

Disc diffusion method was done as described previously in chapter 4 according to NCCLS disc diffusion standard method as modified by Woods *et al.*, (1984).

RESULTS

Of the 233 isolates of coagulase-negative staphylococcci (CoNS) tested for beta-lactamase production, 162(69.5%) produced the enzyme. Results of beta-lactamase production are shown in Table 5.1. The only *S. cohnii* strain did not produce any beta-lactamase while all the strain of both *S. hominis* and *S. lugdunensis* were beta-lactamase producers. Table 5.1 shows beta-lactamase production by different species of CoNS while plates 5.1 illustrates positive and negative reactions obtained by the starch paper technique.

Majority of the CoNS isolates tested were resistant to most of the beta-lactam antibiotics used (Table 5.2). Penicillin showed a 100% resistance by all the species. A reasonable proportion of CoNS species were susceptible to ceftriaxone(85.8%) and imipenem(94.8%). Percentages of resistance obtained with cloxacillin (56.7%)and ampicillin(69.1%) were high for some CoNS species. The beta-lactamase inhibitor, augmentin (amoxycillin/clavulanic acid)(60.1%) demonstrated low *in-vitro* activity against CoNS species.

A high resistance rate of more than 50% was recorded for all CoNS species for methicillin resistance(Table 5.2).

DISCUSSION

In this study, beta-lactamase was detected in 162(69.5%) out of 233 coagulase-negative staphylococci strains. This is in agreement with the study of Udo *et al.*, (1997) who detected beta-lactamase in most of their CoNS species. In all, beta-lactamase production was detected in different species except for the only strain of *S. cohnii.*

Beta-lactam antibiotics are still useful in this environment and in developing countries for the treatment of bacterial infections. These compounds like many other antibiotics are subject to enzymatic modifications and degradations, thus resulting in a reduction or loss of the antibiotic activity. In this study, most of these CoNS strains are resistant to most of these beta-lactam antibiotics; Penicillin (100%), methicillin (75%), ampicillin (69.1%), amoxycillin/clavulanic acid (60.1%) and cloxacillin (56.7%). Penicillin, in this study, has been shown to be very inactive against CoNS. Cloxacillin(56.7%) and ampicillin (69.1%) showed low efficacy against CoNS in this study. The beta-lactamase inhibitor amoxycillin/clavulanic acid(75.1%) had a limited spectrum of activity. A reasonable proportion of the CoNS strains was resistant to this antibiotic. This finding is not in agreement with other studies (Phillipon *et al.*, 1989 and Wiedemann *et al.*, 1989). In their studies, some species of CoNS and some other bacteria were inhibited by amoxycillin/clavulanic acid. The fact that all non-beta-lactamase were not inhibited by the various beta-lactam antibiotics is an indication that beta-lactamase production is just one of the resistance mechanism possessed by these organisms.

The broadest spectrum commercially available parenteral antimicrobial imipenem (5.2%)is very active against CoNS strains in this study, while the third-generation cephalosporin, ceftriaxone(14.2%) is very effective too. Coagulase-negative staphylococci isolates are said to exhibit multiple resistant to commonly used antibiotics including beta-lactams (Kloos and Bannerman, 1994; Pfaller and Herwaldt, 1988). There is hope in the use of imipenem and ceftriaxone for serious infections caused by CoNS species in this environment.

Penicillinase-resistant penicillins of which methicillin is the prototype are primarily effective against penicillinase-producing staphylococci (Gravenkemper *et al.*, 1965). In this study most of the penicillinase (beta-lactamase) producers were resistant to methicillin. About 75% of the total CoNS strains were resistant to methicillin. This is in agreement with the report of Kariuki and Hart (1997). Kernodle *et al.*, (1988) reported that methicillin-resistant coagulase-negative staphylococci may be present in low numbers on the skin of patients and emerge in hospital as predominant flora and potential pathogens, especially after surgical prophylaxis. This is very similar to the epidemiology of *Enterobacter* infection (Flynn *et al.*, 1988). Widespread distribution of methicillin resistance within CoNS may be initiated by the transfer of mec A gene among the CoNS and *S. aureus*. Archer and Scott (1991) found a conjugative (*tra* gene) in the CoNS species, *S. epidermidis, S. haemolyticus, S. hominis, S. simulans, S. warneri, S. saprophyticus* and *S. capitis*. They reported that transfer genes (*tra* gene) are usually found on plasmids that encode gentamicin resistance in multiresistant isolates of CoNS.

The present findings and previous reports (Varaldo *et al.,* 1984; Marsik and Brake, 1982; Richardson and Marples, 1982) suggest a progressively increasing spread of staphylococcal resistance to methicillin. Significant incidences of methicillin resistance among clinical isolates of coagulase-negative staphylococci have also emerged in this study. Nosocomial infections caused by methicillin-resistant staphylococci pose a serious problem for health care institutions (Kloos and Bannerman, 1994). The detection of resistance in these isolates has been reported to be hampered by the variability in standard techniques used in determinig methicillin resistance (Kloos and Bannerman, 1995). The resistant strains are said to be often heteroresistant to beta-lactam antibiotics in that two subpopulations (one susceptible and the other resistant) coexist within a culture (Chambers, 1988). The findings in this study suggest that the problem of methicillin resistance in coagulase-negative staphylococci, about which little information is available in our environment deserves more extensive investigation.

CONCLUSION

Most of the coagulase-negative staphylococci isolates produce beta-lactamase. Coagulase-negative staphylococci are a major component of the normal flora of the cutaneous ecosystem. Therefore great caution must be excercised in the use of beta-lactam antibiotics for infections caused by CoNS in view of the high rate of beta-lactamase production and very high resistance to methicillin recorded for CoNS in this environment.

Table 5.1. Beta-Lactamase Detection in Coagulase - Negative Staphylococci (CoNS) Strains.

Species	No. of Strains Tested	Beta-Lactamase Production. No. (%) Positive
Staphylococcus capitis	22	19(86.4)
Staphylococcus cohnii	1	0(0)
Staphylococcus epidermidis	104	69(66.3)
Staphylococcus haemolyticus	24	21(87.50)
Staphylococcus hominis	5	5(100)
Staphylococcus lugdunensis	2	2(100)
Staphylococcus saprophyticus	36	20(55.55)
Staphylococcus simulans	24	14(58.33)
Staphylococcus warneri	6	5(83.33)
Staphylococcus xylosus	6	4(66.67)
Unclassified	3	3(100)
TOTAL	**233**	**162(69.5)**

Table 5.2: Resistance Patterns of Coagulase-negative Staphylococci strains to some Beta-lactam Antibiotics

Coagulase-negative Staphylococci species	No. of Strains tested	Beta lactamase Activity	Penicillin (1 unit)	Cloxacillin (10µg)	Ampicillin (10µ	Amoxycillin/ Clavulanic Acid (30ug)	Imipenem (10µg)	Ceftriaxone (30µg)	Methicillin (5µg)
Staphylococcus capitis	22	19^{+}	100	52.6	57.9	84.2	0	0	89.5
		3^{-}	100	66.7	66.7	33.3	0	0	0
Staphylococcus cohnii	1	1^{-}	100	100	100	100	0	0	100
Staphylococcus epidermidis	104	69^{+}	100	68.1	91.3	55.1	8.7	20.3	88.4
		35^{-}	100	40.0	60.0	34.3	5.7	5.7	62.9
Staphylococcus haemolyticus	24	21^{+}	100	76.2	90.5	85.7	0	19.0	71.4
		3^{-}	100	33.3	66.7	0	0	66.7	66.7
Staphylococcus hominis	5	5^{+}	100	0	80.0	60.0	0	0	80.0
Staphylococcus lugdunensis	2	2^{+}	100	0	50.0	100	0	0	50.0
Staphylococcus saprophyticus	36	20^{+}	100	55.0	45.0	95.0	10.0	15.0	85.0
		16^{-}	100	31.3	6.3	12.5	6.3	18.8	50.0
Staphylococcus simulans	24	14^{+}	100	85.7	78.6	92.9	0	14.3	64.3
		10^{-}	100	60.0	80.0	40.0	10.0	10.0	70.0
Staphylococcus warneri	6	5^{+}	100	0	60.0	60.0	0	0	80.0
		1^{-}	100	0	0	100	0	0	0
Staphylococcus xylosus	6	4^{+}	100	75.0	50.0	75.0	0	50.0	75.0
		2^{-}	100	50.0	50.0	50.0	0	0	50.0
unclassified	3.	3^{+}	100	100	66.7	100	33.3	0	100

Key:

\+ Beta-lactamase

\- Non-Beta-lactamase

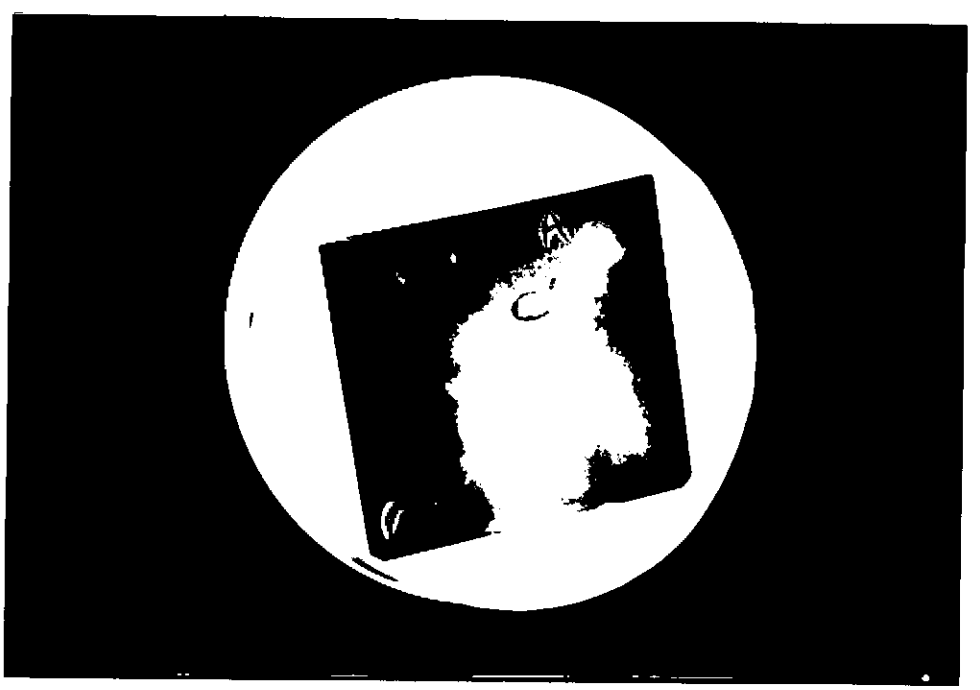


Plate 5.1 **Beta - Lactamase detection on starch paper.**

A - *Staphylococcus aureus* ATCC 29213 (positive control)
B - *Enterococcus faecalis* ATCC 29212 (negative control)
C - Test organism (positive)
Spots with white halo positive result.

CHAPTER 6

DETERMINATION OF PLASMID PROFILES OF SOME COAGULASE-NEGATIVE STAPHYLOCOCCI ISOLATES IN LAGOS.

INTRODUCTION

Plasmids are autonomous self-replicating extrachromosomal DNA elements (Lederberg, 1952; Lewin, 1987). They are organised like that of bacterial chromosomes with the genes which ensure replication, maintenance and distribution to daughter cells during division of the host cells (Tompkins 1985). They are not essential for normal bacteria growth but are infectious and can be transferred between bacteria of the same or different genera. The infectious nature of plasmids was a key feature in their discovery (Falkow, 1975). Usually all functions required for plasmid transfer including pili synthesis are encoded by genes on the plasmids. The presence of plasmids in bacteria is non-essential for their survival except in the face of hostile environment such as antibiotics. This factor contributes to the stability of plasmid -mediated resistance in bacteria. Plasmids can carry genes conferring virulence or pathogenicity as well as antimicrobial resistance (Falkow, 1975; Hardy, 1981). The presence of different types of antimicrobial resistance genes on plasmids and their potential for transmission suggest that plasmids are major vectors in the dissemination of resistance genes through bacterial populations. Genetic elements such as plasmids and transposons are mainly responsible in the spread of resistance in the hospitals as well as community ecosystems (O'Brien, 1997).

Plasmids are being observed with increasing frequency in a number of bacterial species. Study on staphylococcal plasmids started with the study of plasmids of *Staphylococcus*

aureus in relation to penicillin resistance. *Staphylococcus aureus* was one of the earliest bacteria species in which extrachromosomal inheritance was recognised (Barber, 1949). However, little attention was paid to plasmids occurring in coagulase-negative staphylococci. This was as a result of: the confusion over the classification and identification of CoNS (Feltham, 1974; GoodFellow *et al.,* 1981), the absence of readily identifiable plasmid phenotypes other than resistance to antibiotics and heavy metal ions (Novick *et al.,* 1977); and the absence of a rapid, convenient but reproducible method for screening for staphylococcus plasmids. Plasmid profiles in CoNS were first used to distinguish different strains of coagulase-negative staphylococci by Parisi and Hecht (1980). They were able to demonstrate the existence of a common strain of *S. epidermidis* causing infection among infants in a neonatal unit which was being passed from infant to infant. Studies on coagulase-negative staphylococci, *S. aureus* and *Enterococcus faecalis* demonstrated the transfer of antibiotic resistance plasmids among these organisms (Archer *et al.,* 1985; Forbes and Scaberg, 1983, Forse *et al.,* 1979; Jaffe *et al.,* 1980; Scaberg and Zervos, 1986). These studies suggest that apart from being a significant pathogen, multiple resistant coagulase-negative staphylococci may be a reservoir of resistance for other organisms (Pfaller and Herwaldt, 1988).

In Nigeria, Olukoya *et al.,*(1994) reported only on the diverse nature of plasmids extracted from some strains of *S. aureus* isolated from various hospitals in Lagos. They also showed the high resistance-pattern of these strains to commonly prescribed antibiotics. There is no information on plasmid profiles of CoNS in Lagos. The genetic basis of antibiotic resistance in *Staphylococcus* has been a subject of interest and speculation amongst microbiologists. This work therefore investigated the plasmid DNA profiles of some strains

of coagulase-negative staphylococci isolated in Lagos and their relationship to antibiotic resistance.

MATERIALS AND METHODS

Bacterial Isolates Tested:

A total of 233 clinical isolates of coagulase-negative staphylococci collected from the Microbiology laboratories of Lagos University Teaching Hospital, Idi-Araba and General Hospital Ikeja were characterised using established methods of Kloos and Schleifer (1975) and ID 32 STAPH commerical kit. These isolates were obtained over 18 months period.

Antimicrobial Susceptibility Testing:

Antibiotic susceptibility patterns of 233 coagulase-negative staphylococci isolates were determined by the disc diffusion method of Bauer *et al.*, (1966) as described previously in chapter 4.

Antibiotics Discs:

The following antibiotic discs were used (AB Biodisk) ampicillin(10mcg); chloramphenicol (10mcg); cloxacillin(10mcg); erythromycin (5mcg); gentamicin (10mcg); penicillin (1unit); streptomycin (10mcg); tetracycline (10mcg); ceftriaxone (30mcg-Oxoid); ofloxacin (10mcg-Oxoid).

Plasmid Isolation:

Initial attempts made to screen for plasmid from coagulase-negative staphylococci (CoNS) isolates identified using various methods, did not give satisfactory results. A modification

of the method of Takahashi and Nagano (1984) was used.

Chemicals, Reagents:

The lysostaphin solution consisted of 12ml of lysostaphin (Sigma Chemical Co., St. Louis, Mo) per ml, 100mM NaCl 40mM Tris-NaOH and 50mM disodium EDTA (pH 6.9). This solution was stored in the freezer. Lysing solution for the CoNS and standard *E. coli* strain V517 consisted of 4% sodium dodecyl sulfate (SDS) in 100 mM Tris stock. For use, an equal volume of freshly prepared 0.4M NaOH was added to the stock solution.

Buffer A consisted of stock buffer solution containing 400 mM Tris-acetic acid and 20 mM disodium EDTA (pH 8.0). For use this solution was diluted 10 times with distilled water. Buffer B consisted of 3M sodium acetate acid (pH 5.5). Buffer C consisted of 10mM Tris-acetic and 2 mM disodium EDTA (pH 8.0). All buffers were autoclaved at 115^{0}C for 10 minutes and stored at 4^{0}C. Absolute ethanol used was stored in the frezeer. Eppendorf tubes and polypropylene micropipette tips were washed, autoclaved at 115^{0}C for 10 minutes and dried before use.

Bacterial Strains and Plasmids:

Plasmids of known molecular weights were used as control, *E. coli* V517 and phage Lambda DNA digest. Forty-four clinical isolates of CoNS isolates from various sources and resistant to more than two antibiotics were screened for plasmid.

Growth Media:

Mueller-Hinton agar was employed for culturing all strains.

Plasmid DNA Isolation Procedure:

Colonies were scraped from Mueller-Hinton agar (Oxoid), into eppendorf tubes containing 200ml of lysostaphin solution or buffer A depending on the organism (CoNS or *E. coli* V517). The cell pellet was thoroughly suspended and subsequently incubated at 37°C for 10 minutes after addition of lysing solution (400ml). A volume of 300ml of cold buffer B was added and gently mixed. This was maintained at 0°C for 5 minutes. The tubes were subsequently centrifuged at room temperature and maintained at 0°C for 10 minutes. The resultant salt-precipitated material was again centrifuged at 0°C. the supernatant was decanted to another tube. An equal volume of chloroform was added to the supernatant. This was emulsified and centrifuged at 0°C to break the emulsion. The upper aqueous phase about 500ml was carefully transferred to another tube. Ethanol precipitation was done by the addition of 1ml cold ethanol (-20°C) and maintained at 0°C for 5 minutes. The precipitate was collected by centrifugation at 0°C while the supernatant was removed by decantation. The resultant pellet was dissolved in 100ml of buffer C. The resultant plasmid DNA solution was subjected to electrophoresis.

Agarose gel electrophoresis:

This was carried out using 0.7% agarose in Tris borate on a horizontal apparatus. Twenty-five microlitre (25 ml) of sample was mixed with 5ml of tracking dye glycerol in water and applied to the wells. Electrophoresis was performed at 10V/cm(voltage/cm) for 2 hours.

Gels were stained with ethidium bromide (0.5mg/ml) for 45 minutes, observed and photographed under UV-light.

Molecular weight estimation:

The correlation of molecular weights to relative mobilities obtained is shown in Fig. 6.1. Using known molecular weight plasmids harboured in *E. coli* V517, the equation for the regression line was obtained. Relative mobilities were correlated to known molecular weights.

RESULTS

The resistance patterns of the CoNS isolates tested to some commonly prescribed antibiotics are shown in Table 6.1. About 82% of CoNS strains were resistant to two or more antibiotics while 2.1% were resistant to all the antibiotics used in this study. Thirty-seven different antibiotics resistance patterns were observed among the CoNS. (Table 6.2).

Plasmid analysis showed that 20 out of the 44 CoNS strains harboured one or more plasmids of different molecular weights (Table 6.3). The plasmid sizes ranged between 0.76 and 13.5 kilobases (Plates 6.1 - 6.3). Table 6.4 shows the drug resistance pattern and plasmids detected in some of the CoNS. Species that harbour plasmids include *S. epidermidis, S. saprophyticus, S. haemolyticus, S. capitis* and *S. simulans.* Most of these strains are mainly from blood, wound swabs and urethral swabs. Multiple plasmids occurred mostly in *S. saprophyticus* group.

DISCUSSION

Plasmid DNA profile analysis of some of these CoNS isolates revealed that, most of the plasmids are low molecular weight plasmids. This is also similar to the study of Kloos *et al.,* (1980) in which most of the plasmids obtained in their study consisted more of small sized plasmids. Several studies have indicated the presence of plasmids in variety of *Staphylococcus* species (Kloos *et al.,* 1979, Kloos, 1990).

This study has shown that the plasmid contents of CoNS in our environment were diverse in nature and the antimicrobial resistance patterns were also diverse. Kloos, (1990) reported that except for *S. auricularis* and *S.lugdunensis* most other human CoNS species carry multiple plasmids. Although multiple plasmids were observed in this study but the percentage was lower than 50%. Archer *et al.,* (1985) showed the diverse nature of *S. epidermidis* plasmids among isolates from different patients, from the same site on a given patient at different sampling times and from different sites on a given patient at the same sampling time.

In this study about 7(35%) had 8.5 kilobase plasmid and two of the strains that harbour this plasmid had the same antibiotic resistance pattern (Pen Amp Tet). In the absence of curing experiments, the possibility that this plasmid could be coding for the same antibiotics resistance could be concluded. Most of the strains that harbour multiple plasmids were also resistant to many of the antibiotics tested. Although, multiple plasmids can have multiple functions, multiple antibiotic resistance obtained in this study could be one of the functions.

CONCLUSION

Plasmid profile analysis of the isolates revealed that some of the strains that were resistant to more than two antibiotics harboured plasmids. These plasmids are low molecular weight plasmid and these might act as replicons. Thus the presence of plasmids might be one of the causes of the high level of antibiotic resistance encountered amongst coagulase-negative staphylococci in this environment.

Table 6.1: Antibiotic resistance patterns of coagulase- negative Staphylococcal species to some commonly used antibiotics.

Species	No. of Strains	NUMBER (% RESISTANCE)									
		Ampicillin (10µg)	Chloramphenicol (10µg)	Cloxacillin (10µg)	Erythromycin (5µg)	Gentamicin (10µg)	Penicillin (1 unit)	Streptomycin (10µg)	Tetracycline (30µg)	Ceftriaxone (10µg)	Ofloxacin (10µg)
S. capitis	22	13(59.1)	-	12(54.5)	11(50.0)	5(22.7)	22(100)	4(18.2)	9(40.9)	-	-
S. cohnii	1	1(100)	-	1(100)	-	1(100)	1(100)	1(100)	-	-	-
S. epidermidis	104	84(76.9)	33(31.7)	61(58.6)	42(40.4)	40(38.5)	104(100)	65(62.5)	54(45.8)	16(15.4)	8(7.7)
S. haemolyticus	24	21(87.5)	6(25.0)	17(70.8)	13(54.2)	17(70.8)	24(100)	14(58.3)	11(45.8)	6(25.0)	5(20.8)
S.. hominis	5	4(80.0)	-	-	-	4(80.0)	5(100)	3(60.0)	-	-	-
S. lugdunensis	2	1(50.0)	1(50.0)	-	1(50.0)	2(100)	2(100)	1(50.0)	-	-	-
S. saprophyticus	36	10(27.8)	11(30.5)	16(44.4)	7(19.4)	18(50.0)	36(100)	23(63.9)	22(61.1)	6(16.7)	4(11.1)
S. simulans	24	19(79.2)	14(58.3)	18(75.0)	16(66.7)	8(33.3)	24(100)	20(83.3)	18(75.0)	3(12.5)	5(20.8)
S. warneri	6	3(50.0)	2(33.3)	-	2(33.3)	-	6(100)	5(83.3)	4(66.7)	-	-
S. xylosus	6	3(50.0)	4(66.7)	4(66.7)	4(66.7)	2(33.3)	6(100)	4(66.7)	4(66.7)	2(33.3)	-
Unidentified	3	2(66.7)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	-	-
Total	233	161(69.1)	74(31.7)	132(56.7)	99(42.5)	100 (42.9)	233(100)	143(61.4)	125(53.6)	33(14.2)	22(9.4)

Table 6.2: ANTIMICROBIAL RESISTANCE PATTERNS OF COAGULASE- NEGATIVE STAPHYLOCOCCAL SPECIES

ANTIMICROBIAL RESISTANT PATTERN									NUMBER OF ISOLATES SHOWING PATTERNS.											
									S. capitis	*S. cohnii*	*S. epidermidis*	*S. haemolyticus*	*S. hominis*	*S. lugdunensis*	*S. saprophyticus*	*S. simulans*	*S. warneri*	*S. xylosus*	Unclassified	Total
PEN									7	-	4	1	-	-	3	2	-	2	-	19
PEN	AMP								-	-	1	-	1	-	1	1	1	-	-	5
PEN	GEN								-	-	6	-	1	1	-	-	-	-	-	8
PEN	STR								-	-	6	1	-	-	-	-	1	-	-	8
PEN	TET								1	-	-	-	-	-	6	-	-	-	-	7
PEN	STR	TET							-	-	6	-	-	-	-	-	2	-	-	8
PEN	STR	GEN							1	-	2	-	-	-	5	-	-	-	-	8
PEN	AMP	TET							1	-	1	5	-	-	-	-	-	-	-	7
PEN	AMP	CHL							-	-	-	-	-	-	3	-	-	-	-	3
PEN	AMP	CXC	GEN						-	-	4	4	-	-	-	-	-	-	-	8
PEN	AMP	CXC	TET						1	-	8	-	-	-	-	-	-	-	-	9
PEN	AMP	CHL	TET						-	-	4	-	-	-	-	-	-	-	-	4
PEN	AMP	CXC	ERY						1	-	6	-	-	-	-	-	-	-	-	7
PEN	AMP	GEN	STR						-	-	-	-	3	-	-	-	-	-	-	3
PEN	CHL	CXC	STR						-	-	-	-	-	-	2	-	-	-	-	2
PEN	CXC	GEN	STR	TET					-	-	-	-	-	-	6	-	-	-	-	6
PEN	CHL	ERY	GEN	STR					-	-	-	-	-	1	-	-	-	-	-	1
PEN	AMP	CXC	ERY	STR					-	-	7	-	-	-	-	-	-	-	-	7
PEN	AMP	CXC	GEN	STR					-	1	-	-	-	-	-	-	-	-	-	1
PEN	AMP	CXC	ERY	TET					6	-	-	-	-	-	-	-	-	-	-	6
PEN	AMP	CXC	STR	TET					-	-	4	-	-	-	1	5	-	-	-	10
PEN	AMP	CHL	ERY	OFX	STR				-	-	-	-	-	-	-	2	-	-	-	2
PEN	AMP	CXC	ERY	GEN	STR				4	-	-	7	-	-	-	1	-	-	-	12
PEN	AMP	CHL	GEN	STR	TET				-	-	5	-	-	-	-	-	-	-	-	5
PEN	AMP	CHL	ERY	STR	TET				-	-	6	-	-	-	-	-	2	-	-	8
PEN	AMP	CXC	GEN	STR	TET				-	-	7	-	-	-	1	-	-	-	-	8
PEN	CHL	CXC	ERY	STR	TET				-	-	-	-	-	-	-	1	-	1	1	3
PEN	AMP	CRO	ERY	GEN	STR	TET			-	-	-	-	-	-	2	-	-	-	-	2
PEN	AMP	CXC	CRO	OFX	STR	TET			-	-	4	-	-	-	-	1	-	-	-	5
PEN	AMP	CRO	CXC	ERY	GEN	STR			-	-	5	-	-	-	-	-	-	-	-	5
PEN	AMP	CHL	CXC	ERY	STR	TET			-	-	5	-	-	-	-	3	-	1	-	9
PEN	AMP	CHL	CXC	ERY	GEN	STR	TET		-	-	4	-	-	-	-	6	-	-	2	12
PEN	AMP	CHL	CXC	ERY	OFX	STR	TET		-	-	2	-	-	-	2	-	-	-	-	4
PEN	CHL	CRO	ERY	GEN	STR	TET	CXC		-	-	-	1	-	-	2	-	-	-	-	3
PEN	CHL	CRO	CXC	OFX	STR	TET	GEN		-	-	-	-	-	-	2	2	-	-	-	4
PEN	AMP	CHL	CRO	CXC	ERY	GEN	STR	TET	-	-	7	-	-	-	-	-	-	2	-	9
PEN	AMP	CHL	CRO	CXC	ERY	GEN	STR	OFX	TET	-	-	-	5	-	-	-	-	-	-	5
						TOTAL				22	1	104	24	5	2	36	24	6	6	3	233

KEYS: AMP=AMPICILLIN; CHL=CHLORAMPHENICOL; CXC=CLOXACILLIN; ERY= ERYTHROMYCIN; GEN=GENTAMICIN; PEN=PENICILLIN; STR=STREPTOMYCIN; TET=TETRACYCLINE; CRO=CEFTRIAXONE; OFX=OFLOXACIN.

Table 6.3: Plasmid-Containing Coagulase-negative Staphylococcal Isolates and their Molecular Weights (N=20).

Isolates Number	Number of Plasmids	Plasmid size (Kilobase)
2293	1	12.9
1913	3	2.6, 2.9, 8.95
2577	1	9.1
391	1	8.6
2088	1	0.76
2941	3	1.17, 4.5, 8.07
673	1	8.8
2975	2	1.04, 1.48
2268	1	1.47
4545	2	1.0, 1.48
4649	2	8.5, 13.5
2729	3	2.62, 7.88, 8.5
2921	3	1.26, 1.58, 8.8
P003	1	7.6
2898	1	8.5
2021	1	8.5
PA 550	1	8.5
2100	1	8.5
2203	2	7.6, 13.5
2201	1	13.5

Table 6.4: Drug Resistance pattern and plasmids detected in some coagulase-negative staphylococci isolates.

Species (Number of isolates)	Drug Resistance Pattern								Source	Number of Plasmids	Molecular weight (Kilobase)
S. epidermidis	Pen	Amp	Cxc	Gen					Blood	1	12.9
S. epidermidis	Pen	Amp	Tet						Urine	2	8.5, 13.5
S. epidermidis	Pen	Amp	Cxc	Tet					Wound swabs	1	8.5
S. epidermidis	Pen	Amp	Tet						HVS	1	8.5
S. epidermidis	Pen	Amp	Chl	Tet					Urethral swabs	1	13.5
S. epidermidis	Pen	Amp	Cxc	Chl	Gen	Tet	Str	Cro	Blood	3	2.6, 2.9, 8.95
S. epidermidis	Pen	Amp	Cxc	Ery	Gen	Str			Blood	1	0.76
S. saprohyticus	Pen	Chl	Cxc	Gen	Str	Tet	Cro	Ofx	HVS	1	1.47
S. saprohyticus	Pen	Amp	Tet						Urine	2	1.0, 1.64
S. saprohyticus	Pen	Tet							Urethral swabs	3	2.62, 7.88, 8.5
S. saprohyticus	Pen	Amp	Ery	Gen	Str	Tet	Cro		Wound swabs	3	1.26, 1.58, 8.8
S. saprohyticus	Pen	Chl	Cxc	Gen	Str	Tet	Cro	Ofx	Seminal fluid	2	7.6, 13.5
S. haemolyticus	Pen	Amp	Gen	Str					Blood	1	8.6
S. haemolyticus	Pen	Amp	Gen	Str					Wound swabs	3	1.7, 4.5, 8.07
S. haemolyticus	Pen	Str							Wound swabs	2	1.04, 1.48
S. capitis	Pen	Str	Gen						Urine	1	9.1
S. capitis	Pen	Amp	Cxc	Ery	Gen	Str			Urethral swab	1	7.6
S. similans	Pen	Amp	Cxc	Ery	Gen	Str	Tet		Wound swabs	1	8.5
S. similans	Pen	Amp	Cxc	Ery	Gen	Str	Tet		Wound swabs	1	8.5
Unidentified	Pen	Chl	Cxc	Ery	Str	Tet			Seminal fluid	1	12.9

KEYS: Amp=AMPICILLIN; Chl=CHLORAMPHENICOL; Cxc=CLOXACILLIN; Ery= ERYTHROMYCIN; Gen=GENTAMICIN; Pen=PENICILLIN; Str=STREPTOMYCIN;

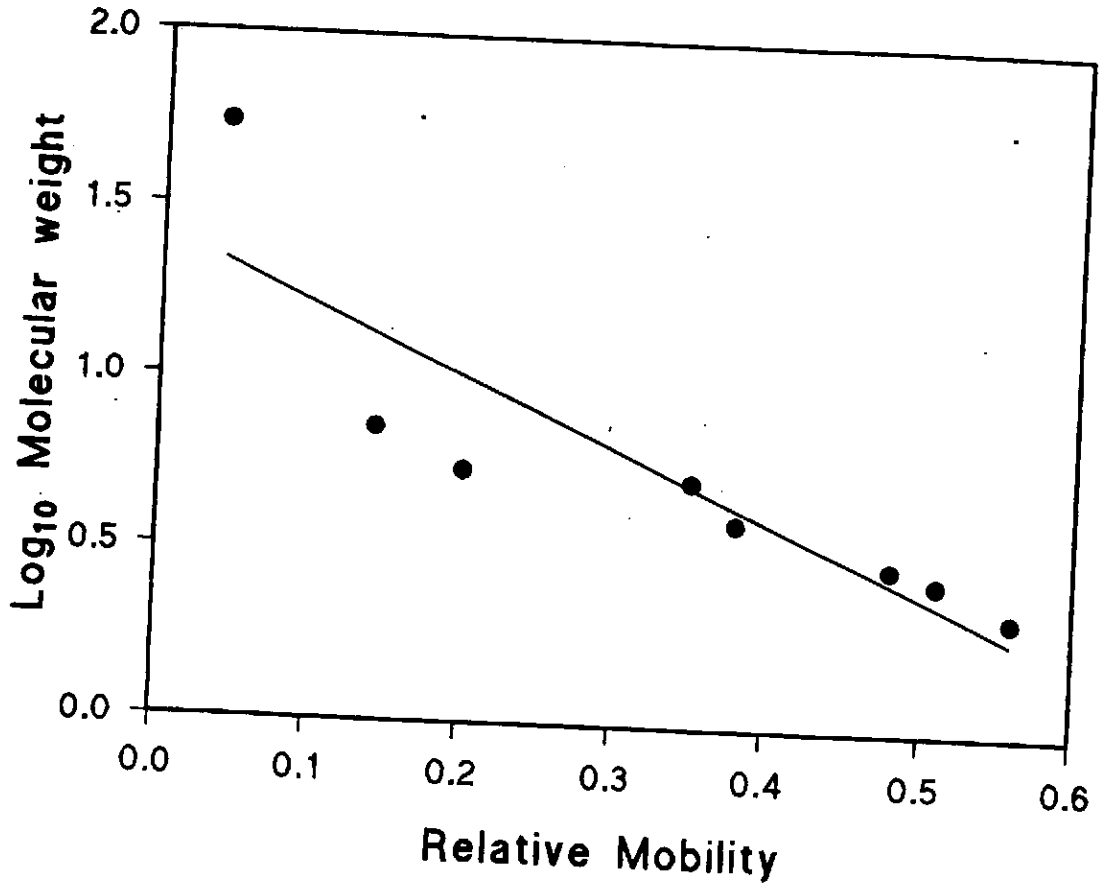


Figure 6.1. A typical standard curve for molecular weight determination using E. coli V517.

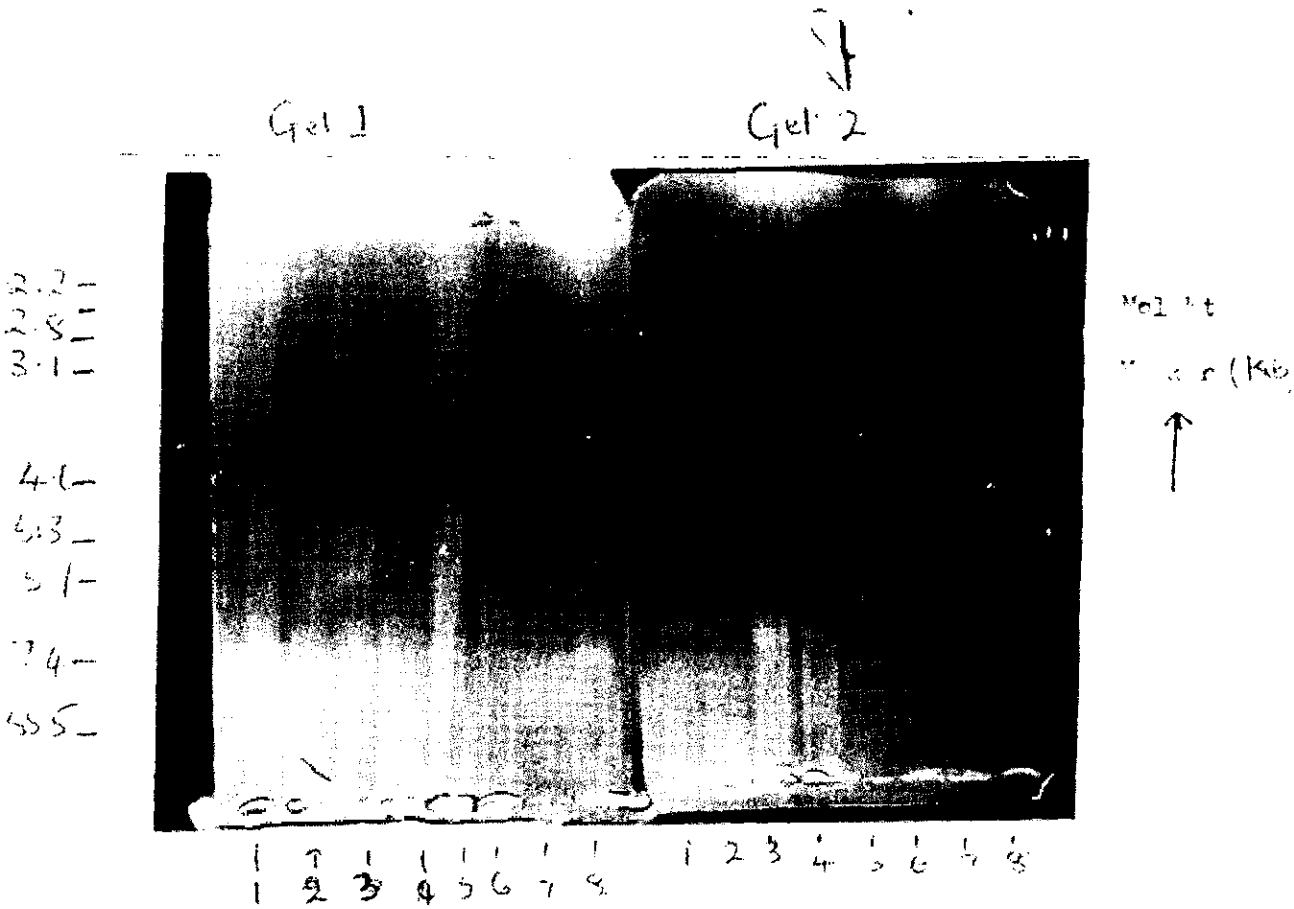


Plate 6.1 Plasmid patterns of coagulase-negative staphylococci strains.

Gel I. Lane 1 E.coli V517 standard

Lane 6 Staphylococcus saprophyticus

Gel 2. Lane1 E.coli V517 standard

Lane 3 Staphylococcus saprophyticus

Lane 4 Staphylococcus epidermidis

Lane 6 Staphylococcus haemolyticus

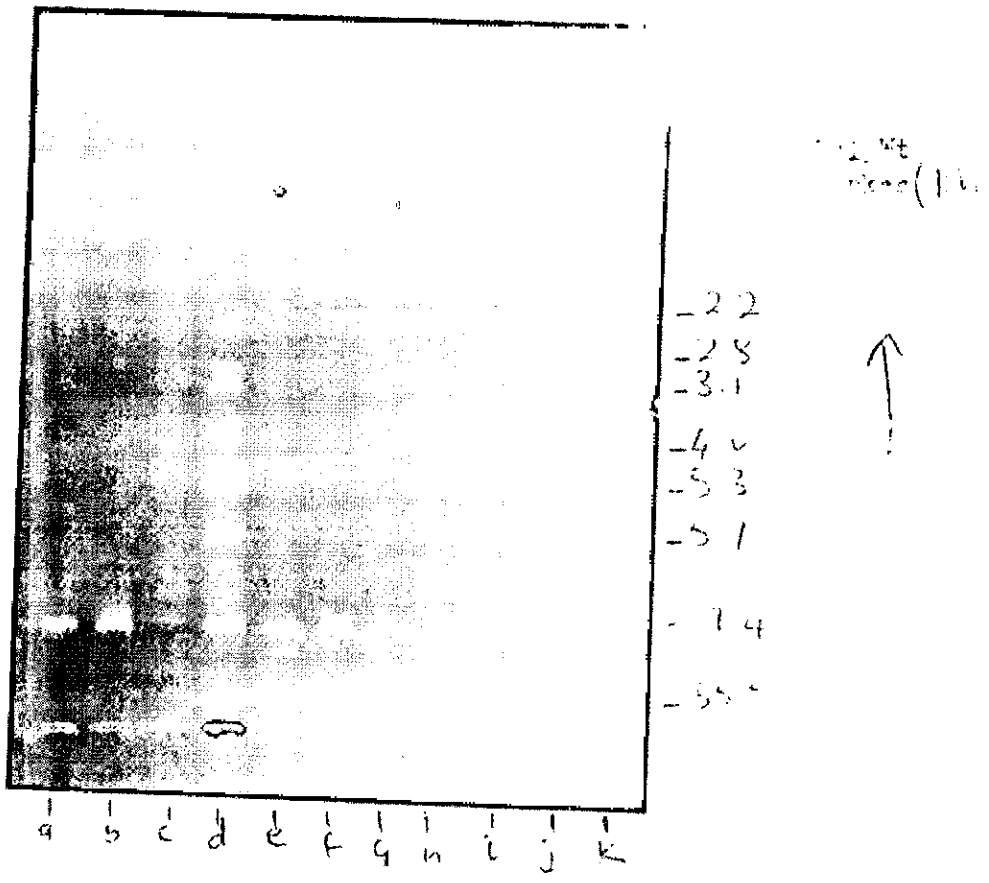


Plate 6.2 Plasmid patterns of coagulase-negative staphylococci strains.

Lane d E. coli V517 Standard molecular weight markers;

Lanes, a, f, h, i, and j Staphylococcus epidermidis,

Lanes, b and e Staphylococcus haemolyticus;

Lanes, c and k Staphylococcus capitis,

Lane, g Staphylococcus simulans;

Lane, d E.coli IV517 Standard molecular weight markers).

Gel 4

Plate 6.3 Plamid patterns of coagulase-negative staphylococci.

Lanes 2,3 and 6. Staphylococcus epidermidis

Lane 8 Lambda digest standard.

INTRODUCTION

Coagulase-negative staphylococci (CoNS) were considered in the past as laboratory contaminants or members of the normal bacterial flora in man(Pfaller and Herwaldt, 1988). They are now well established as the causative organisms of many diseases especially diseases relating to prosthetic heart valves, ventricular peritonial shunts, arthritis associated with artificial joints, endocarditis, and urinary tract infections (Pfaller and Herwaldt, 1988; Kloos and Bannerman 1994). Together with the coryneforms, CoNS form the major part of the skin flora of man (Noble and Somerville, 1974) including the mucous membranes.

In the cutaneous ecosystem, coagulase-negative staphylococci have a benign relationship with their host and function as commensal or saprophytic organisms. These orgaisms can gain entry into the host through damage to the cutaneous organ system by trauma, inoculation by needles, or by direct implantation of foreign bodies (Kloos and Bannerman, 1994). Some reports suggested that the mechanism by which CoNS gain entry into the host might be endogenous from the skin or mucosal (pulmonary or alimentary tract) surfaces of the patients or exogenous from the hospital environment or the hands of hospital personnel (Dandalides *et al.*, 1986; Dunne *et al.*, 1987; Gahrn-Hansen, 1987). Thus the CoNS may develop the style of a pathogen depending upon their ability to adhere to host or foreign body surfaces thereby causing colonization, and breach or avoid the host habitats such as the anterior nares, axillae, inguinal and perineal areas may reach densities of 10^3 to 10^6 cfu/cm^2 of surface and in dry habitats or the extremities may reach 10 to 10^3 cfu/cm^2 (Kloos

and Bannerman, 1994). Contact spread has added importance in hospital where a large proportion of the staff and patients carry CoNS species and antibiotic resistant strains in the nose or on the skin etc. (Narayani *et al.*, 1990). The major reserviors of common hospital pathogens are health care personnel, patients and visitors (Mulhausen *et al.*, 1996).

The aim of this study was to determine the prevalence of CoNS from some body sites of apparently healthy individuals and to compare the isolates obtained from the sites with those obtained from clinical specimens.

MATERIALS AND METHODS

Bacterial Strains and sampling population:

A total of one hundred and ninety-four specimens were obtained from apparently normal population from Lagos University Teaching Hospital (LUTH), General Hospital, Ikeja and some staff of the Nigerian Institute of Medical Research (NIMR), between September and November, 1996). The sampling population consisted of medical personnel; doctors (3) and nurses (8); mothers (10) and babies (10); and NIMR staff 25. The specimens collected included axillary swabs from babies; while hand, nasal, axillae and skin (forehead) swabs were from others.

Isolation and Characterisation Procedure:

Sterile swab sticks moistened in sterile normal saline were used to obtain specimens from axillae, hand, nasal and skin. All specimens were inoculated unto blood agar, nutrient agar, and MacConkey agar and incubated aerobically at 37^0C for 18 - 24 hours. All gram-positive, catalase-positive clustering cocci were identified using the standard methods as described in Appendix one. Coagulase production (both slide and tube tests) were determined as described in appendix one. Differentiation of the isolates from micrococci was performed based on tests recommended by Schleifer and Kloos (1975)(Appendix one).

All gram-positive, catalase-positive, coagulase-negative Staphylococci were further characterised using the method of Kloos and Schleifer (1975) and ID 32 STAPH kit as

described previously in Chapter 2 (Appendix one). Beta-lactamase and slime production were determined as described in Chapters 5 and 3 respectively.

Resistance to various antimicrobial agents were determined on Muellar-Hinton agar (oxoid) by the disc diffusion method of Bauer *et al.*, (1966) as described previously in Chapter 4. Diameters of zone of inhibition were measured in millimetres using a ruler, and results interpreted according to standard techniques (NCCLS, 1993a). Antimicrobial discs used were Gram-positive AB Biodisk containing the following antibiotics: ampicillin (10mg), chloramphenicol (10mg), cloxacillin (10mg), erythromycin (5mg), gentamicin (5mg), penicillin (1 unit), streptomycin (10mg) and single discs of ceftriaxone (30mg), norfloxacin (10mg), ofloxacin(10mg), amoxycillin/clavulanic acids (30mg), imipenem (10mg) and pefloxacin (5mg). Control organisms used were; *Staphylococcus aureus* ATCC 29213, *S. epidermidis* ATCC 14990, *S. saprophyticus* ATCC 15305.

RESULTS

Pure and mixed growth of bacteria were obtained from the 194 swabs specimens from apparently normal populations. These consisted of mixed growths of both gram-positive organisms, gram-negative bacteria and pure growths of gram-positive cocci. A total of 85 coagulase-negative staphylococci were obtained. This included 55 pure cultures of CoNS and 30 mixed cultures of CoNS with other bacteria.

Characterisation of the 55 pure cultures of coagulase-negative staphylococci to species level gave the following species distribution: *S. epidermidis* (18); *S. saprophyticus* (5); *S. xylosus* (8); *S. hominis* (10); *S. haemolyticus* (7); *S. capitis (4); S. simulans* (3)- Table 7.2.

A total of 26(47.3%) were slime producers while 40(72.7%) produced beta-lactamase enzyme (penicillinase)-Table 7.3. Of the 55 isolates of CoNS 100% were resistant to penicillin ; Ampicillin (98.2%), Cloxacillin (81.8%), erythromycin (83.6%), chloramphenicol (61.8%), amoxycillin\clavulanic acid (78.2%) and methicillin (89.1%) were also highly resistant to these isolates. Pefloxacin (5.5%), gentamicin (7.3%), Imipenem (7.3%), and norfloxacin (9.1%) were the most effective drugs against these isolates while ceftriaxone (18.2%); ofloxacin (27.3%), and streptomycin (36.14%) had good *in-vitro* efficacy against these isolates -Table 7.4

DISCUSSION

In this study, seven types of coagulase-negative staphylococci (CoNS) were obtained from different sites among normal population with *S. epidermidis* (32.7%) being the most prevalent. The prevalence rates of the other six species were *S. hominis* (18.2%), *S. xylosus* (14.5%), *S. haemolyticus* (12.7%), *S. saprophyticus* (9.1%), *S. capitis* (7.3%) and *S. simulans* (5.5%). In the characterisation of CoNS from various clinical specimens in this study, 10 different species were obtained with *S. epidermidis* (44.3%) being the most prevalent. The prevalence rates of the other species were *S. saprophyticus* (14.6%), *S. capitis* (11.0%), *S. haemolyticus* (9.8%), *S. simulans* (9.8%), *S. warneri* (2.8%), *S. xylosus* (2.4%), *S.hominis* (2.0%), *S. lugdunensis* (0.8%) and *S. cohnii* (0.4%).

In the two studies, *S. epidermidis* was the most prevalent. This is in agreement with the studies of several authors which had attributed *S. epidermidis* as the most CoNS species encountered species in clinical specimens (Kloos and Bannerman, 1994; Pfaller and Herwaldt, 1988) and most widely distributed over the body surfaces (Kloos, 1986; Kloos and Musselwhite, 1975). The distribution of the other species of CoNS in this study was at variance with the study on the characterisation of CoNS in clinical specimens. *S. hominis* 10 (18.2%) was the second most prevalent species in this study whereas, it accounted for only 5(2%) strains out of 244 strains obtained from clinical specimens. In fact, it was among the group of least isolated species. This might be attributed to the sources of specimens because *S. hominis* are reported to be numerous on skin sites where apocrine

glands (example, axillae, inguinal) are found. There were differences in the isolation rates from clinical specimens and the normal population. In the study of CoNS from clinical specimens, isolates were obtained from a wider spectrum of sites which included wounds from different sites, blood, urine, urethral swabs, ear, eye, bone etc.

Beta-lactamase was detected in 40(72.7%) out of 55 CoNS. This was comparable to beta-lactamase detected in 162(69.5%) out of 233 CoNS from clinical specimens. This might also account for the high resistance patterns obtained in this study for the beta-lactam antibiotics, penicilin (100%); ampicillin (98.2%); methicillin (89.1%), cloxacillin (81.8%) amoxycillin/clavulanic acid (78.2%). The finding shows that most strains of CoNS were beta-lactamase producers and consequently resistant to beta-lactam antibiotics.

Slime production was detected in most of the species and slime producers accounted for 26(47.3) of CoNS in this study while among the clinical specimens 96(40.5%) were slime producers. In both studies *S. epidermidis* accounted for the greatest slime producers. Slime production has been attributed as part of the microbial factors associated with virulence among CoNS species especially *S. epidermidis* (Kloos and Bannerman, 1994; Farber *et al.*, 1990; Christensen *et al.*, 1985). The first step in the process of foreign body (intravascular catheter, prosthetic devices etc.) infection involves the adhesion of bacteria to biomaterials (mainly synthetic polymers. Polysaccharide-adhesin (PS/A) appears to enhance the very early stages of colonization of biomaterials (Kloos *et al.*, 1992). They suggested that long-term colonizing strains on the skin of healthy individuals also produce significant amounts of PS/A and biofilm.

In this study most of the CoNS were resistant to commonly used antibiotics, especially the beta-lactams. But gentamicin one of the commonly used aminoglycosides showed a good *in-vitro* efficacy of 92.7%, unlike the CoNS strains from clinical specimens which showed 57.1% sensitivity. The effectiveness of the newer drugs were comparable to those obtained from strains from clinical specimens. Thus the multiresistance reported in this study was associated with the excessive use or abuse of drugs in this environment (Odugbemi, 1981).

CONCLUSION

Staphylococcus epidermidis has been shown to be the most prevalent both on the skin and from clinical specimens. The isolation of multidrug-resistant CoNS from apparently healthy population demonstrate the extent of antibiotic usage in this environment. Since CoNS are common inhabitant of the skin, they might be a source of antibiotic resistant for other bacteria of other genera.

Table 7.1: Distribution of sampling Population and Specimens Collected.

Sampling Population	Number	Hand Swabs	Skin	Nasal	Axillary
Doctors	3	3	3	3	3
Nurses	8	8	8	8	8
Mothers	10	10	10	10	10
Babies	10	-	-	-	10
NIMR Staff	25	25	25	25	25
Total	**56**	**46**	**46**	**46**	**56**

Table 7.2: Distribution of Coagulase-negative Staphylococci (CoNS) Isolated from Different Sampling Specimens.

		No. of Coagulase-negative Staphylococci Species						
	Total No.(%) CoNS Isolates	***Staphylococcus epidermidis***	***Staphylococcus saprophyticus***	***Staphylococcus xylosus***	***Staphylococcus hominis***	***Staphylococcus haemolyticus***	***Staphylococcus capitis***	***Staphylococcus simulans***
Hand Culture (46)	13(28.3)	5	1	2	2	2	-	1
Skin Culture (46)	18(39.1)	7	2	4	2	-	3	-
Nasal Culture (46)	10(21.7)	2	1	1	2	3	-	1
Axillary (56)	14(25.0)	4	1	1	4	2	1	1
TOTAL (194)	55(100)	18(32.7)	5(9.1)	8(14.5)	10(18.2)	7(12.7)	4(7.3)	3(5.5)

Table 7.3. Beta-Lactamase Detection and slime production among coagulase-negative strains.

Species	No. of Strains Tested	Beta-Lactamase Production.	Slime production
Staphylococcus capitis	4	3 (75.00)	1 (20.0)
Staphylococcus epidermidis	18	15 (83.3)	11 (61.1)
Staphylococcus haemolyticus	7	5(71.4)	4 (57.1)
Staphylococcus hominis	10	6 (60.0)	4 (40.0)
Staphylococcus saprophyticus	5	3 (60.0)	2 (40.0)
Staphylococcus simulans	3	2(66.7)	1 (33.3)
Staphylococcus xylosus	8	6 (75.0)	3 (37.5)
TOTAL	**55**	**40 (72.7)**	**26 (47.3)**

Table 7.4: Antibiotic resistance patterns of coagulase- negative Staphylococcal species from normal population.

Species	No. of Strains	NUMBER (% RESISTANCE)														
		Ampicillin	Chloramphenicol	Cloxacillin	Erythromycin	Gentamicin	Penicillin	Streptomycin	Tetracycline	Ceftriaxone	Ofloxacin	Pefloxacin	Norfloxacin	Imipenem	Amoxycillin/ Clavulanic acid	Methicillin
		Number (% Resistance)														
S. capitis	4	4 (100)	4(100)	3 (75.0)	4 (100)	-	4(100)	1 (25.0)	4 (100)	-	1 (25.0)	-	-	-	3 (75.0)	4 (100)
S. epidermidis	18	18 (100)	13 (72.2)	15(83.3)	16 (88.9)	2 (11.1)	18(100)	8 (44.4)	15 (83.3)	5 (27.8)	6 (33.3)	1 (5.6)	2 (11.1)	1 (5.6)	15(83.3)	17 (94.4)
S. haemolyticus	7	7 (100)	5 (71.4)	6 (85.7)	6 (85.7)	-	7(100)	1 (14.3)	7 (100)	1 (14.3)	-	-	1 (14.3)	1 (14.3)	4 (57.1)	6 (85.7)
S.. hominis	10	9 (90.0)	7 (70.0)	8 (80.0)	7 (70.0)	1 (10.0)	10(100)	3 (30.0)	8 (80.0)	-	2 (20.0)	1 (10.0)	-	2 (20.0)	9 (90.0)	8 (80.0)
S. saprophyticus	5	5 (100)	3 (60.0)	4 (80.0	4 (80.0)	-	5 (100)	3 (60.0)	3 (60.0)	1 (20.0)	1 (20.0)	-	2 (40.0)	-	3 (60.0)	3 (60.0)
S. simulans	3	3 (100)	1 (33.3)	2(66.7)	3 (100)	-	3 (100)	1 (33.3)	3(100)	1 (33.3)	2 (66.7)	-	-	-	3 (100)	3 (100)
S. xylosus	8	8 (100)	1 (12.5)	7(87.5)	6 (75.0)	1 (12.5)	8 (100)	3 (37.5)	5(62.5)	2 (25.0)	3 (37.5)	1 (12.5)	-	-	6 (75.0)	8 (100)
Total	55	54 (98.2)	34(61.8)	45 (81.8)	46 (83.6)	4 (7.3)	55 (100)	20 (36.4)	45(81.8)	10 (18.2)	15 (27.3)	3 (5.5)	5 (9.1)	4 (7.3)	43 (78.2)	49 (89.1)

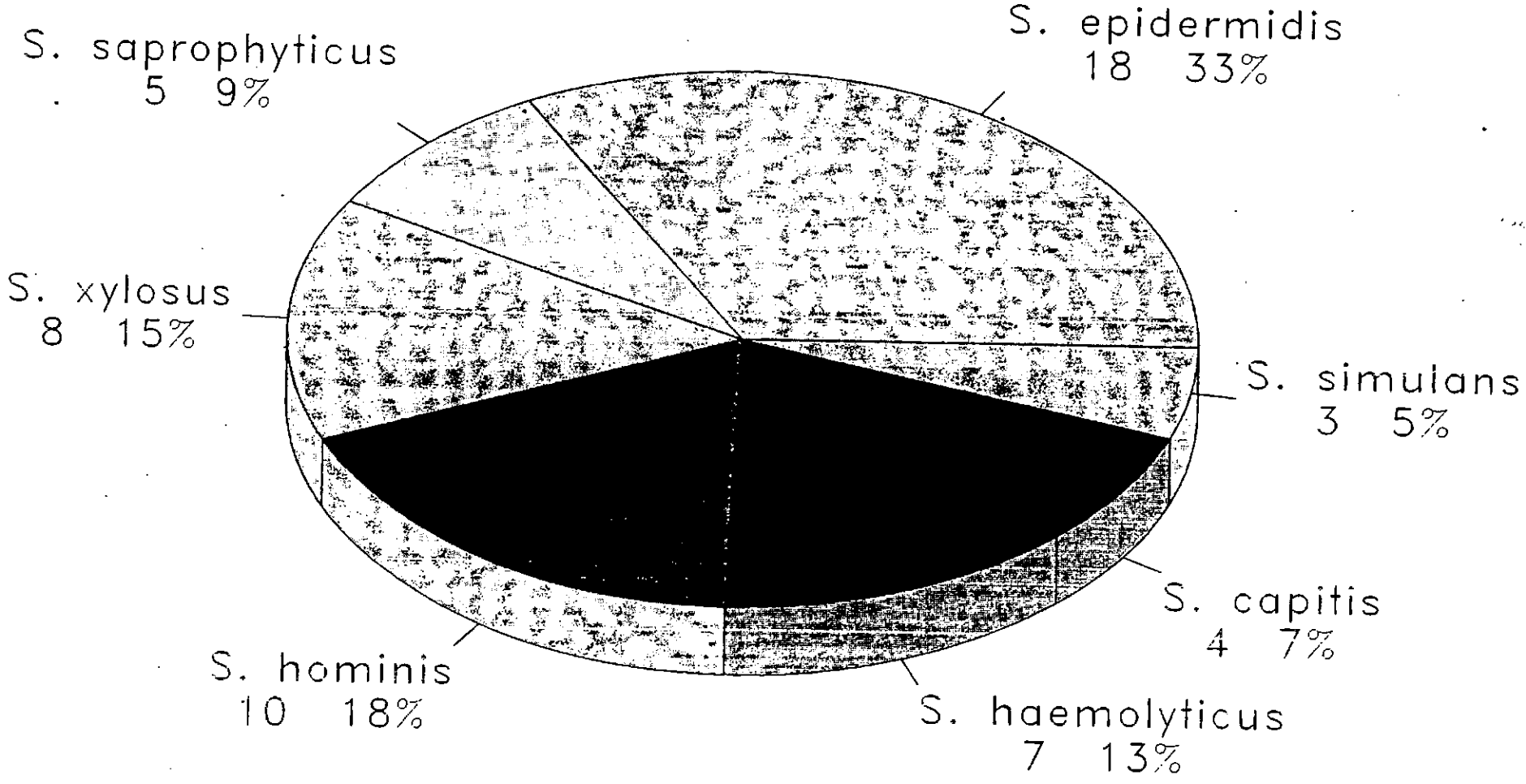


Fig. 7.1. Species distribution of coagulase-negative staphylococci isolates from normal population.

CHAPTER 8

GENERAL DISCUSSION

The present work was undertaken to look at the possible involvement of coagulase-negative staphylococci (CoNS) in clinical infections in our environment. This therefore necessitated an in-depth study into characterization, detection of extracellular products and plasmids and antibacterial susceptibility profiles of these group of organisms isolated from different clinical specimens. Based on the results obtained using both conventional methods and the rapid commercial API Kit (ID 32 STAPH), 10 different species of coagulase-negative staphylococci were obtained.

Between the Conventional methods of characterization of Kloos and Schleifer, 1975 and the rapid API Kit, there was no statistical significant difference in the number of isolates characterised. However, there was a major significant difference in the types of CoNS species identified. The Kloos and Schleifer conventional method was unable to identify some new species for example, the newly described human species of CoNS *Staphylococcus lugdunensis.* This was characterised only by the API Kit. Although, most of the important prevalent human species of CoNS were identified in this study using conventional methods, it is necessary to use the Kloos and Bannerman scheme of 1995 because it contains additional biochemical tests. This will take care of newly described human species of CoNS.

Staphylococcus epidermidis was the most prevalent in all groups of infections. The predominance of this group of CoNS in most clinical specimens is consistent with other studies on species characterization of CoNS that relate *Staphylococcus epidermidis* to be a major species of clinical relevance (Kloos and Bannerman, 1994; Udo *et al.*, 1997).

All the other species of CoNS obtained were implicated at low incidence in a variety of infections, with *Staphylococcus saprophyticus* being the most prevalent and it was found mostly in cases of urinary tract infections. These findings are not surprising in view of the widespread distribution of staphylococci over the body surface. Thus, their isolation in pure cultures from clinical specimens makes them opportunistic pathogens in our environment. Some of the newly described species *S. lugdunensis* identified in this study was isolated only from wound specimen. About 50% of this CoNS species possess lipase enzyme.

The possession of some extracellular products for example, enzymes have been linked to virulence in a number of microrganisms. A total of 40.50% of CoNS showed lipase activity with greatest activity being shown by *S. haemolyticus* (54.17%). Other enzymes detected among CoNS were as follows: Dnase 11.8%; gelatinase 11.00%, protease 8.9%. These enzymes are recognised virulence factors and similar to those produced by *Staphylococcus aureus*(Gemmell, 1986; 1987). Available evidence also suggests that at least part of the human virulence of CoNS can be attributed to their ability to elaborate some of these exoproteins (Gemmell and Roberts, 1973, Gemmell, 1987). Some of these CoNS have been shown to produce extracellular slime which creates a protective microenvironment for

CoNS strain and a barrier to antibiotic action. Approximately 69.5% of the coagulase-negative staphylococci tested in this study were also beta-lactamase producers. Beta-lactamase production is the predominant resistance mechanism of microorganisms to beta-lactam antibiotics (Livermore, 1991). This has led to consequent therapeutic failure of some beta-lactam antibiotics particularly penicillin (Garau, 1994). It is not surprising that the results showed 100% resistance to penicillin by all the CoNS species, with 75% resistance to methicillin. Coagulase-negative staphylococci tested showed over 50% resistance to ampicillin, cloxacillin and amoxycillin/clavulanic acid while very low resistance was recorded for imipenem (5.2%) and ceftriaxone (14.2%). Imipenem is, however, not rountinely used in this environment. It is also not recommended for use in small infants due to its greater propensity for eliciting seizures compared to other beta-lactam agents (Wong et al., 1988). It has been found that CoNS can show wide variability in their antibiotic resistance patterns and be resistant to many antibiotics (Ang *et al.*, 1985, Deighton *et al.*, 1988; Pal and Ayyagari 1989, Kloos and Bannerman, 1994). Apart from a large proportion of CoNS being resistant to many beta-lactam antibiotics, many were resistant to commonly prescribed antibiotics (Tetracycline, Streptomycin, Gentamicin, Erythromycin in our environment. The quinolones, pefloxacin, ofloxacin and norfloxacin used in this study were highly effective against CoNS.

Multiple drug resistance were encountered especially with isolates from pus and other skin sites. These results are not surprising in view of the high level of antibiotic abuse/poor hygiene in certain areas in Nigeria. Although resistance to the expensive broad spectrum antibiotics notably the cephalosporins and quinolone is still low, their heavy use may

precipitate the development of resistant strains with time. However, because of the lower virulence of CoNS, therapy of CoNS infections should await the result of drug susceptibility testing.

As the antimicrobial resistance patterns of CoNS in our environment were diverse in nature so were their plasmid contents. Most of these plasmids were of low molecular weight. Most of the strains that harboured multiple plasmids were also resistant to many of the antibiotics tested. Thus the multiple plasmids might have accounted for the multiple antibiotic resistance obtained.

CHAPTER 9

GENERAL CONCLUSION

It is clear from this study that one would expect to isolate a variety of species and many different strains of coagulase-negative staphylococci (CoNS) in our laboratories. Coagulase negative staphylococci are widely spread on the human body, distinguishing the aetiologic agent/agents from contaminating flora is a serious challenge to the clinical laboratory. The solution to this problem will be facilitated by the quality of the specimen obtained from the patient and determining accurately the infecting agent.

This study has also identified the possession of some enzymes like DNase, lipase, gelatinase by CoNS. Some of these exoproteins are recognised virulence factors and similar to those produced by *S. aureus*. Slime production has been shown to be an important factor in the colonization and infection of prosthetic devices and some of the CoNS in this study produced slime.

This study has also identified the production of Beta-lactamase enzyme by CoNS. Detailed information on the antibiotic susceptibility patterns, and minimum inhibitory concentrations of antibiotics against different species have been provided. Therapeutic usage of these antibiotic especially the commonly prescribed ones in our environment should be based on good *in vitro* efficacy, since most of the CoNS were resistant to them. The presence of Beta-lactamase enzyme calls for great caution in the use of Beta-lactam antibiotics.

The antibiotic multidrug-resistant nature of CoNS and their plasmid profiles were established. The isolation and characterization of the CoNS from skin , axillae, hands, and nose showed also that some Staphylococcus demonstrate habitat or niche preferences on their particular hosts. Since CoNS are common inhabitants of the skin and mucous membranes, multiple drug resistant CoNS may be a reservoir of resistance for other organisms. Caution should also be exercised in the indiscriminate use of antibiotics.

Finally the results of this study should be useful to the laboratory personnel and clinicians who still have been querying the isolation of CoNS as contaminants. This will guide the clinicians in their treatment schedule especially in neonates, seriously ill and immuno-compromised patients. This is the first detailed report on coagulase-negative staphylococci (CoNS) in Lagos.

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APPENDICES

APPENDIX ONE

PROCEDURES USED FOR SOME BIOCHEMICAL TESTS CARRIED OUT DURING THE STUDY

MEDIA USED

The following media used for the study were prepared according to the manufacturer's or author's instructions. All media were sterilised by autoclaving at 121°C for 15 minutes, cooled to 50°C and poured aseptically into sterile petri dishes or final containers and stored at 4°C except where otherwise stated.

1) P agar (Naylor and Burgi, 1956)

Peptone	-	10.0g
Yeast extract	-	5.0g
Sodium chloride	-	5.0g

Glucose	-	1.0g
Agar	-	15.0g
Distilled water	-	1 litre

pH 7.4

2) P SHEEP BLOOD AGAR

Same as in No. 1 P - agar was cooled to 45-50°C, 5% sheep blood added to it, mixed with gentle rotation and poured into sterile petri dishes. One plate was incubated at 37°C to ascertain purity.

3) NUTRIENT BROTH (OXOID)

Lab-Lemco Powder (Oxoid L37)	-	1.0g
Yeast Extract Powder (Oxoid L37)	-	2.0g
Peptone (Oxoid L37)	-	5.0g
Sodium chloride	-	5.0g
Distilled water	-	1 litre.

pH 7.4

4) NUTRIENT AGAR (OXOID)

Lab-Lemco Powder (Oxoid L37)	-	1.0g
Yeast Extract Powder (Oxoid L37)	-	2.0g
Peptone (Oxoid L37)	-	5.0g
Sodium chloride	-	5.0g

Agar	-	15.0g
Distilled water	-	1 litre.

pH 7.4

5) PHOSPHATE-BUFFERED SALINE (OXOID)

Sodium chloride	-	8.0g
Potassium chloride	-	o.2g
Disodium hydrogen phosphate	-	1.15g
Potassium dihydrogen phosphate	-	0.2g
Distilled water	-	1 litre.

pH 7.3 approx

6) MacCONKEY AGAR (BIOTEC)

Selected Peptone mixture	-	15.0g
Sodium chloride	-	5.0g
Neutral red	-	0.05g
Lactose	-	10.0g
Bile salts	-	5.0g
Agar No. 2	-	12.0g
Distilled water	-	1 litre.

pH 7.4

7) BLOOD AGAR BASE (OXOID)

Proteose peptone	-	15.0
Liver digest	-	2.5g
Yeast extract	-	5.0g
Sodium chloride	-	5.0g
Agar	-	12.0g
Distilled water	-	1 litre

8) BLOOD AGAR

The blood base was cooled to 45-50oC, 10% human blood added to it, mixed with gentle rotation and poured into sterile petri dishes. One plate was incubated at 37oC to ascertain purity.

9) MANNITOL SALT AGAR (OXOID)

Lab-Lemco powder	-	1.0g
Peptone	-	10.0g
Mannitol	-	10.0g
Sodium chloride	-	74.0g
Phenol red	-	0.025g
Agar	-	15.0g
Distilled water	-	1 litre

pH 7.5 approx.

10) TROPTONE SOYA AGAR (TSA) OXOID

Tryptone	-	15.0g
Soya peptone	-	5.0g
Sodium chloride	-	5.0g
Agar	-	15.0g
Distilled water	-	1 litre

pH 7.4

11) MUELLER-HINTON BROTH (OXOID)

Beef dehydrated infusion	-	6.0g
Casein hydrolysate	-	17.5g
Starch	-	1.5g
Distilled water	-	1 litre

pH 7.4

12) MUELLER-HINTON AGAR (OXOID)

Beef dehydrated infusion	-	6.0g
Casein hydrolysate	-	17.5g
Starch	-	1.5g
Agar	-	10.0g
Distilled water	-	1 litre

13) PHYSIOLOGICAL SALINE

Sodium chloride	-	8.5g
Distilled water	-	1 litre.

14) PHENOLPHTHALEIN PHOSPHATE AGAR.

Phenolphthalein diphosphate, sodium salt 1% aqueous solution Sterilze by filtration and store at 4oC

Phenolphtalein phosphate solution	-	10ml
Nutrient agar	-	1000ml

The nutrient agar was melted and cooled to 45-50°C.

Phenolphthalein phosphate solution was added aseptically, mixed and distributed into petri dishes.

15) NITRATE BROTH (DIFCO).

Beef Extract	-	3.0g
Peptone	-	5.0g
Potassium nitrate	-	1.0g
Distilled water	-	1 litre.

16) THIOGLYCOLLATE MEDIUM BROTH (OXOID).

Lab-Lemco (L29)	-	1.0g
Yeast extract	-	2.0g

Peptone	-	5.0g
Dextrose	-	5.0g
Sodium chloride	-	5.0g
Sodium thioglycollate	-	1.1g
Methylene blue	-	0.002g
Agar No. 1	-	1.0g
Distilled water	-	1 litre

pH 7.3

17) DNase TEST AGAR (DIFCO)

Tryptose	-	20.0g
Deoxyribonucleic acid	-	2.0g
Sodium chloride	-	5.0g
Agar	-	15.0g
Distilled water	-	1 litre

pH 7.3

18) TRYPTONE YEAST EXTRACT GLUCOSE AGAR OXOID

Tryptone	-	20.0g
Yeast extract	-	2.0g
Glucose	-	10.0g
Agar	-	10.0g

Distilled water	-	1 litre

pH 7.2

19) PURPLE AGAR BASE

Peptone	-	10.0g
Beef extract	-	1.0g
Sodium chloride	-	5.0g
Bromocresol purple	-	0.02g
Agar	-	15.0g
Distilled water	-	1 litre

pH 6.8±0.2.

20) CARBOHYDRATE AGAR

Carbohydrate agar was prepared by adding an appropriate sample of a filter-sterilized carbohydrate stock solution to an autoclave-sterilized purple agar base (Difco) medium cooled to 45-50°C. The final carbohydrate concentration in the agar medium was 1%.

21) MEDIA FOR ACID PRODUCTION FROM GLYCEROL.

Ammonium hydrogen phosphate	-	1.0g
Potassium chloride	-	0.2g
Magnesium sulphate	-	0.2g
Yeast extract	-	2.0g

Glycerol (Sigma)	-	10.0ml
Bromocresol purple	-	0.04g
Agar	-	9.0g
Distilled water	-	1 litre

After the medium was autoclaved and allowed to cool, 0.4ml of a 1000mg/ml solution of erythromycin (sigma) was added and allowed to mix. The medium was dispensed into petri dishes.

GRAM-STAIN

Gram-staining of pure culture, of isolates was performed as follows; A thin film or smear of an 18-24 hours old pure culture was prepared on a clean grease-free slide. The film was air dried and heat-fixed. It was subsequently flooded with crystal violet stain and allowed to remain for 30 seconds. The stain was then poured off and the remainder rinsed with water. The slide was subsequently flooded with Lugol's iodine (mordant) for another 30 seconds, and rinsed off with running tap water. Alcohol-acetone was then applied for precisely 10 seconds.

The slide was immediately rinsed with tap water, counterstained with safranin for 30 seconds and washed off with tap water. the slide was blotted dry in between filter paper and observed under oil immersion objective.

Organisms that are purple in colour are recorded as gram-positive while those organisms

that took up the counterstain (Safranin) and were red in colour were recorded as gram-negative.

COAGULASE TEST

SLIDE TEST: A drop of physiological saline was placed on each end of a glass slide. A colony of the test organism (from nutrient or blood agar plate) was emulsified in each of the drops to make two thick suspensions. A drop of plasma was added to one of the suspensions and mixed gently. A positive result was shown by clumping of the organism within 10 seconds. No plasma was added to the second suspension in order to differentiate any granular appearance of the test organism from true coagulase clumping.

TUBE TEST: The plasma was diluted 1 in 10 in physiological saline. About 0.5 ml of diluted plasma was separately pipetted into 3 test tubes labelled test organism(s), positive control and negative control respectively. Five drops (about 0.1 ml) of each test organism culture (18-24 hours) was added to the tubes labelled for test organisms. Five drops of a culture of *Staphylococcus aureus* control strain ATCC 29213 (18-24 hours) was added to the tube labelled for the positive control. Five drops of sterile broth (nutrient broth) was added to the tube labelled for negative control. After mixing gently, the tubes were incubated at 37 degrees centigrade. The tubes were examined after 1 hour for clotting. If no clotting had occurred, the tubes were examined at 30 minutes intervals for up to 6 hours. Coagulum was observed from positive tubes by tilting the tubes at an angle. Negative coagulase control organism was *Staphylococcus epidermidis* ATCC 14990.

CATALASE TEST

Using a sterile glass rod a good growth of the test organism was removed from a 24 hour old culture on hutrient agar slant. This was emulsified in 0.5 ml of 3% hydrogen peroxide solution. A positive reaction was indicated by the rapid production of gas bubbles. Staphylococcus strain ATCC 29213 was used as positive control organism while Streptococcus strain was used as negative control.

PRODUCTION OF ACID FROM GLYCEROL (Schleifer and Kloos, 1975)

Three to four colonies of the test organism were used to make a streak on the surface of the medium (appendix 1.21). A positive result was indicated by the appearance of a yellow halo surrounding the streak after 18 to 24 hours of incubation. Organism that did not produce acid were incubated for a total of 72 hours before being recorded as negative for acid production from glycerol.

LYSOZYME AND LYSOSTAPHIN SUSCEPTIBILITIES.

An agar overlay was prepared by adding 0.1 ml of saline cell suspension (containing approximately 10^7 colony-forming units (cfu/ml) to a tube containing 3 ml of fluid, soft P agar (0.75% agar). The contents were gently mixed and poured on the surface of a dry P agar plate. Using a 2 ml syringe a drop of sterile lysozyme solution (400 mg/ml) was placed on the inoculated agar overlay plate. On the same inoculated agar overlay a sterile lysostaphin solution 200 mg/ml was placed too but several centimeters apart. After the

application of lysozyme and lysostaphin the plates were incubated for 48 hours and examined. Growth inhibition around the spots were recorded accordingly: susceptible - complete growth inhibition; slightly resistant - partial growth inhibition, and resistant - no visible growth inhibition.

OXIDASE TEST

A smear of an 18-24 hours old culture of test isolate was made on a clean Whitman No.1 filter paper previously dipped in oxidase reagent (1%aqueous solution of tetramethyl-p-phenylamine dihydrochloride) with the aid of a sterile platinum loop. The appearance of a dark-purple colour at the region of the smear within 5-10 seconds confirmed a positive result.

ANAEROBIC GROWTH IN A THIOGLYCOLLATE MEDIUM (AEROBIC REQUIREMENT)

The aerobic requirement of each strain was estimated by using the semi-solid thioglycollate medium described by Evans and Kloos (1972). A loopful of a culture of test organism was suspended in 2 ml of physiological saline. About 0.1 ml sample (approximately 10^7 cfu/ml) of the cell suspension was then used to inoculate a tube containing 8 ml of fluid (52 °C) thioglycollate medium, (Brewers fluid thioglycollate medium plus 0.3% agar) inoculated tubes were allowed to solidify at room temperature and then were incubated for 5 days at 35 °C. Growth characteristics were observed within this periods. Strains demonstrating uniform anaerobic growth show this properly within 24-48 hours and require no further

incubation. Strains producing a gradient of dense to light growth down the medium or those which failed to show evidence of anaerobic growth within 48 hours were incubated for the entire 5 days. This was to allow for the possibility of discrete colony development.

HAEMOLYSIS ON BLOOD AGAR

P-sheep blood agar plates were prepared (Appendix 1.2), streaked with the appropriate test organism and incubated at 37 °C for 24 hours.

Haemolytic reactions were recorded as follows, a clear colourless wide zone surrounding the colony of the organism was indicating strong haemolysis, a faint colourless zone around the colony of the organism indicate trace haemolysis and negative by the absence of any apparent haemolysis.

NITRATE REDUCTION

Sterile nitrate broth (0.5 ml) was inoculated with a heavy growth of a 24 hour old culture of a test organism and incubated at 37 °C for 5 days as described by Cowan and Steel (1985).

Positive result was recorded if red colouration was produced on the addition of 1 drop of sulphanilic acid reagent (sulphanilic acid - 8g in 1 litre of 5 N Acetic acid) and 1 drop of alpha naphthylamine reagent (alpha- naphthylamine - 5 g in 1 liter of 5 N Acetic acid). This showed that nitrate was reduced. Powdered zinc dust was added at 5 mg/ml (knife point) of cultures to tubes not showing a red colour and allowed to stand for a few minutes.

Production of red colour was indicative of the presence of unreduced nitrate confirming the inability of the organism to utilize nitrate while absence of red colour means the nitrate have been utilized.

PHOSPHATASE TEST

Phenolphthalein phosphate agar was lightly inoculated with the test organism to obtain discrete colonies and incubated for 48 hours at 37 °C. About 0.1 ml ammonia solution (sp. gr. 0.880) was placed on a lid of the petri dish while the inoculated medium was inverted above it. Free phenolphthalein liberated by phosphatase reacts with the ammonia and phosphatase-positive colonies become bright pink. *Staphylococcus aureus* ATCC 29213 was used as positive control while *Staphylococcus saprophyticus* ATCC 15305 was used as negative control.

ACETOIN PRODUCTION (Davis and Hoyling, 1973)

The test organism was patch inoculated onto tryptone, yeast extract, glucose agar. After 48 hours of incubation at 30 °C, a 1 cm disk of Whatman 3 M paper freshly soaked in 10% sodium pyruvate solution was placed on each growth patch. The plates were reincubated for 3 hours. Acetoin production was detected by spotting one drop of 40% potassium hydroxide, one drop of 1% creatine, and one drop of 1% alpha naphthol (alcoholic) onto each disk and observed for a pink-red colour change for 1 hour at room temperature for positive result.

NOVOBIOCIN SUSCEPTIBILITY

Novobiocin susceptibility was determined by the disc diffusion method of Bauer et al (1966) with an inoculum suspension of 10^7 cfu/ml in normal saline equivalent to a 0.5 Mcfarland turbidity standard on Mueller-Hinton agar plates using a sterile non-toxic swab stick. Five microgramme disc of Novobiocin was used. Incubation and interpretation of result is as described in Chapter 4.

AEROBIC ACID PRODUCTION FROM CARBOHYDRATES

Culture streaks of test organisms were made by lightly inoculating a 1-cm line on the surface of a carbohydrate agar (appendix 1.20) plate with a loopful of cells. Four to eight streaks could be made on a plate. Different carbohydrates were tested. Cultures were incubated and then at 24 and 72 hour. Reactions were interpreted as follows. Moderate to acid strong acid production was shown by yellow indicator colour extending out from the culture streak into the surrounding medium within 72 hours; weak acid production was shown by distinct yellow indicator colour under culture streak but not extending into the surrounding medium within 72 hours; no acid production was shown by very faint to no yellow indicator colour under the culture streak within 72 hours. Strong acid production was detected within 24 hours and moderate acid production of most strains was detected within 48-72 hours.

APPENDIX TWO

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

Preparation of materials and reagents

The microdilution trays used in the microdilution broth test were usually sterilized under ultra-violet (U-V) light for 24 hours (usually overnight) before use.

The pipettes and glass wares were usually sterilized in the hot air oven at 160 degrees Celsius for 2 hours, while the micro pipette tips and McCartney bottles were sterilized in the autoclave at 121 degrees Celsius for 15 minutes or as found appropriate.

Mueller-Hinton broth medium was used as the growth medium for the antibiotic sensitivity testing of the isolates, as suggested by the National Committee for Clinical Laboratory Standards (NCCLS) disc diffusion standard (1993b).

The Mueller-Hinton broth (Appendix 1.11) was prepared according to specification of the manufacturer and dispensed in 25 ml equal volume into McCartney bottles before sterilization. This is done to limit the risk of contamination of the medium. The broth was stored in the refrigerator at 4 °C until needed.

Preparation of inoculum

Pure cultures of clinical isolates on TSA slant were usually subcultured in sterile nutrient

broth. This was incubated at 37 °C overnight and used for preparing the inoculum for the sensitivity testing as follows:

The overnight culture of the organisms with detectable growth was evident from the turbidity of the medium was centrifuged at 250 rpm for 5 minutes. The supernatant was discarded while the pellet of cells was resuspended in physiological saline (0.85% NaCl) and centrifuged again. This was repeated twice to remove any excess broth medium mixed with cell mass.

The pure cell mass was finally suspended about 5 ml physiological saline and adjusted to 0.5 Mcfarland standard.

The resulting standardized suspension of cells was then diluted ten-fold to give 10^7 cfu/ml (1:10 dilution). This was used as the inoculum for performing the test.

3. Antibiotic dilution procedure

The solvent used for the various antibiotic powder and tablets/solutions was sterile normal saline, the same solution was as the diluent.

Various concentrations of the antibiotic as required were prepared in double strength, since the Mueller-Hinton broth medium (basal medium) was also prepared in double strength. Both the antibiotic and the basal medium were mixed in equal volume to bring their concentrations back to single strength.

4. Inoculation of test plates

With an adjustable micro-pipette, 50 ml of the sterile double strength Mueller-Hinton broth was pipetted into the microtitre plate wells (U-shape). Then 50 ml of the already prepared antibiotics of various concentrations were added into all the wells except control well. Also 25 ml of the standardized 10^7 cfu/ml inoculum was added to the mixture of the antibiotics and growth medium (Mueller Hinton broth) in the wells.

Two control wells were maintained for each tests. These included wells containing the antibiotic and the growth medium without any inoculum (antibiotic control)and well containing only the growth medium and physiological saline, but inoculated with the test organism (organism control).

The plates were incubated overnight at 37 °C for 18-24 hours after which the results were read. The MIC was taken as the lowest concentration of the antibiotic that completely inhibited the growth of the organisms as detected by the unaided eye.

Comparison of growth (by visible turbidity) was made with the organism and antibiotic control wells as reference standards for positive and negative results respectively.

Reference organism *Staphylococcus aureus* ATCC 25923 was used.

APPENDIX THREE

32 500

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For in vitro diagnostic use

ID 32 STAPH

Identification system for staphylococci

ID 32 STAPH is an identification system for the genera *Staphylococcus, Micrococcus, Stomatococcus* and *Aerococcus*, using standardized and miniaturized biochemical tests with a specially adapted database.
The complete list of those bacteria that it is possible to identify with this system can be found in the identification table (p. 22 of this instruction manual).
Reading and interpretation can be carried out automatically or manually.

PRINCIPLE

The ID 32 STAPH strip consists of 32 cupules, 26 of which are used as test cupules and contain dehydrated test substrates.
After 24 hours of incubation, reading can be performed either using the ATB instruments, or visually.
Identification is obtained by referring to the Analytical Profile Index or with the corresponding identification software.

REAGENTS

Kit contents (25 tests) :

- 25 ID 32 STAPH strips
- 25 incubation lids
- 25 result sheets
- 1 instruction manual

Additional products (not included in the kit) :

- Suspension Medium, 2 ml (ref. 70 600) or 3 ml (ref. 70 630) if the ATB Inoculator is used
- Reagents : VP A (ref. 70 570)
  VP B (ref. 70 580)
  NIT 1 (ref. 70 440)
  NIT 2 (ref. 70 450)
  FB (ref. 70 560)
- Mineral oil (ref. 70 100)
- ATB Electronic Pipette (ref. 99 040) or Inoculator (ref. 15 740) and Tips (ref. 15 710)
- ATB Densitometer (ref. 15 500) or McFarland Standard (ref. 70 900)
- ATB Instruments + Identification software (consult bioMérieux) or ID 32 STAPH Analytical Profile Index (ref. 32 590)
- Ampoule stand (ref. 70 200)
- Air-tight box or anaerobic jar

Required laboratory equipment :

- 37°C incubator
- Refrigerator
- Bunsen burner
- Marker pen

STORAGE

The ID 32 STAPH strips, FB, VP B and NIT 2 reagents should be stored at 2-8°C until the expiration date indicated on the packaging.
The NIT 1 and VP A reagents should be stored at room temperature.
The reagents may be kept for up to 1 month after the ampoules have been opened.
The FB reagent is very sensitive to light : wrap the bottle in aluminium foil. The reagent should be destroyed as soon as it turns amber.

COMPOSITION OF MEDIA AND REAGENTS

Suspension Medium 2 or 3 ml	Demineralized water	
VP A reagent	KOH Water	20 g 100 ml
VP B reagent	α naphthol Organic solvent	12 g 100 ml
NIT 1 reagent	Sulfanilic acid Acetic acid 5 N	0.8 g 100 ml
NIT 2 reagent	N-N-dimethyl-1-naphthylamine Acetic acid 5 N	0.6 g 100 ml
FB reagent	Fast Blue BB Organic solvents	0.35 g 100 ml

INSTRUCTIONS FOR USE

Specimens and bacterial cultures should be considered infectious and handled appropriately by trained and competent technicians.
Aseptic technique and usual handling precautions for the bacterial group studied should be observed throughout this procedure (Refer to Biosafety in Microbiological and Biomedical Safety, US Department of Health and Human Services, 1988 or to the Regulation of each country).

Selection of the colonies

Harvest the colonies obtained on blood agar, P Agar, Mannitol Salt agar, Baird Parker or MacConkey medium.

Preparation of the strip

- Remove the strip from its packaging.
- Discard the desiccant.
- Place the lid on the strip.
- Record the strain reference on the elongated tab of the strip.

Preparation of the inoculum

- Open an ampoule of Suspension Medium, 2 ml (or 2 ml of sterile distilled water without additives). If the inoculator is used, Suspension Medium, 3 ml is required (or 3 ml of sterile distilled water without additives).
- Remove several identical colonies and make a suspension having a turbidity equal to 0.5 McFarland measured with the aid of the ATB 1550 Densitometer or compared with a turbidity standard (McFarland Standard).

Note : If the strip is to be read AUTOMATICALLY, it is ESSENTIAL to use the DENSITOMETER to adjust the turbidity of the bacterial suspension.

Inoculation of the strip

55 µl / cupule

- AUTOMATIC inoculation
  - On a tray of the ATB Inoculator, place a strip, the inoculated ampoule of Suspension Medium and an ATB tip.
  - The inoculator will automatically homogenize the medium and distribute 55 µl into the cupules.
- MANUAL inoculation
  - Homogenize the inoculated ampoule of Suspension Medium using the ATB Electronic Pipette.
  - Inoculate the strip by distributing 55 µl of suspension into each cupule with the ATB Electronic Pipette.

Overlay tests URE, ADH and ODC with 2 drops of mineral oil (cupules 1.0, 1.1, 1.2).
Place the lid on the strip.
Incubate at 37°C for 24 hours in aerobic conditions.

NOTE : Some ventilated incubators may completely dehydrate the medium in the cupules. In this case, place the strip in an air-tight box with a receptacle containing a small volume of water. The humid atmosphere created will avoid dehydration of the tests.

Reading the strip

Develop the reactions in row 0 by adding 1 drop of the following reagents :

- NIT test (cupule 0.0) : NIT 1 and NIT 2 reagents.
- VP test (cupule 0.1) : VP A and VP B reagents.
- Tests βGAL to PyrA (cupules 0.2 to 0.5) : FB reagent.

Read after 5 minutes (do not exceed 10 minutes) :

- AUTOMATIC reading :
  using the ATB instruments. The reader records the colours of each cupule and transmits the information to the computer.
- VISUAL reading :
  refer to the Reading Table. Record the results on the result sheet.

Identification

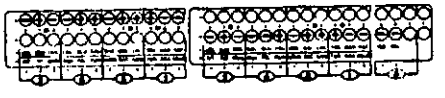
Identification can be made :

- AFTER AUTOMATIC READING :
  the results are transmitted to the computer and interpreted by the corresponding identification software.
- AFTER VISUAL READING :
  - use the Analytical Profile Index after having coded the results into a numerical profile.

    The tests on the result sheet are separated into groups of three. Each result is assigned a value of 1, 2 or 4 when positive and the values are added together within each group.

    The first 24 tests (left part of the strip) enable the construction of an 8-digit profile, by combining the 4 digits of the upper row (1) and the 4 digits of the lower row (0).

    The other tests (right part of the strip) are additional tests to be used when indicated in the Analytical Profile Index in cases of low discrimination.



2661 2461 0 *S. haemolyticus*

  - use the identification software by entering the 9-digit numerical profile via the keyboard : preceding profile plus a 9th digit for additional tests RIB and CEL (cupules 1.C and 1.D).

QUALITY CONTROL

The strips are systematically controlled at various stages of their manufacture. For those who wish to perform their own quality control tests, it is recommended to use the following stock cultures :

	URE	ADH	ODC	ESC	GLU	FRU	MNE	MAL	LAC	TRE	MAN	RAF	NIT	VP	βGAL	ArgA	PAL	PyrA	NOVO	SAC	NAG	TUR	ARA	βGUR	RIB	CEL
1.	-	-	-	-	+	+	-	V	-	+	-	-	-	-	-	+	-	V	-	V	-	-	-	-	-	-
2.	-	+	-	-	+	-	-	+	-	+	-	-	+	+	-	-	-	+	-	+	+	-	-	+	-	-
3.	+	-	-	-	+	+	+	+	+	+	+	-	+	-	+	-	+	V	-	+	+	-	-	-	+	-
4.	+	-	+	-	+	+	+	+	+	+	-	-	+	+	-	-	-	+	-	+	+	-	-	-	-	-
5.	-	-	-	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	+	+	+	-	+	+
6.	+	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	V	-	V	+	+	V	V	-	-	+

1. *Staphylococcus auricularis* ATCC 33753
2. *Staphylococcus haemolyticus* ATCC 29970
3. *Staphylococcus intermedius* ATCC 29663
4. *Staphylococcus lugdunensis* ATCC 49576
5. *Staphylococcus sciuri* ATCC 49575
6. *Staphylococcus lentus* ATCC 49574

ATCC : American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, USA.

DISPOSAL OF USED MATERIAL

After use, all ampoules, pipettes, tips and strips should be incinerated, autoclaved or immersed in a disinfectant for decontamination prior to disposal.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Qualified laboratory personnel should use aseptic technique and established precautions for infectious agents.
- Do not pipette specimens or reagents by mouth.
- Do not use reagents past the expiration date.
- Upon removal from refrigerator, allow reagents to come to room temperature (20-30°C).
- All inoculated products should be considered infectious and handled appropriately.
- After completing test, reading and interpretation, all specimens, spills and inoculated products must be autoclaved, incinerated or immersed in a germicide prior to disposal.
- Interpretation of the test results should be made by a competent microbiologist who should also take into consideration the patient history, the source of the specimen, colonial and microscopic morphology and, if necessary, the results of any other tests performed, particularly the antimicrobial susceptibility patterns.

LIMITATIONS

The ID 32 STAPH system is designed uniquely for the identification of the species included in the database.

RECOMMENDATIONS

To obtain the best results with the ID 32 STAPH strip, it is important to scrupulously respect the following points of the procedure :

- use the isolation medium recommended in this instruction manual (see § Selection of the colonies) ;
- precisely adjust the inoculum to 0.5 McFarland (the Densitometer must be used if the strip is to be read and interpreted by the ATB instruments) ;
- distribute exactly 55 µl per cupule with the ATB Electronic Pipette or the inoculator (imperative if the strip is to be read and interpreted by the ATB instruments) ;
- respect the incubation time (24 hours) and the reading time (5-10 minutes after addition of reagents) ;
- the reagents should be of good quality : check the expiration date and storage conditions and use within one month of opening the ampoules.

READING TABLE

CUPULE	TEST	REACTION/SUBSTRATE	RESULT	
			NEGATIVE	POSITIVE
1.0	URE	UREase	yellow	orange red-violet
1.1	ADH	Arginine DiHydrolase	yellow	orange-red
1.2	ODC	Ornithine DeCarboxylase		
1.3	ESC	ESCulin (Hydrolysis)	colourless-pale grey	brown-black
1.4	GLU	GLUcose	red red-orange	yellow yellow-orange
1.5	FRU	FRUctose		
1.6	MNE	MaNnoseE		
1.7	MAL	MALtose		
1.8	LAC	LACtose (Fermentation)		
1.9	TRE	TREhalose		
1.A	MAN	MANnitol		
1.B	RAF	RAFfinose		
1.C	RIB	RIBose		
1.D	CEL	CELobiose		
1.E		Empty cupules		
1.F				
			NIT 1 + NIT 2 (5 min < 10 min)	
0.0	NIT	NITrates (Reduction)	colourless	pink-purple
			VP A + VP B (5 min < 10 min)	
0.1	VP	Acetoin Production	colourless	pink-red
			FB (5 min < 10 min) β GAL ⇒ PyrA	
0.2	βGAL	β GALactosidase	colourless pale purple pale orange	purple
0.3	ArgA	Arginine Arylamidase	colourless pale orange	orange
0.4	PAL	ALkaline Phosphatase	colourless pale purple pale orange	purple
0.5	PyrA	Pyrrolidonyl Arylamidase	colourless pale orange	orange
0.6	NOVO	NOVObiocin (Resistance)	red red-orange	yellow yellow-orange
0.7	SAC	Sucrose (Fermentation)		
0.8	NAG	N-Acetyl-Glucosamine (Fermentation)		
0.9	TUR	TURanose (Fermentation)		
0.A	ARA	ARAbinose (Fermentation)		
0.B	βGUR	β GlucURonidase	colourless	yellow
0.C		Empty cupules		
0.D				
0.E				
0.F				