CHARACTERIZATION OF STAPHYLOCOCCI ISOLATED FROM MAN AND ANIMALS IN NIGERIA

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A thesis in the Department of Veterinary Microbiology and Parasitology

Submitted to the Faculty of Veterinary Medicine, in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF IBADAN

MARCH 1984

DEDICATION

Dedicated to the eternal memories of my late father who died on 22-11-80 and my helpful, lovely and understanding wife who passed away suddenly on 23-3-84.

ABSTRACT

A combination of physiological, biochemical and molecular characteristics was used in the study of 291 strains of staphylococci isolated from man, goats, sheep, poultry and other animals. Novobiocin-resistant strains were more prevalent amongst the coagulase-negative staphylococci examined. Of the 110 caprine strains of coagulase-negative staphylococci, 91 (87.7 per cent) were novobiocin-resistant. The strains identified to species level were found to be Staphylococcus sciuri, Staphylococcus lentus, Staphylococcus xylosus, Staphylococcus gallinarum, Staphylococcus cohnii and Staphylococcus saprophyticus. Staphylococcus sciuri and Staphylococcus lentus were easily differentiated from other novobiocin-resistant staphylococci by a positive-oxidase reaction. Morphologically, Staphylococcus sciuri and Staphylococcus lentus appeared indistinguishable. Staphylococcus lentus appeared more biochemically active than Staphylococcus sciuri and indeed other coagulase-negative staphylococci isolated from goats with the exception of Staphylococcus gallinarum.

Differentiation of Staphylococcus sciuri from Staphylococcus lentus was facilitated by the inability of several strains of Staphylococcus sciuri to produce acid from melibiose. Novobiocin-resistant staphylococcal species were the only ones isolated from sheep and they had almost identical physiological and biochemical characteristics with those of caprine origin. Staphylococcus gallinarum, a new species of coagulase-negative staphylococci isolated from animals was found amongst the present collection of coagulase-negative staphylococci. Staphylococcus gallinarum resembled Staphylococcus lentus in its acid production from several carbohydrates used but its oxidase-negative reaction was used in its differentiation from Staphylococcus lentus. Staphylococcus gallinarum strains commonly produced acid from fructose, maltose, D-(+)-mannose, D-(-)-ribose, sucrose and D-(+)-trehalose. Most of the novobiocin-resistant, coagulase-negative staphylococci from animals were physiologically active as they produced a variety of extracellular products. Weak desoxyribonuclease activities

were commonly found amongst Staphylococcus sciuri and Staphylococcus lentus and the strong reaction produced by Staphylococcus hyicus was found to be of diagnostic use. One strain of Staphylococcus sciuri produced thermostable nuclease and another strain of Staphylococcus lentus produced staphylokinase. Whilst intense protease production was commonly associated with Staphylococcus sciuri and Staphylococcus lentus, none of these strains produced lipolytic effects on egg-yolk medium whilst lipolytic effects were seen in strains of Staphylococcus xylosus and Staphylococcus cohnii. The characteristics of the novobiocin-resistant, coagulase-negative staphylococci isolated from humans did not differ from those of animal strains.

The strains of Staphylococcus aureus isolated from humans and animals had similar physiological and biochemical characteristics. The production of hyaluronidase and acetoin from glucose was helpful in differentiating Staphylococcus aureus from other coagulase-positive Staphylococcus intermedius and Staphylococcus hyicus strains isolated from various hosts. The strain of

Staphylococcus intermedius isolated from a man having a pet dog had similar characteristics as the other strains of Staphylococcus intermedius of canine origin. The human strain of Staphylococcus intermedius produced proteinase, phosphatase, desoxyribonuclease and thermostable nuclease but it failed to hydrolyze Tween 80 and did not produce hyaluronidase and acetoin from glucose. Acid production from a variety of carbohydrates by the human strain of Staphylococcus intermedius was however similar to that of Staphylococcus aureus of human origin but the strain did not produce acid from D-(+)-turanose.

In the course of phage typing Staphylococcus aureus strains, a caprine phage was isolated from one strain (UI 150). Three caprine strains of Staphylococcus aureus that were untypable by using the International sets of phages for typing human strains of Staphylococcus aureus were susceptible to this caprine phage G1. Staphylococcus aureus strains isolated from humans and goats which were susceptible to the phage 80/81 complex had similar antibiograms.

Whilst several of the human strains of Staphylococcus aureus were found to be of human biotype, the caprine strains

of Staphylococcus aureus belonged to biotype C.

Resistance to tetracycline was found in some strains of Staphylococcus aureus of caprine origin whilst a higher percentage of human strains of Staphylococcus aureus produced penicillinase. Sensitivity to the cephalosporins was commonly found amongst all the Staphylococcus aureus strains examined.

With human factor sera, all the Staphylococcus aureus strains isolated from humans were serotypable. Of the 14 caprine strains of Staphylococcus aureus examined, 10 were serotypable. Agglutinogens a_5, b_1, o and h_2 were found amongst the Staphylococcus aureus strains of human and animal origins. Two Staphylococcus intermedius strains isolated from humans and dogs were serotypable and they had k_1k_2 agglutinogen in common. All the 3 Staphylococcus aureus strains isolated from giant rats (Cricetomys gambianus) were serotypable with a_5 and p agglutinogens being common to them.

Some isolates were obtained which could not be identified

The application of chemotaxonomic and molecular characterization procedures however provided a relationship to some of the newly-described species of staphylococci. Some relatively uncommon teichoic acids were found amongst some strains. A caprine strain of coagulase-positive staphylococcus had glycerol and glucosamine teichoic acids whilst a strain of coagulase-negative staphylococcus of caprine origin possessed glycerol, glucose, galactose and N-acetylglucosamine teichoic acids. The guanine plus cytosine (G + C) content of the deoxyribonucleic acid (DNA) of some of the strains tested ranged between 32.7 and 34.6 per cent. DNA-DNA hybridization values amongst some of the coagulase-negative staphylococci revealed some relationships to Staphylococcus gallinarum, Staphylococcus lentus and Staphylococcus sciuri.

ACKNOWLEDGEMENT

I am sincerely grateful to Professor M. Ola. Ojo who, despite his engagements at home and abroad, still had enough time to offer useful advices at every stage involved in the preparation of this thesis. The planning and supervision of this thesis are the brain-child of Professor Ojo. I thank him for his interest. I am happy that some aspects of this thesis are currently being investigated by Professor Ojo.

I am also profoundly indebted to Dr. Luc. A. Devriese of the Faculty of Veterinary Medicine, State University of Ghent, Gent, Belgium for his active interest and valuable contributions towards the preparation of this thesis. Dr. Devriese not only served as my external supervisor but also became a real gem of a friend. He donated his own blood used for some of the tests described herein. Furthermore, he saw to my confort during my stays in Ghent, Brussels and Munich. My visit to Lyon, France was due to the personal efforts of Dr. Devriese. The excellent assistance offered by Arlette, Messrs Lucien Serraes and

Luc Derijcke is highly appreciated. I also wish to thank Professor Albert Devos and the staff of the Department of Veterinary Microbiology, University of Ghent, Belgium for their co-operation during my stay in their laboratory.

My visits to the Institut Pasteur de Brabant, Brussels, Belgium not only facilitated the phage typing of my strains but also provided an opportunity to isolate for the first time, a caprine phage for routine use. I am thus very grateful to Dr. C. Godard and Mr. Lambert Antoine for their co-operation.

Many thanks are due to Professor J. Fleurette, Dr. Y. Brun and Mrs. J. Reat of the Laboratoire de Bacteriologie-Virologie, Universite Claude Bernard, Lyon, France for the excellent assistance offered me during my stay in Lyon. The serotyping of my strains of S. aureus was done in Lyon, France.

The completion of the molecular characterization of some of the strains studied at the Technische Universitat Munchen, was due to the personal interest of Professor Dr. K.H. Schleifer. At this juncture, I must express my thanks to Mrs. Schleifer, Dr. F. Gotz, Mr. Gunter Krause,

Miss. G. Fischer, Miss Pomper and Mrs. Huber for making my stay in Munich a memorable one.

I take this opportunity to thank also my late wife, Mrs. Shola Adegoke, Dr. A.I. Adetosoye, Dr (Mrs) Aghomo and Mr. D.A. Bamgboye of the Faculty of Veterinary Medicine, University of Ibadan for their assistance and encouragement. I thank Messrs Matthew O. Oladele and Isaac A. Obiwale for typing the draft and manuscript.

Finally, the study leave granted by the University of Ibadan and the scholarships offered me by the governments of Belgium, West Germany and France not only facilitated a detailed study of all my strains but also enhanced an early completion of my thesis and for these grants I am eternally grateful.

CERTIFICATION PAGE

I certify that this work was carried out by Mr. G.O. Adegoke at the Department of Veterinary Microbiology and Parasitology, University of Ibadan and also partly at the Faculty of Veterinary Medicine, State University of Ghent, Gent, Belgium; Laboratoire de Bacteriologie, Faculte de Medecine, Alexis-Carrel, Universite Claude Bernard, Lyon, France and Lehrstuhl fur Mikrobiologie, Technische Universitat Munchen, Munich, West Germany.

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ABBREVIATIONS USED IN THE TEXT AND TABLES

S. aureus	=	Staphylococcus aureus
ATCC	=	American Type Culture Collection
NCTC	=	National Collection of Type Cultures
CCM	=	Czechoslovakia Collection of Microorganisms.
PBS	=	Phosphate Buffer Saline
c.f.u./ml	=	Colony forming unit per millilitre
Tox^+	=	Toxigenic
Tox^-	=	Non toxigenic
Pen	=	Penicillin
Ox	=	Oxacillin
CF	=	Cefalothine
K	=	Kanamycin
GM	=	Gentamycin
NN	=	Tobramycin
C	=	Chloramphenicol
TE	=	Tetracycline
MI	=	Minocycline
D	=	Deoxycline
E	=	Erythromycin

L	=	Lincomycin
PR	=	Pristinamycin
FF	=	Fosfomycine
RA	=	Rifampicin
VA	=	Vancomycin
MA	=	Cefamandol
SXT	=	Trimethoprim
FOX	=	Cefoxitin
CXM	=	Cefuroxim
CTX	=	Cefotaxim
RTD	=	Routine Test Dilution
DNA	=	Deoxyribonuclei Acid
poly AB	=	B-N-acetyl glucosaminyl ribitol teichoic acid
DNase	=	Deoxyribonuclease
α-haemolysin	=	alpha-haemolysin
B-haemolysin	=	beta-haemolysin
δ-haemolysin	=	delta-haemolysin
E	=	Radioactivity count
c.p.m.	=	Counts per minute
SSC	=	Standard sodium citrate buffer

Sp.A	=	Special Radioactivity Count.
Tm	=	Melting point
CTAB	=	N-cetyl-N, N, N-trimethyl ammonium bromide
mm Ø	=	Diameter in millimetres
mcg/ml	=	Micrograme per millimetre

CHAPTER 1

INTRODUCTION

Nigeria, which occupies about 14 per cent of the land area of West Africa, lies approximately between latitudes 4°N and 14°N and longitudes 3°E and 15°E. As at 1963, the population of Nigeria was put at about 56 million and by current conservative estimates, the population will be approaching 100 million. Much as there are people in the country, a variety of livestock also abounds. For example, the population of goats in Nigeria is put at about 21.2 million (FAO, 1965). Other livestock include cattle (12 million), sheep (7.2 million), pigs (0.8 million), camels (16,000), poultry (80 million) and horses (431,000). Of the total rural households having livestock, goats account for about 52.1 per cent. Only poultry (67.5 per cent of households) surpasses goats' population per household. The percentage of sheep and cattle per household are 2.13 and 9.5 per cent respectively (Federal Office of Statistics, 1966). Goats are important animals in the rural areas of Nigeria.

There is no taboo attached to goat meat and thus the animal can be used to supply animal protein (Ojo, 1976). Upton (1967) showed that 103 out of 158 farmers in 6 divisions in the South and North of River Niger kept goats. Dwarf goats, common in the Southern States of Nigeria, are characterized by their compact bodies and sturdy and short legs. The Red Sokoto, important for the quality of the hide and skin are predominantly found in the northern parts of the country. In addition to being a good source of animal protein, goats are of economic importance in Nigeria. According to the FAO Report (1970), 7.2 million pieces of goats' hides and skins, 1.7 million pieces of sheep hides and skin were exported by Nigeria.

Goats suffer from a lot of diseases including viral and parasitic diseases. Thus gastro-enteritis, pneumo-enteritis (pestes des petits ruminants) and pneumonia often affect goats (Ojo, Obi and Durojaiye, personal communication). Addo, Chineme and Eid (1980) in a survey of the incidence of chronic mastitis in goats in some states of Nigeria noted that staphylococci were

the most common isolates and also the organisms were found in apparently normal milk. With the mammoth population of man, it is conceivable that bacterial infections will abound. Thus Hurn (1951), Waddy (1957) reported epidemics with high morbidity and mortality rates in the former Northern Nigeria. Klebsiella septicaemia (Alausa, 1977) and pyogenic meningitis (Montefiore, Alausa and Sobayo, 1978) have also been reported amongst people living in Ibadan.

In most technologically-advanced countries of the world, domestic animals excluding pets are adequately housed and penned where appropriate. In Nigeria, however, most of the domestic animals live in close proximity to man. It is thus a common occurrence to see at times some animals moving freely in villages, towns and cities. Infact open-transportations of livestock are practised.

The close association of man and animals often leads to zoonotic infections. Live (1972) reported the transmission of staphylococci between man and dogs living together. In Nigeria, the impact of human strains of staphylococci associated with hospital epidemics upon

the animal populations is yet to be ascertained (Adekeye, 1979) as only few laboratories do undertake epidemiological/epizootiological studies of staphylococci. Invariably, identification of staphylococci rests solely on the application of relatively few phenotypic markers. Perhaps the conspicuous lack of infrastructure and essential microbiological materials are responsible for the inability to characterize fully the various strains of staphylococci hitherto isolated from man and animals in Nigeria (Onile, Alausa and Montefiore, 1976; Adekeye, 1980; Mohan, 1980. Adekeye (1980) found that of the 52 isolates of Staphylococcus aureus from man, 17 (32.6 per cent) were enterotoxigenic. The implications of enterotoxigenic strains of Staphylococcus aureus are that, with the care-free attitudes of preparing and serving foods coupled with open-display of consumables, staphylococcal food poisoning may be endemic sooner than expected as staphylococcal food poisoning associated with enterotoxin-producing Staphylococcus aureus has been reported (Wieneke, 1974). Staphylococci should now start to attract the attention of of health officials.

It is of interest to note that the characterization of staphylococci has continued to receive attention after the pioneering works of Rosenbach (1884). Thus with the application of chemotaxonomic and molecular methods in the characterization of staphylococci isolated from man and animals (Elek and Levy, 1950; Oeding, 1952; Baird-Parker, 1962, 1963, 1965a,b; Kloos and Schleifer, 1975a; Schleifer and Kloos, 1975a; Hajek, 1976; Devriese, et al. 1978; Devriese et al. 1983; Kloos and Schleifer, 1983) our present knowledge of the characteristics of staphylococci has greatly improved.

Staphylococci occur as transient as well as resident populations on the skin of man. The greater part of the surface of the healthy skin of man yields large numbers of Staphylococcus epidermidis which appears to grow and multiply in the sebaceous sweat glands and hair follicles. Certain sites on the skin of man, notably the anterior nares, the perineum, groin, axilla may also be colonized by staphylococci. Human beings who have their nasal vestibules colonized by Staphylococcus aureus are potential carriers. Marples (1965), and Noble and Sommerville

(1974) found that staphylococci were the major inhabitants of the skin of man. Kloos and Schleifer (1983) noted that Staphylococcus anicularis predominates in the external human ear. In the anterior nares of man generally regarded as the reproduction sites or "natural habitat" of staphylococci, the carrier rates have been reported by Noble and Sommerville (1974) to range from less than 10 per cent to more than 40 per cent in normal adults outside of hospitals. These authors also found that nasal carrier rates are highest in infants and reach their lowest levels in geriatics. White and Timbury (1963) found that varieties of coagulase - positive staphylococci inhabit the anterior nares of man. Roodyn (1960), Kay (1963), Cameron (1970), Kloos and Musselwhite (1975) found that Staphylococcus aureus can colonize the nares of some individuals for a considerable period of time. In some people however, nasal carriage of Staphylococcus aureus is transient. In addition to carriage by man, staphylococci occur in the soil and fresh water used by man. Staphylococci are also found within the homes and hospitals shared by man and they can be

isolated from utensils, furniture, paper currency and swimming pools (Kloos and Schleifer, 1981).

Previously, in man, amongst the staphylococcal species, the "residency-status" of only Staphylococcus aureus was ascertained. However, it is now known that some of the newly-described species of coagulase-negative staphylococci (Kloos and Schleifer, 1975a; Schleifer and Kloos, 1975a) can colonize the human skin. For example, Staphylococcus haemolyticus is more prevalent on infants than on children or adults (Carr and Kloos, 1977). Staphylococcus warneri is only occasionally found on human skin and then only as small populations. The populations of Staphylococcus simulans, Staphylococcus saprophyticus, Staphylococcus cohnii and Staphylococcus xylosus on the skin of man vary and also depend on the individual carrying the organisms. With the presence of both coagulase-positive and coagulase-negative staphylococci on the skin, ear and external nares, Kloos and Schleifer (1975b) devised a 13 - test dichotomic scheme which leads to a comprehensive understanding of the human staphylococci.

In man, Staphylococcus aureus causes mainly furuncles (boils), pneumonia, osteomyelitis, cervicitis and bacteraemia. The enterotoxins produced by Staphylococcus aureus are important in food poisoning in man (Bergdoll, 1972; Bergdoll and Bennett, 1976; Stersky, Todd and Pivnick, 1980). Coagulase-negative staphylococci are known to cause diseases in man. Brun, Fleurette and Forey (1977) found that Staphylococcus epidermidis was the predominant coagulase-negative species isolated from various infections in France. Acute pyelonephritis or cystitis has also been reported to be caused by coagulase-negative staphylococci - mainly Staphylococcus saprophyticus (Torres-Perreira, 1962; Mortensen, 1969; Bailey, 1973; Oeding and Digranes, 1976).

In animals including birds, the potentially pathogenic Staphylococcus aureus has been studied. Staphylococcus aureus isolated from the nares and udders of sheep are known to belong to biotype C (Hajek and Marsalek, 1976). Unfortunately, not much is known about the carriage of Staphylococcus aureus by goats. However, they carry large cutaneous and nasal populations of Stapylococcus xylosus, Staphylococcus sciuri and Staphylococcus lentus (Adegoke,

Devriese and Van de Kerekhove, to be published). In dogs, the primary coagulase-positive Staphylococcus found in the nares and on the hairy cutaneous areas is Staphylococcus intermedius (Hajek, 1976). Staphylococcus intermedius can occasionally be transferred to the skin of human handlers and is also associated with canine otitis externa. Cattle can carry populations of Staphylococcus aureus on udders and in quarter milk especially if they are suffering from mastitis or are in herds with afflicted animals (Wallace, Quinsberry and Tanimoto, 1962; Hajek and Marsalek, 1969). Coagulase-negative staphylococci may also be associated with bovine mastitis (Stabenfeldt and Spencer, 1966; Lee and Frost, 1970; Holmberg, 1973; Devriese and De Keyser, 1980). Staphylococcus hyicus appears to be associated with many different animal species. It has been found in pigs, poultry, cattle and horses as well. Staphylococcus hyicus has been implicated in exudative epidermitis in pigs (Devriese, 1977).

In Africa, where community-acquired infections still abound, Alausa, Montefiore, Sogbetun, Ashiru, Onile and

Sobayo (1977) have found that Staphylococcus aureus and Klebsiella spp. are the commonest causes of septicaemia. Staphylococcus aureus and other bacteria notably Escherichia coli, Haemophilus influenzae and Streptococcus pneumoniae have been found to be responsible for meningitis in African children (Audu, 1966; Seriki, 1970; Barclay, 1971; Alausa and Osoba, 1974).

In a survey of parasites and bacteria infestation of house rats (Rattus rattus) caught within human habitations in Ibadan, Nigeria, Escherichia coli and Staphylococcus aureus were found to be the most frequently isolated bacteria (Akinboade, Dipeolu, Ogunji and Adegoke, 1981a). Also Akinboade et al. (1981b) found that Staphylococcus aureus strains were the predominant pathogens isolated from rabbit urine. Staphylococcus aureus has been incriminated in bovine fascioliasis (Ogunrinade and Adegoke, 1982) and reduced hatchability in chicken eggs in Nigeria (Akinyemi, Ojeh and Adegoke, to be published).

Generally staphylococci can be readily distinguished from each other by easily realisable phenotypic markers.

In order to differentiate the various strains of Staphylococcus aureus which occur in man and animals, Meyer (1966a) suggested the subdivision of Staphylococcus into varieties. Hajek and Marsalek (1971) also proposed a biotyping scheme in which several Staphylococcus aureus biovars were characterized.

With the sporadic occurrence of staphylococci in man and animals, the need to characterize isolates from any of these hosts is of prime importance as it is now known that both coagulase-positive and coagulase-negative staphylococci may be pathogenic. Thus the present study was undertaken to reveal the characteristics of staphylococci isolated from man and various animals with:

(1) physiological and biochemical character parameters;

(2) biotyping scheme for all strains classified as coagulase-positive and also determination of their antibiotic susceptibility patterns:

(3) serotyping and phage typing Staphylococcus aureus strains as a means of identifying epidemiologically important ones and testing of a newly-developed caprine phage for routine use.

(4) application of current taxonomic criteria such as assay for cell wall teichoic acids, peptidoglycan, lactic acid configuration and determination of the guanine and cytosine content of the deoxyribonucleic acid (DNA) as well as DNA-DNA hybridization studies of the "unclassifiable" strains of staphylococci isolated during the period of the present project.

CHAPTER 2

LITERATURE REVIEW

General: The organisms which today are known as staphylococci were originally called "micrococci" by Von Recklingaushen (1871). The name "Staphylococcus" was first used by Ogston (1882). Rosenbach (1884) is given credit for introducing the generic name Staphylococcus. Staphylococcus aureus Rosenbach is now generally accepted as the validly published name for the nomenclatural type species of the genus Staphylococcus (Editorial Board, 1958).

The characterization of aerobic, Gram-positive, catalase-positive cocci was controversial prior to the introduction of molecular systematics to the taxonomy of the genus Staphylococcus. The recognition of the various coagulase-negative staphylococci as being distinct from each other was not satisfactorily undertaken. For several years, attention was focussed on the differentiation of staphylococci from micrococci. Hucker (1924, 1928), felt that micrococci and staphylococci were indistinct

and therefore placed them together in the genus Micrococcus. Rahn (1929), Abd-El-Malek and Gibson (1948) believed that staphylococci and micrococci had been indiscriminately named. They concluded that strains isolated from milk were all members of a large group of organisms which they referred to as the "Staphylococcus - Micrococcus complex". Evans, Bradford and Niven (1965) were not satisfied with the lumping of staphylococci and micrococci into a single genus or complex. They proposed the separation of Staphylococcus and Micrococcus on the basis of their relation to oxygen. Species which were facultative aerobes were then classified as Staphylococcus and species that were obligate aerobes were referred to as Micrococcus. Breed, Murray and Smith (1957) and Baird-Parker (1963) were in support of the separation of staphylococci and micrococci based on anaerobic utilization of glucose. Mortensen and Kocur (1967), Klesius and Schuhardt (1968), however, criticised the separation of staphylococci from other cluster-forming, Gram-positive and catalase-positive cocci on the basis of anaerobic utilization of gluoose. They noted that under certain

growth conditions, some Gram-positive and catalase-positive cocci were able to grow slowly anaerobically and to ferment glucose slowly. Furthermore, Kloos and Schleifer (1975a), Schleifer and Kloos (1975a) found that certain species of staphylococci did not produce acid from glucose under anaerobic conditions and that micrococci may produce acid from glucose anaerobically. The ability of staphylococci to produce acid from glycerol aerobically in the presence of erythromycin was then suggested by Schleifer and Kloos (1975b) for the separation of staphylococci from micrococci. Peny and Buissiere (1970) suggested that the degradation of DL-alanyl-B-naphthlamide and the decarboxylation of arginine are useful indices for differentiating staphylococci from micrococci. Morrison, Tornabene and Kloos (1971) noted that the presence of aliphatic hydrocarbons in neutral lipids extracted from micrococci distinguishes them from staphylococci.

Lysostaphin produced by a coagulase-negative Staphylococcus possesses antibiotic and lytic properties against other members of the genus (Schindler and

Schuardt, 1964). Klesius and Schuhardt (1968) tested the lysostaphin susceptibility of several gram-positive, catalase-positive cocci and found that lysostaphin susceptibility was the closest approximation to a single characteristic except for deoxyribonucleic acid base ratio analysis that can differentiate staphylococci from micrococci. Presently, the most reliable criteria for the separation of staphylococci and micrococci are the deoxyribonucleic acid (DNA) base composition and the cell wall composition. Several authors using molecular systematics have presented convincing results to show that the genera Staphylococcus and Micrococcus are quite different. Silvestri and Hill (1965), Auletta and Kennedy (1966), Rosypal, Rosypalova and Horejs (1966) found that members of the genus Staphylococcus have a guanine plus cytosine (G + C) content in deoxyribonucleic acid (DNA) within the range of 30 to 39 mol per cent and that members of the genus Micrococcus have a G + C content in DNA within the range of 63 to 73 mol. per cent. The findings that staphylococci possess normal menaquinones whilst micrococci possess dehydrogenated ones is also

very useful thus confirming biochemical differences between these genera (Jeffries, Cawthorne, Harris, Cook and Diplock, 1969). Staphylococci and micrococci have different cell wall compositions. The cell wall of staphylococci contains either ribitol-teichoic acid or glycerol in teichoic acid (Davison and Baddiley, 1963; 1964; Davison, Baddiley, Hofstad, Losnegard and Oeding, 1964). On the other hand, Tornabene, Morrison and Kloos (1970) found that the cells of micrococci contain aliphatic hydrocarbons whilst those of staphylococci do not. Thus differences in the aliphatic hydrocarbon content of a cell can be used in the differentiation of staphylococci from micrococci. Furthermore, cytochrome pattern (Faller, Gotz and Schleifer, 1980); fructose-1, 6-diphosphate (FDP) aldolase class (Gotz, Nurnberger and Schleifer, 1979) can be convincingly utilized for the differentiation of staphylococci from micrococci. On routine basis, the separation of staphylococci from micrococci can be achieved by employing lysostaphin sensitivity test (Schleifer and Kloos, 1975b), phage adsorption test (Schumacher-Perdreau, Pulverer and Schleifer, 1978), sensitivity to furazolidone (von Rheinbaden and Hadlok,

1981), use of selective media (Curry and Bordvian, 1976; Schleifer and Kramer, 1980), modified oxidase and benzidine tests (Faller and Schleifer, 1981) and serology (Seidl and Schleifer, 1978).

Staphylococci, generally, are facultative anaerobes and they have a diameter of less than 1 µm (Baird-Parker, 1963, 1965). They grow best in the presence of air. They are typical mesophiles growing at temperatures between 6.5 and 46°C (optimum 35 to 40°C) and at pH values of between 4.5 and 9.3 (optimum 7.0 to 7.3). They are Gram-positive, catalase-positive cocci producing a variety of extracellular enzymes and toxins. The metabolism of staphylococci is both respiratory and fermentative. Oxygen is generally the terminal electron acceptor although some strains can grow anaerobically utilizing nitrate as an electron acceptor.

Staphylococci are ubiquitous in nature and are therefore encountered on the skin and the upper respiratory tracts of man and animals. These areas serve as a potential reservoir for infection which varies according to host animal, strains being carried and

environmental conditions. Staphylococci may also be found in the throat, mammary glands and intestinal tract. Staphylococci together with diptheroids account for most of the indigenous flora of the skin of man (Hartman, 1978). The highest carrier rates of staphylococci (Staphylococcus aureus) are usually found in newborn infants as almost all of them may acquire staphylococci within a few days of birth. Thereafter, the nasal carrier rate declines so that children between six months and 2 years of age commonly have rates of about 20 per cent (Cunliffe, 1949; Hurst, 1957; Rycroft and Williams, 1960). The carrier rate increases during childhood to the adult rate of approximately 30 per cent. It appears that there are three main classes of carriers: the persistent carriers, the persistent non-carriers and the intermittent carriers. The last group may be further subdivided by separating off the occasional carriers who yield [redacted] positive cultures from time to time. Some 10 to 20 per cent of normal adults seem practically never to yield staphylococci from the nose even on repeated swabbing. A further 10 to 20 per cent yield

staphylococci usually in large numbers at all examinations and such people can harbour a single phage type of staphylococci for a very long period of even up to 7 years (Roodyn, 1960). The intermittent carriers commonly carry staphylococci from periods of a few weeks at a time and are then free from staphylococci for sometime. They often carry different phage types in successive periods of carriage which suggests that it is in fact the carriage of the staphylococci that is intermittent and not the ease of recovery of the staphylococci from a persistent carrier (Gould and Cruichshank, 1957). Similarly, persistent carriers of a single type do occasionally yield negative results.

Whilst the mechanism of the acquisition of staphylococcal disease is unclear, it is possible that airborne staphylococci may infect exposed burns and open wounds. However, more intimate contact may be necessary under conditions where trauma is not involved (Morse, 1980). In nature, man constitutes the principal reservoir of staphylococci. Men and women do carry staphylococci either in the nose or nn the skin. However, no appreciable difference in the nasal carrier rate of

Staphylococcus aureus by men and women has been reported (Miller, Galbraith and Green, 1962). A lower carrier rate of Staphylococcus aureus has been found amongst Africans in West Africa (Findlay and Abraham, 1946; Williams, 1963). Despite the close contact that the infant has with the mother, Barber, Wilson, Rippon and Williams (1953), noted that babies do not often acquire their mothers' staphylococci. Thus of the 128 babies swabbed on discharge from hospital, the authors found that only 25 (20 per cent) carried the staphylococal type that their mother had had on admission to hospital. In another survey, Williams (1961), found that only 10 per cent of the 502 babies examined had staphylococci of the same phage type as their mother on discharge from the hospital.

Staphylococci are polytypic in nature. Thus both coagulase-positive and a variety of coagulase-negative strains occur in nature. Usually stringent criteria based on temporal studies and population size are therefore often applied in determining the major habitats of these bacteria in man and other hosts. After detailed studies of the distribution and persistence of various Micrococcaceae on the skin of man, Kloos and Schleifer

(1975a); Kloos and Musselwhite (1975); Carr and Kloos (1977) found that Staphylococcus epidermidis and Staphylococcus hominis occur predominantly in the axillae and perineum. Practically, these organisms are carried by most human beings. Staphylococcus simulans, Staphylococcus saprophyticus, Staphylococcus cohmii and Staphylococcus xylosus occur only occasionally on the human skin. Although man and animals live with pathogenic staphylococci from birth until death, they become infected only when their susceptibility is appreciably affected. The mere presence of staphylococci is not sufficient reason for the development of infection. Local conditions at the site where staphylococci lodge contribute significantly to the initiation of infection (Soltys, 1963). Thus, when pathogenic staphylococci are found in the environment, it is often believed that they have been derived from man and animals. However, Morrison, Fair and Kennedy (1961), found a high incidence of coagulase-positive, antibiotic-resistant Staphylococcus aureus strains amongst cattle, horses, dogs and cats. Since these animals live in association with man, these authors

suggested that domestic animals may be a source of staphylococci which are pathogenic for man.

Staphylococcal diseases:

Staphylococci cause a variety of diseases in man and animals. In man, the general characteristic of staphylococcal disease is a suppuration. The most frequent lesion is a cutaneous abscess or boil which begins as an infection of sebaceous glands or hair shafts. At full development, the centre of the abscess shows liquefaction necrosis (pus) consisting of dead bacteria, phagocytes and fluid surrounded by a firm wall of fibrin, inflammatory cells and viable bacteria. Staphylococcus aureus may cause acute hematogenous osteomyelitis, endocarditis and pneumonia in man (Morse, 1980). Onile, Alausa,and Montefiore (1976) in their study of 117 patients whose ages ranged from four years to above five years, with purulent conjunctivitis at the University College Hospital, Ibadan, Nigeria, found that Staphylococcus aureus accounted for a third of all the cases examined. Staphylococcus aureus has also been found to be responsible for two-thirds of cases of ocular infections

in New York, United States of America (Lacatcher-Khorado, Sullivan and Gutierez, 1967). Staphylococcus aureus has also been incriminated in urinary tract infections in some Nigerian males (Alausa, Montefiore and Sobayo, 1979). In the gastro-intestinal tract, special mechanisms control staphylococcal diseases. The proliferation of staphylococci in the gastro-intestinal tract is controlled by the antagonistic action of the many other bacteria species like the Enterobacteriaceae present. Thus an upset in the balance, for example, by a broad-spectrum antibiotic such as tetracycline, facilitates the emergence of a high population of antibiotic - resistant staphylococci in the faeces. Under these circumstances, staphylococci sometimes invade the bowel wall and produce acute staphylococcal enterocolitis which may be fatal. Many of the strains of Staphylococcus aureus involved produce enterotoxin B which may also play a causative role in staphylococcal enterocolitis. Generally about 50 per cent of Staphylococcus aureus strains produce enterotoxins which are a major cause of food poisoning (particularly types A and D) and

staphylococcal toxic-shock syndrome (Type F). Enterotoxins in suspected food are demonstrated by immunological techniques such as gel diffusion with specific antiserum. At present, there are six serologically distinct enterotoxins. They are enterotoxins A (Casman, 1960); B (Bergdoll, Surgalla and Dack, 1959); C (Bergdoll et al., 1965); D (Casman, Bennett, Dorsey and Issa, 1967); E (Bergdoll et al., 1971) and F (Bergdoll et al., 1981). Foods commonly implicated in staphylococcal food poisoning include custards, salad dressing, sliced meats and meat products contaminated by food handlers who have open lesions due to enterotoxin-producing Staphylococcus aureus or who are asymptomatic carriers. Enterotoxin-producing strains of Staphylococcus aureus have been isolated in Nigeria (Adekeye, 1980). However, manifestations of staphylococcal food poisoning in both man and animals are yet to be documented in Nigeria. Although in humans, Staphylococcus aureus enterotoxins are the most common causes of food poisoning, dairy products like cheese and milk are involved in most of the large-scale outbreaks of Staphylococcus aureus food intoxications (Anderson and Stone, 1955; Armijo, Henderson, Timothee and Robinson,

1957; Allen and Stovale, 1960). Staphylococci are common in the udder and as milk is easily exposed to these organisms, they may induce mastitis in cows (Thatcher and Simon, 1956; Garcia, Moreno and Bergdoll, 1980; Olsvik, Berdal, Fossum and Omland, 1981). Thus cheese and other dairy products made from contaminated milk may induce food -poisoning when ingested (Weed, Michael and Herger, 1943; Hendricks, Belknap and Hausler, 1959; Ikram and Leudecke, 1977). However, clear indications for animals as the origin of strains implicated in food poisoning have not been described to date. Moreover, the strains have not been biotyped and many foods may become contaminated by strains of human origin during processing or conservation (Devriese, personal communication). Staphylococcal mastitis in cows may vary from a very acute to a very mild form in which no general and very few local signs of disease are produced (Smith, 1959). The acute form usually occurs shortly after parturition. Constitutional disturbances are marked. The body temperature is raised soon after the onset of the disease often to 41^{o}C. Anorexia, stiffness and pains

are present. In fatal cases, an increasing dullness is noted. The temperature commences to fall to subnormal levels and death may occur within 1 to 4 days of the onset of the disease. The chronic form in some respects resembles that caused by Streptococcus agalactiae. It is characterized by an area of induration often relatively small, commencing near the base of the teat of the affected quarter. The milk may be a little altered in appearance although it has a high leucocyte count.

Histologically, the teat canal and ducts are usually thickened by fibrous tissue and some destruction of the secretory cells and replacement by fibrin is common. In Nigeria, in addition to Staphylococcus aureus, Ojo (1978), found that Escherichia coli, beta-haemolytic streptococci and Corynebacterium pyogenes were associated with mastitis. However, the incidence and economic effects of mastitis in dairy animals in Nigeria are yet to be properly documented (Ojo, 1978).

Staphylococcal disease in poultry occurs in fairly young birds and affects joints and tendon sheaths.

Clinically, it assumes acute or chronic form. The acute form is characterized by severe arthritis and diarrhoea. The chronic form is associated with chronic arthritis and emanciation. Staphylococcosis in poultry is similar to tick pyaemia in lambs (Soltys, 1963). Staphylococcus aureus derives its importance in poultry meat hygiene from its potential production of enterotoxin which may cause food poisoning in humans. Although Hajek and Marsalek (1973) found that very few strains of Staphylococcus aureus from live poultry produced enterotoxins, Gibbs, Patterson and Harvey (1978a,b) found that many of their poultry biotype strains produced enterotoxin A. Perhaps the methodologies employed were responsible for the divergent results. Devriese (1980) however, has noted that staphylococci from live poultry have not yet been associated on firm grounds with human infections or food intoxication. Whilst the carriage of Staphylococcus aureus in man has been documented (Williams, 1963; Solberg, 1965), there is a paucity of data on the carrier state of these organisms in poultry.

Staphylococci, generally, form a relatively well-defined genus on the basis of numerical phenetic studies

(Feltham, 1979), protein homology (Schleifer and Kloos, 1975a) and molecular characteristics (Goodfellow, Mordarski, Tkacz, Syzba and Pulverer, 1966). In medical and veterinary bacteriology, Gram-positive and catalase-positive cocci are subdivided into coagulase-positive staphylococci (Staphylococcus aureus) and coagulase-negative staphylococci (Staphylococcus epidermidis) or more correctly coagulase-negative Micrococcaceae. Furthermore, coagulase-positive staphylococci are differentiated on the basis of biotyping (Hajek and Marsalek, 1971) whilst coagulase-negative staphylococci are differentiated from each other on the basis of chemotaxonomic criteria and DNA - DNA homology studies (Schleifer and Kloos, 1975a).

Extracellular products

Most of the extracellular products and toxins released by staphylococci during growth are useful in characterizing the different strains from different hosts. Coagulase, an extracellular product of staphylococci for example, is known to be produced as early as the lag phase and continues throughout the logarithmic phase

(Duthie, 1954; Marstron and Fahlberg, 1960). Coagulase production is generally thought to be linked with pathogenicity. Loeb (1903) was the first to demonstrate the ability of staphylococci to clot plasma. Various animal plasmas are coagulated by Staphylococcus aureus. Rabbit, human, horse and pig plasmas are coagulated by strains of Staphylococcus aureus from man and animals. Bovine plasma (Meyer, 1967; Grun, 1968) and sheep plasma (Hajek, Marsalek and Cerna, 1968) are usually coagulated only by strains originating from animal sources. The coagulase reaction is a peculiar characteristic of Staphylococcus aureus (Subcommittee, 1965) and various methods have been used for the test. Blobel and Berman (1960) used fluorescent anticoagulase for differentiation between coagulase-positive and coagulase-negative staphylococci but they could not establish absolute specificity of their technique. Borchardt, Pierce and Shaffer (1963) used Oucherlony immunodiffusion methods to assay for coagulase and found that there were problems with heterogeneity of the reactants (antigens and/or antibodies). A number of

reasons may therefore be adduced for the non-detection of some strains of Staphylococcus aureus producing coagulase. These may include growth under unsuitable conditions, use of plasmas deficient in fibrinogen or the production of staphylokinase and/or coagulase-destroying enzymes (Lominski, Smith and Morrison, 1953; Munch-Petersen, 1961). Antibodies to coagulase may also be present in the sera of many normal individuals and this can be responsible for false negative coagulase tests (Duthie and Lorenz, 1952).

Pure preparations of staphylocoagulase are essential before its biological nature, mode of action and contributions towards pathogenicity can be elucidated. Thus Zolli and San Clemente (1963) obtained an immunological and electrophoretic homogeneity of staphylocoagulase by using three cycles of dialysis in ethanol - water mixtures followed by column chromatography through Sephadex G - 200. Siwecka and Jeljaszewich (1968) obtained highly active and electrophoretically homogeneous preparations of staphylocoagulase which they suggested contained sugars as a structural component of the enzyme

with an iso-electric point at pH 8.5. Coagulase can be oxidized and consequently inactivated by several chemicals notably bromine, iodine, hydrogen peroxide, ascorbic acid, propylene glycol and ammonia (Lieb, 1962). In vitro coagulase system could also be delayed by the action of some antibiotics. Penicillin G, streptomycin, tetracycline, chloramphenicol, kanamycin, vancomycin, methicillin and cloxacillin could decrease the in vitro activity of the coagulase clotting mechanism (Castagneri and Morci, 1965). Some physiological factors may also affect the biosynthesis of coagulase. Tirunarayanan (1966) showed that glucose and bicarbonate had a stimulatory effect on the formation of coagulase. Pariza and Iandolo (1969) noted that active protein synthesis stimulated by ribosomal regeneration and not growth was necessary for the production of coagulase.

At least four antigenically distinct coagulase and antibodies for these antigens exist (Duthie and Lorenz, 1952). Elek (1959) found that staphylocoagulase was not only antigenic but antibodies to the antigen could be produced in vivo and measured in vitro. Thus it is easy

to assume that coagulase may act either to produce intravascular clotting or thrombosis or to lay down a fibrin barrier which contributes to the development of the lesion. However, there is no good evidence that in vivo clotting or thrombosis is exceptionally more prevalent in staphylococcal than in other microbial diseases or that fibrin network is related directly to the action of the enzyme. Therefore, it is conceivable that coagulase is of primary importance to the staphylococci only in the very early stages of infection, that is, during the time they are attempting to establish a foothold in the tissues. Once this goal has been achieved, the role of coagulase becomes less important (Blair, 1962). Invariably, whilst coagulase test has been the best single test to determine pathogenicity (Baird-Parker, 1965), Abramson (1972); Meyer and Schleifer (1978) and Adegoke (1981) have suggested that the test should not be considered an absolute criterion.

Clumping factor, also an extracellular product of staphylococci, has been found to be a protein, trypsin-sensitive and easily destroyed by ultrasonic treatment

but resistant to ultrasonic light (McNeil, 1968). Clumping factor (CF), is also believed to be a polypeptide localized in the cell wall of Staphylococcus aureus, where it is covalently bound (Elkins, Hyde and Kelly, 1970; Umeda, Ikebuchi and Amako, 1980). To identify Staphylococcus aureus, the clumping factor is determined by the plasma agglutination reaction. However, since the method has been found inadequate in view of the fact that staphylococci may react with some plasma constituents other than fibrinogen, such as immunoglobulins, Flandrois and Carret (1981) proposed staphylococcal affinity to fibrinogen by passive hemagglutination for screening Staphylococcus aureus. Adegoke, Devriese, Godard, Fleurette, Brun and Ojo (1983a) found affinity to fibrinogen useful for screening caprine strains of Staphylococcus aureus. Prior to the introduction of the use of passive haemagglutination by Flandrois and Carret (1981), Duthie (1954); Bruckler, Schaeg and Blobel (1974); Switalsky (1976) had suggested the method to detect the clumping factor of Staphylococcus aureus extracts during purification experiments. Generally clumping factor

(Tager and Drummond, 1965) or slide coagulase test, can be performed with several animal plasmas. Zablocki, Rolicka and Kokocinska (1957), Lewis and Wilson (1973) noted that plasmas obtained from opossum, guinea-pig, elephant, sheep and goat did not produce staphylococcal clumping. However, Adegoke (1981) found that goat's plasma was suitable for performing clumping factor reaction with caprine strains of Staphylococcus aureus. Perhaps the structural differences between the fibrinogen (Lewis and Wilson, 1973) may be responsible for the non-clumping of staphylococci in plasma of some animal species. Clumping factor, usually used as a screening test (Cadness-Graves, Harper and Miles, 1943) is different from coagulase (Duthie, 1954; Rotter and Kelly, 1966). Blackstock, Hyde and Kelly (1968) however, have suggested that coagulase and clumping factor may be closely related. The production of clumping factor occurs between 90 and 120 minutes of logarithm - phase growth in brain heart infusion broth (McNeil, 1966). Clumping factor is not the factor responsible for the virulence of staphylococci but it is a relatively useful index of pathogenicity

(Kapral and Li, 1960; McNeil, 1968; Yoshida and Takeuchi, 1970). However, the prototype of the virulent Staphylococcus can be taken as the strain isolated from typical staphylococcal lesion and pyogenic abscess (Burns and Holtman, 1960). Epidemiologically, there are differences in virulence among coagulase-positive and coagulase-negative strains of staphylococci. The ability of these micro-organisms to produce disease as opposed to the ability to spread it may, therefore, be due to separate characteristics of the strains. Burns and Holtman (1960), suggested that more often than not, a series of factors were associated with virulent strains of staphylococci. Prominent amongst these factors are enterotoxin, dermonecrotoxin, alpha and delta haemolysins, fibrinolysin (staphylokinase), coagulase, leucocidins, hyaluronidase, deoxyribonuclease, phosphatase, lipase, pigment, gelatinase production, mannitol fermentation and tellurite reduction. Jay (1966); Grossgebauer, Schmidt and Langmaach (1968) believed that lysozyme was also an important "pathogenicity factor".

In differentiating a pathogenic staphylococcus, Zak, Jeljaszewich and Kedzia (1967), suggested that no single

test should be regarded as a sole criterion. Jeffries and Wailes (1961), Osowieki (1962) had earlier thought that coagulase and deoxyribonuclease were good indices of pathogenicity of staphylococci. The production of deoxyribonuclease and phosphatase by non-pathogenic strains of staphylococci (Beerens, Catsaras and Tahon, 1967; Sperber, 1977) makes the tests useless for screening pathogenic staphylococci. DiSalvo (1958), Chesbro and Auburn (1967), found that there was a correlation between the production of staphylococcal deoxyribonuclease and coagulase. Brandesh and Willis (1970) therefore suggested that nuclease and coagulase production be used as an index of pathogenicity of staphylococci. Although Chesbro and Auburn (1967) suggested that nuclease production be used as an indicator for the detection of Staphylococcus aureus in foods because of its close relationship to the production of enterotoxin in contaminated foodstuffs, Adegoke and Ojo (1982) noted that deoxyribonuclease activity could not be regarded as an index of pathogenicity of Staphylococcus aureus when it is realised that few caprine strains of staphylococci produced deoxyribonuclease.

In bovine mastitis however, examination of nuclease activity in quarter samples is a valuable diagnostic procedure (Gudding, 1981). Invariably, interest in the nuclease of Staphylococcus aureus is related to both its diagnostic value and its role in the pathogenicity of the organism (Abramson, 1972). Deoxyribonuclease could be demonstrated in a defined medium (Jeffries, Holtman and Guse, 1957), on trypticase - soya agar plate with added calcium and deoxyribonucleic acid (DiSalvo, 1958) or by the viscosimetric method of Weckman and Catlin (1957) as modified by Osowiecki and Pakula (1962) using purified thymus deoxyribonucleic acid (DNA) substrate at pH 7. Thiamine has been found to be essential for the production of deoxyribonuclease (Osowiecki and Pakula, 1962). These authors also noted that the highest deoxyribonuclease activity was produced when staphylococci were grown at 37^{o}C in Todd - Hewitt media supplemented with yeast extracts and aeration. Some differences however are known to exist between nucleases studied by several authors. Timasheff and Bernardi (1970) found that acid deoxyribonuclease, pancreatic deoxyribonuclease and

staphylococcal nuclease conformations differed as measured by optical rotary dispersions, circular dichroism and infrared spectra. Staphylococci nucleases were found to contain in solution about 20 to 30 per cent alpha helix with the remainder mostly unordered. Thus the findings of Timasheff and Bernardi (1970) were generally in agreement with the crystal structure data of staphylococcal nuclease. Furthermore, these authors found that acid and pancreatic deoxyribonucleases both contained little or no alpha helix and their secondary structures were predominantly in the unordered and beta conformations. In essence, therefore, the ability of a microorganism to produce a nuclease is of diagnostic value only when it is heat-resistant (Sticker and Freestone, 1971). Thermostable nuclease production by Staphylococcus aureus was first recognized by Chesbro and Auburn (1967). Staphylococcus epidermidis and other organisms are believed to produce thermolabile nucleases (Lachica, Hoeprich and Genigeorgis, 1971). Sloan, Robinson and Kloos (1982), however, reported the isolation of Staphylococcus staphylolyticus which produced a heat-

stable deoxyribonuclease. Whilst this may be unusual for a coagulase-negative Staphylococcus, Gramoli and Wilkinson (1978) had earlier on isolated heat-stable deoxyribonuclease-positive strains of Staphylococcus simulans.

In man, studies on staphylococci isolated from a variety of infections have revealed several coagulase-negative strains with respect to their biochemical properties and production of extracellular factors and enzymes (Dornbush, Nord, Olsson and Wadstrom, 1976; Oeding and Digranes, 1977; Durham and Kloos, 1978). With the pioneering works of Kloos and Schleifer (1975a), Staphylococcus simulans is now believed to be probably related to Staphylococcus aureus. Thus with the good correlation between coagulase and thermostable nuclease production by staphylococci, Dornbusch et al. (1976) suggested that strains producing only nuclease may belong to an intermediate group sharing the characteristics of both Staphylococcus aureus and Staphylococcus epidermidis. Serologically, there are differences in the nucleases of human, bovine and canine origins (Scharmann and Blobel,

1968). In the comparative study of several biochemical characteristics of two serologically different nucleases of Staphylococcus aureus of human and canine origins, Scharmann and Blobel, (1969) found that they had similar pH optima and similar requirements for calcium and copper, also both were inhibited by polyvinyl sulphate. The authors further found that citrate stimulated the activity of human nuclease but inhibited that of canine enzyme and whilst heating at 100°C for 2 min. caused canine nuclease a loss of 90 per cent of its activity, human nuclease lost only 40 per cent.

Hyaluronidase (Schwabacher, Cunliffe, Williams and Harper, 1945; Rajchert-Trzpi, 1961) is commonly produced by coagulase-positive staphylococci. Coagulase-negative staphylococci (Jeljaszewich, 1960; Vogelsang, Wormnes and Ostervold, 1962) generally are deficient in the production of hyaluronidase. Interestingly, however, 11 of the 57 strains of coagulase-negative staphylococci examined by Abramson and Friedman (1966) produced hyaluronidase. A similar finding was reported by Tauraso and White (1963). However, the only coagulase-positive staphylococcal strain

at present known to be hyaluronidase-negative is Staphylococcus intermedius. Hyaluronidase (Durans-Reynals, 1942; McClean, 1943) is believed to contribute to the infective process of bacteria by depolymerizing the hyaluronic acid -present in the intracellular ground substance of connective tissue thereby allowing the spread of bacterial toxic metabolic products. Elek (1959) also suggested that hyaluronidase reduces the effect of the infecting agent by dilution thereby rendering the invader more vulnerable to local defences. Physiological activities of hyaluronidase may be affected by a variety of chemicals. Thus Schmidt (1966), noted that the ions of iron, silver, copper, mercury, zinc and lead as well as rutin, heparin, potassium permanaganate, hydrogen peroxide and formalin have pronounced inhibitory effects on staphylococcal hyaluronidase the production of which occurs maximally at 30°C after 48 h of incubation (Abramson, 1972). The earlier methods for the detection of hyaluronidase were cumbersome and expensive (Lachica, Weiss and Deibel, 1969). Devriese and Hajek (1980) therefore proposed a modified test for the detection of

hyaluronidase with the use of a capsulated strain of Pasteurella multocida type A as the indicator strain spot inoculated onto suitably prepared lawn of bacterial growth on serum-trypticase soya agar plate. Staphylococcal hyaluronidase (Blair, 1962) may contribute to the severity of mixed infections with other bacterial or viral agents by spreading both the organisms and their products. Furthermore, Blair (1962), suggested that whilst not all strains of staphylococci produce a potent dermonecrotic lethal toxin, the intense necrosis that follows the intradermal injection of many staphylococcal culture filtrates in experimental animals and the rapidly fatal result of intravenous injection, give rise to the speculation that toxin may play a part either in the evolution of an infection or in the clinical manifestation of staphylococcal disease.

Staphylococcus aureus, Staphylococcus epidermidis and Staphylococcus haemolyticus are the three major species in the genus Staphylococcus that do produce haemolysins (toxins) (Schleifer and Kloos, 1975a). Staphylococcus aureus produces at least four different membrane damaging

agents, namely, alpha-, beta-, gamma-, and delta-haemolysins (Wadstrom and Mollby, 1972; Wiseman, 1975). Human strains of Staphylococcus aureus produce predominantly alpha-haemolysin (Bryce and Rountree, 1936). Marks (1952) even suggested that the production of alpha-haemolysin was indicative of the virulence of human strains of Staphylococcus aureus. Hinton and Orr (1957) corroborated Mark's findings and they also noted that the production of alpha and delta haemolysins and staphylokinase were characteristic of the more virulent strains of coagulase-positive staphylococci. Staphylococcal alpha toxin can be defined as a protein uniting haemolytic, lethal and dermonecrotic activities and also exhibiting a wide variety of other biological properties (Jeljaszewich, 1972). Alpha haemolysin of Staphylococcus aureus destroys cells from many animal species including rabbit erythrocytes and it has got a membrane - binding region (Lo and Fackrell, 1979). The sensitivities of erythrocytes from different animal species, however, correlates with the amount of toxin specifically bound (Wiseman and Caird, 1972; Cassidy and Harshman, 1973).

Rabbit erythrocytes are more susceptible to the action of alpha toxin while the red cells from other animals are less active. The susceptibility of erythrocytes from different individuals of the same species may differ five-fold or more (Bernheimer, 1965). Invariably, erythrocyte susceptibility to alpha toxin may depend on the amount of substances released by alpha toxin from the membranes of erythrocytes and the level of natural proteolytic activity typical for a particular animal species (Wiseman and Caird, 1970). Staphylococcal alpha toxin may play an important role at certain stages of a generalised infection or during the establishment of a staphylococcal lesion. Interaction with other biologically active staphylococcal products may be essential for this activity.

Animal strains of Staphylococcus aureus are characterized by the production of beta haemolysin (Williams, 1947). In support of this finding, Elek and Levy (1950) noted that on an average, ten times as many strains from animal sources produced beta haemolysin as did strains isolated from man. However, poultry strains of Staphylococcus aureus are usually negative

(Devriese, personal communication). Markham and Markham (1966) however found that between 85 to 100 per cent of cultures from cattle, sheep, pigs, hedgehogs and guinea-pigs yielded beta-haemolysin. Beta haemolysin is believed to be a separate haemolysin (Glenny and Stevens, 1935). It is a phospholipase C which is specific for sphingomyelin. The content of sphingomyelin in erythrocyte phospholipids governs the susceptibility of red cells of different species to beta haemolysin. The effects of Staphylococcus aureus sphingomyelinase C on erythrocytes has been well characterized. In the erythrocytes, the choline containing phospholipids are located in the outer layer and the choline-free phospholipids in the inner layer (Verkleij, Zwaal, Roelofsan, Comfurius, Kastelijn and Deenen, 1973; Bergelsch and Barsakov, 1977). Staphylococcus aureus sphingomyelinase C (Beta-haemolysin) degrades sphingomyelin only in the outer phospholipid layer of the erythrocyte membrane. The in vivo effects of beta haemolysin though may be largely unknown, however, important disturbances of the delicate membrane functions probably arise from the sphingomyelin depletion as certain

cells in the infectious processes like monocytes, neutrophils and mast cells are affected by beta-haemolysin. Delta haemolysin produced by staphylococci is a surface active polypeptide (Thelestan, Mollby and Wadstrom, 1973, Colacicca, Basu, Buckelew and Bernheiner, 1977). The mechanism of haemolytic activity of delta toxin is not clear (Jeljaszewich, 1972). Bernheimer (1970) however, had suggested that delta lysin resembles streptolysin S in its lack of antigenicity, inhibition by phospholipids and ability to lyse wall-less bacteria.

Amongst staphylococci, Christie and Wilson (1941); Rountree (1947); Willis, Jacobs and Goodburn (1963) noted that concomittant production of beta haemolysin and staphylokinase has not been frequent. However, Adegoke and Ojo (1981) isolated some strains of staphylococci from both healthy and sick goats which produced both beta haemolysin and staphylokinase. Furthermore, in addition to Staphylococcus aureus, some of the newly described species of staphylococci, notably Staphylococcus hyicus subsp. hyicus, Staphylococcus simulans, Staphylococcus sciuri and Staphylococcus xylosus do

produce staphylokinase. Staphylokinase (staphylococcal fibrinolysin or Muller's factor) is an extracellular protein that converts plasminogen into the active proteolytic and fibrinolytic enzyme plasmin (Lack, 1948; Gerheim and Fergusson, 1949). Elek (1959) in the study of the characterization of Muller factor used heated serum as substrate and did not differentiate staphylokinase from protease. However, Elek is credited with associating the Muller factor to staphylokinase. Quie and Wannamaker (1962) further substantiated that the Muller factor was indeed staphylokinase when they demonstrated the presence of an inhibitor in human serum which they identified as antistaphylokinase. This thermostable inhibitor of the Muller factor was found in the gamma-globulin fraction of serum and was absent from agamma-globulinaemic serum. Furthermore, normal rabbit sera were found to be deficient in the inhibitor but the latter could be induced by immunization with a concentrate of staphylococcal extracellular products. Quie and Wannamaker (1962) also noted that the inhibitor induced in rabbits was found in the gamma-globulin fraction of

the serum proteins and was also able to inhibit staphylokinase and Muller fractor activity. Staphylokinase and streptokinase are however different (Neter, 1937). Even, Davidson (1960), serologically, differentiated streptokinase from staphylokinase by showing that streptokinase required a proactivator found in human serum, a product not necessary for staphylokinase activity. The authors also noted that streptokinase without proactivator would not activate plasminogen of guinea-pig, rabbit or bovine origins while staphylokinase activated plasminogen from these three sources. Staphylokinase is believed to be of unique value in the differentiation of Staphylococcus aureus of human origin from strains of other animals (Madison and Dart, 1936; Christie and Wilson, 1941; Meyer, 1966; Hajek and Marsalek, 1971). However, the testing and interpretation of staphylokinase activities (Devriese and Van de Kerkchove, 1980) demand great care as conflicting results on the production of staphylokinase by staphylococci abound in the literature. Perhaps an important cause of variations in staphylokinase testing may be due to differences in the reactivity of

plasminogen of different animal species with staphylokinase (Lack and Glanville, 1970) and confusion between staphylokinase and protease.

Phosphatases though not widely employed for taxonomic purposes are however useful for the detection of Staphylococcus aureus (Hobbs, Kendall and Gilbert, 1968). Phosphatase is thought to have no significant role in the pathogenicity of staphylococci (Dlabac and Rusinko, 1962; Choudhuri and Chakrabarty, 1969) since other strains of staphylococci as well as other organisms produce phosphatase (Baird-Parker, 1963). Elek (1959) tried to link coagulase and phosphatase to a single protein. However, Drummond and Tager (1962) successfully separated staphylocoagulase from acid phosphatase enzyme activity by cellulose column-zone electrophoresis. San Clemente and Zolli (1963) also separated staphylocoagulase from acid phosphatase by gel filtration using sephadex G-200 and eluting with a sodium acetate buffer. Electron microscopy (Sawashige, 1967) showed that the activity of acid phosphatase is localized in the cytoplasm and cytoplasmic membrane of staphylococci.

Biochemical Characteristics

Previously, characterization of staphylococci based on acid production from carbohydrates was not widely accepted (Elek, 1959). Presently, however, biochemical characterization of staphylococci isolated from man and animals is generally regarded as a valuable taxonomic criterion. Thus Kloos and Schleifer (1975a); Schleifer and Kloos (1975a); Kloos, Schleifer and Smith (1976); Hajek (1976); Devreise, Hajek, Oeding, Meyer and Schleifer (1978); Devriese et al. (1983) and Kloos and Schleifer (1983) employed a variety of carbohydrates in their novel descriptions of staphylococci isolated from man and animals.

In addition to the application of biochemical characterization in the taxonomy of staphylococci, cell wall composition and molecular studies are of immense use in the descriptions of species of staphylococci. Peptidoglycan, a complex macromolecule found only in micro-organisms, maintains the strength and shape of the bacterial cell wall. There is a great variation in the composition and structural arrangement of the peptido-

glycan of Gram-positive and Gram-negative bacteria. The staphylococcal cell wall is made up of 40 to 60 per cent by weight of peptidoglycan. Teichoic acids and protein A make up the other major components of the staphylococcal wall. The peptidolycan is a hetero-polymer made up of polysaccharide chains which are cross-linked through short peptides. The glycan moiety is relatively uniform in all bacteria and it resembles chitin. It consists of B, 1, 4-linked units of N-acetylglucosamine which is similar to that of chitin. Each alternate glucosamine residue or muramic acid unlike chitin, is substituted by a lactic acid ether. Usually the carboxyl group of muramic acid is substituted by a peptide containing alternating L- and D- amino acids and the free amino group of the diamino acid in position 3 of the peptide subunit forms a peptide linkage with c-terminal D-alanine of an adjacent peptide subunit. In several Gram-positive bacteria, an interpeptide bridge can be inserted (Schleifer and Kandler, 1972). Furthermore, these authors also noted that the different composition of the peptide moiety and in particular of the interpeptide bridge, serves as a useful differentiating

characteristic in the taxonomy of Gram-positive bacteria isolated from man and animals. Thus after extensive studies of the characteristics of Staphylococcus aureus, Staphylococcus hyicus subsp. hyicus, Staphylococcus simulans, Staphylococcus cohnii and Staphylococcus xylosus, Kloos and Schleifer (1975a), Schleifer and Kloos (1975a), Devriese et al. (1978) found that the peptidoglycan type L-Lys-Gly_{5-6} is commonly found in these organisms. Also Staphylococcus intermedius, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus warneri, Staphylococcus capitis and Staphylococcus saprophyticus (Kloos and Schleifer, 1975a; Schleifer and Kloos, 1975a; Hajek, 1976; Schleifer, Schumacher-Perdreau, Gotz and Popp, 1976) are known to contain peptidoglycan type L-Lys-Gly_{3-5} L-Ser. Notwithstanding the rather tight structure of peptidoglycan (Ghuysen, Strominger and Tipper, 1968) a number of antibiotics can inhibit its biosynthesis. For example, D-cycloserine and o-carbamylserine can inhibit the biosynthesis of the nucleotide peptide precursors of the cell wall. Vancomycin, ristocetin and bacitracin

inhibit the utilization of these precursors for the synthesis of linear glycopeptide. Penicillins and cephalosporins inhibit the formation of cross-linkages in the glycopeptide.

Staphylococci are known to produce many inhibitory substances which may show a bacteriolytic (Arvidson, Holme and Wadstrom, 1970) or a bactericidal effect (Gardner, 1949; Barrow, 1963; Hsu and Wiseman, 1967) on other strains of staphylococci. Generally, bacteriocins are high-molecular-weight bacterial substances produced by various species of bacteria and are usually active against the same related species. However, Staphylococcus aureus strains of phage type 71 are known to produce a bacteriocin which inhibits streptococci, coryneforms and actinomycetes (Noble, 1980). This finding is in agreement with those of Jetten, Vogels and de Windt (1972) who found that the inhibitory effect of staphylococcin 1580 was not restricted to staphylococci as a number of gram-positive organisms were sensitive to it. However, the authors noted that none of the Gram-negative bacteria they examined was sensitive to staphylococcin 1580.

Bacteriocins produced by Gram-positive organisms are known to have a much wider action spectrum than bacteriocins produced by Gram-positive organisms (Frederique, 1946; Hamon and Peron, 1963; Gagliano and Hinsdill, 1970). The specificity of the actions of bacteriocins and their nature distinguish them from most of the "classical" antibiotics. The best studied of the bacteriocins are the colicins, bacteriocins produced by Escherichia coli. There is however a paucity of data on the nature, genetics and mode of action of staphyloccins. This may be due to the difficulty encountered in the isolation and production of staphylococcins (Hough, Jones and Wadman, 1950; Ryan, Fried and Mukai, 1955; Dajani and Wannamaker, 1969; Arvidson, Holme and Wadstrom, 1970; Jetten, Vogels and de Windt, 1972).

Like peptidoglycans, cell wall teichoic acids are reliable and probably stable characteristics of Staphylococcus aureus. They are useful chemotaxonomic products in the differentiation of staphylococci (Davison and Baddiley, 1963; 1964). Cell wall teichoic acids are important for the different serological

behaviour of staphylococci (Oeding, 1966).and adsorption of phages (Charterjee, 1969). The immunological properties of the cell wall peptidoglycans and teichoic acids are also useful for the serological differentiation of Staphylococcus species (Oeding, 1974; Digranes and Oeding, 1975; Seidl and Schleifer, 1978). Nearly 100 per cent of human strains of Staphylococcus aureus have poly AB (β - N - acetyl-glucosaminyl ribitol teichoic acid) (Haukenes, 1962; Hofstad, 1965). Although the presence of ribitol teichoic acids has been considered for coagulase-positive staphylococci, Hajek (1976), Schleifer, Schumacher-Perdreau, Gotz and Popp (1976) have found some exceptions in the form of glycerol teichoic acids in Staphylococcus intermedius. Adegoke, Ojo, Devriese, Godard, Fleurette and Schleifer (1983c) also isolated a caprine strain of Staphylococcus aureus which had glycerol and glucosamine teichoic acids.

Jensen (1958) reported an antigen (antigen A) obtained from Staphylococcus aureus of Cowan I type which reacted with all of 400 human sera in the serum-gel double diffusion test. Lofkvist and Sjoquist (1962);

Oeding, Grov and Myklestad (1964) found that antigen A was a protein. The name protein A was suggested by Grov, Myklestad and Oeding (1964) and it has generally been referred to by this name ever since. Protein A is found in over 90 per cent of Staphylococcus aureus strains although individual strains may differ in the amount of protein A produced (Cohen, 1972).

In comparing the general characteristics of endogenous respiration in coagulase-positive and coagulase-negative staphylococci, Ivler (1965) found some differences in the endogenous systems particularly the dehydrogenases. Lactic acid configuration formed by fermentation of glucose is also a useful chemotaxonomic criterion for staphylococci (Schleifer and Kocur, 1973). L-(+)-lactate isomer is commonly formed by Staphylococcus epidermidis, Staphylococcus simulans, Staphylococcus intermedius, Staphylococcus hyicus, Staphylococcus capitis, Staphylococcus xylosus, Staphylococcus saprophyticus, Staphylococcus cohnii and Staphylococcus sciuri. Staphylococcus hominis and Staphylococcus haemolyticus form predominantly D-(-)-lactate isomer whilst Staphylococcus aureus form both

L-(+)- and D-(-)-lactate in reasonable amounts. Lactate dehydrogenase (LDH) enzymes responsible for the formation of lactate in staphylococci are known to exist as different electrophoretic forms or isoenzymes (Gotz and Schleifer, 1975). Lactate, the major end product of anaerobic growth in glucose is paralleled by a high level of lactate dehydrogenase activity (Collins and Lascelles, 1962). Staphylococcal dehydrogenase activity can be assayed by flavoprotein reduction of triphenyl tetrazolium chloride indicator in the electron transport system which according to Mitchell and Moyle (1956) is adjacent to the cell wall containing the A-phage receptor.

Immunological relationship of proteins has been found useful in the estimation of phylogenetic relationships of the various species of staphylococci. Protein homology studies (Champion, Prager, Wachter and Wilson, 1974) have also been used for higher organisms. Schleifer, Meyer and Rupprecht (1979) in their detailed study of the immunological relateness of catalases of several species of Staphylococcus found that the catalases of Staphylococcus epidermidis, Staphylococcus capitis and

Staphylococcus warneri were separated by less than 30 IMD units. The catalases from Staphylococcus xylosus and Staphylococcus saprophyticus were separated by a low immunological distance of 5 IMD units. The catalase of Staphylococcus cohnii was separated from that of Staphylococcus xylosus by a distance of 40 IMD units. Schleifer et al. (1979) further noted that the immunological relationships of catalases of the various species of staphylococci correlated with the genomic relationships as determined by DNA - DNA hybridization studies.

The occurrence of airborne Staphylococcus aureus in hospital environments has been linked with nocosomial infections (Williams, 1966). A variety of coagulase-negative staphylococci, apart from Staphylococcus aureus can act as infectious agents (Meers, Whyte and Sandys, 1975; Oeding and Digranes, 1976; John, Grumling and O'Dell, 1978; Namavar, de Graaff, deWith and Maclean, 1978). Since staphylococci are opportunistic pathogens of man and animals, descriptions of new species are therefore usually based on consistent phenotypic and molecular characters.

Serology: Staphylococci isolated from animals are known

to differ from those of human origin by their physiological properties and also from each other in close dependence upon the animal species in which they multiply (Harry, 1967; Shimizu, 1968). Staphylococcus aureus strains isolated from man and some animals can be distinguished on the basis of specific biochemical, nutritional, phage typing and serological properties (Meyer, 1967; Live, 1972; Oeding, 1972; Rische, Meyer, Tschape, Voigt, Ziomek and Hummel, 1973). Serology was first used for the separation of pathogenic and non-pathogenic staphylococci by Kolle and Otto (1902). Julianelle and Weighard (1934) demonstrated species - specific carbohydrates in pathogenic and non-pathogenic staphylococci by means of precipitation. Cowan (1939) is however credited with the introduction of workable and reproducible serological typing of Staphylococcus aureus. Boiled bacteria were used for the production of antisera, absorption and for agglutination which was then performed on slides with absorbed and unabsorbed sera. Oeding (1952a,b) used formalin-killed bacteria for the production of rabbit immune sera and for absorption.

He performed agglutination on slides with 18 h bacteria. On the basis of cross-absorptions, factor sera were produced and an antigenic scheme established which included 9 antigens denoted by the letters a to K (Oeding and Grov, 1972). There are at least 30 species - specific type agglutinogens possessed by Staphylococcus aureus (Oeding, 1967). Although human-type agglutinogens are found in strains from animal species, biochemical characteristics and phage susceptibility patterns of the strains are usually different. The two main serotyping methods found useful for Staphylococcus aureus are the Cowan - Mercier - Pillet system (Oeding, 1974; Fleurette and Modjadedy, 1976) and the Oeding - Haukenes system (Haukenes, 1967). Pillet's system is based on the recognition of the thermolabile structures on the surface of the bacteria. Oeding's system combines the recognition of the thermolabile and thermostable surface antigens of Staphylococcus aureus by using specific antisera for given agglutinogens (Flandrois, Fleurette and Modjadedy, 1975). Serotyping of Staphylococcus aureus though useful for epidemiological surveys has however not been widely

applied for taxonomic purposes. A number of reasons may be responsible for this: the intricacies involved in the production of specific antisera, the low titre of prepared immune sera coupled with the non-specific interference of the agglutinogens with some group antigens such as protein A (Oeding, 1952a; White, Rattray and Davidson, 1962; Haukenes, 1967; Pillet, Orta and Laveyi, 1969; Cohen, 1969). However, Fleurette and Modjadedy (1976) have proposed a simplified serotyping technique for serotyping Staphylococcus aureus. Adegoke, Devriese, Godard, Fleurette, Brun and Ojo (1983a) used the technique of Fleurette and Modjadedy in serotyping caprine strains of Staphylococcus aureus and found it workable. In addition to the serotypability of caprine strains of Staphylococcus aureus, Adegoke et al. (1983a) found that phages developed for typing human strains of Staphylococcus aureus could also be used for caprine strains of Staphylococcus aureus.

Phage typing: The foundation for phage typing of Staphylococcus aureus was laid by Wilson and Atkinson (1945). The introduction of the systematic use of phages for epidemiological purposes is however credited to Fisk

(1942). Both coagulase-positive and coagulase-negative staphylococci from a variety of hosts can be phage typed with specific phages (Verhoef, Boven and Winkler, 1971; Subcommittee, 1975; Pulverer, Pillich and Klein, 1975; Shimizu, 1977; Parisi, Talbot and Shakam, 1978; Wang, 1978). Phage typing of coagulase-negative staphylococci is of limited application routinely. However, the isolation of lysogenic phages for coagulase-negative staphylococci suitable for typing purposes (Verhoeff et al. 1972; Dean, Williams, Hall and Corse, 1973) provides a means of studying the epidemiology of Staphylococcus epidermidis by phage typing. Generally, most serious infections in humans caused by coagulase-negative staphylococci occur in patients carrying some form of prothases or catheters. In animals, coagulase-negative staphylococci are probably only of importance in sub-clinical mastitis (Devriese, personal communication). Staphylococcus epidermidis has long been usually regarded as a blood contaminant 85 per cent or more of the time. However, an increasing frequency of Staphylococcus epidermidis septicaemia has been noted (Quinn, Cox and

Drake, 1966; Bryan, Sutton, Saunders, Longaker and Smith, 1978; Verhoeff et al. 1978). Generally, endocarditis is believed to be a common but not an essential part of Staphylococcus epidermidis septicaemia. Quinn et al. (1966) reported no cases of Staphylococcus epidermidis endocarditis but the organism was found in all the 9 cases of subacute endocarditis. Thus it is of diagnostic importance if Staphylococcus epidermidis is consistently isolated in blood cultures on each of two separate days.

CHAPTER 3

PHYSIOLOGICAL CHARACTERISTICS

INTRODUCTION

Generally, a clinical or bacterial diagnosis of staphylococcal infection is enhanced by isolation procedures. Many patients but by no means all, with staphylococcal infection discharge large numbers of staphylococci into their environment thus creating a potential cross-infection hazard for their contacts including family members, hospital staff and other patients (Eickhoff, 1972). Invariably the epidemiological risk created by such patients is proportional to the number of staphylococci released into their environment and the route by which such dissemination occurs is also important. Patients with extensive dermatological infection such as major burns or exfoliative dermatitis and patients with staphylococcal pneumonia may disseminate large numbers of staphylococci widely into the environment.

Patients with draining abscesses or wound infections may discharge equally large numbers of staphylococci. Therefore appropriate isolation procedures are undertaken to curtail the varying hazards created by differing foci of infection (Brachman, 1970).

Hitherto, most attention has been focussed on the characteristics of the presumably pathogenic coagulase-positive staphylococci isolated from infections in man and different animals. However, in the descriptions of several new species of staphylococci isolated from man and animals (Schleifer and Kloos, 1975a; Devriese, Hajek, Oeding, Meyer and Schleifer, 1978), a variety of phenotypic markers were employed. These included examination of the morphological characters of colony size, profile, edge, texture, light transmission and pigment, cell size and arrangement. Other physiological characters used included anaerobic growth in a semisolid thioglycollate medium, growth at different concentrations of sodium chloride in agar and at different temperatures and susceptibility to lysostaphin and novobiocin.

Most strains of staphylococci will grow readily in

a chemically defined medium containing glucose, salts, amino acids, thiamine and nicotinic acid. Staphylococci can grow on a variety of simple laboratory media including blood agar, MacConkey and nutrient agar. However, the growth on some special media such as P agar (Kloos, Tornabene and Schleifer, 1974) is often used for differential diagnostic works. Staphylococci generally demonstrate some degree of variations in their phenotypic characteristics. There may be differences in the cultural characters, antibiograms and susceptibility to phages. Although staphylococci are readily killed by most antiseptics and disinfectants at the appropriate concentrations in the absence of serum, pus or albuminous materials, their ubiquitous nature does not appear to affect their viability, as laboratory cultures of staphylococci can survive for months and freeze-dried cultures in broth or serum will keep for years. Infact most strains of Staphylococcus aureus are resistant to slow drying and may survive in the dust in the absence of direct sunlight for months.

Under aerobic conditions, catalase is usually produced by staphylococci. However, the occurrence of

catalase-negative staphylococci have been reported (Lucas and Seeley, 1955; Everall and Stacey, 1956; Tu and Palutke, 1976; Schumacher-Perdreau, Peters and Kocur, 1981). Jensen and Hyde (1963) suggested that catalase-negative staphylococci might be producing apocatalase, a molecule which is serologically identical to catalase but is biologically inactive.

Staphylococci can be isolated from the nasopharynx and skin of animals and humans. Occasionally man may be a source of infection to domestic animals and pets. However, animals generally appear to be colonized by varieties of staphylococci peculiar to them. Skin carriage seems to be important in sheep (Smith, 1959, Ojo, personal communication). Skin carriage is also important in dogs (Ojo, 1972), whereas chronic udder infection is an important reservoir for spread of bovine mastitis. Domestic animals, wild animals and man often carry coagulase-positive or coagulase-negative staphylococci in the nose and throat (Hajek and Marsalek, 1969; Oeding et al., 1970) and most of the strains often associated with pigs, cattle and poultry can be identified to species

level by using growth characteristics and conventional biochemical tests (Devriese, In press) as was done in this present study.

MATERIALS AND METHODS

Isolates: The sources and numbers of strains examined in this present study are shown in Table 3.1a. Sixty strains were isolated from clinical cases of boils and wound from patients who attended the University College Hospital, Ibadan, Nigeria. One hundred and twenty strains were isolated from the nares, eye and rectal swabs of goats from 29 farms located all over the country, 70 strains were isolated from the nares of sheep whose ages ranged between 6 months and 3 years from the University of Ibadan Teaching and Research Farm. Twenty three strains were isolated from the trachea of healthy and sick chickens.

Cultivation techniques: Swab samples from the respective clinical cases were seeded onto 5 per cent sheep blood agar, nutrient and MacConkey agar plates. They were incubated in air at 37°C for 18 - 24 h. Suspected colonies were stained by Gram's technique and preserved

on trypticase soya agar slopes until required for subsequent tests after which they were freeze-dried.

Novobiocin susceptibility test: Each strain was grown in brain heart infusion broth (Oxoid) for 18-24 h at 37°C. The concentration of the bacterial growth was then adjusted with phosphate buffered saline (Appendix 2) to match half the density of a McFaland No. 1 standard (Lennette, Ballows, Hausler and Truant, 1980). The adjusted broth culture was then swab-inoculated onto sterile Diagnostic Sensitivity Test (DST) agar or Trypticase Soya Agar (Difco, Detroit, USA) plates. The plates were allowed to air-dry after which novobiocin (30 mcg) susceptibility disk (Upjohn Co., Michigan, USA) was placed on the inoculated agar surface with sterile forceps. The disk was gently pressed down to ensure even contact and the plates were incubated within 30 min. at 37°C for 18 - 24h. The diameters of the zones of inhibition were read off with a metre rule. Zone sizes of more than 25 mm. were interpreted as indicating sensitivity (Upjohn, Co., Michigan, U.S.A.). Staphylococcus aures (ATCC, 25923) and Staphylococcus gallinarum

(CCM, 3573) were employed as susceptible and resistant controls respectively.

Anaerobic growth in thioglycollate medium: Brewer's thioglycollate medium maintained at 50-55°C was seeded with overnight brain heart infusion broth culture using a straight wire. The medium was allowed to cool down after which it was incubated at 37°C for 18-24 h. Uniformly dense, tiny or scattered colonies were taken as anaerobic growth. The same controls as for novobiocin susceptibiligy test were used.

Growth on P agar: Pin-point inoculation of P agar and brain heart infusion agar with each strain of staphylococcus was made by using a sterile straight wire. The plates were incubated in air at 37°C for 24 h after which they were examined for colonial characteristics. The plates were incubated further for 3 days at room temperature (22-26°C) and later re-examined.

Lysostaphin Sensitivity Test: Poured plates containing 10 mcg/ml of sterile lysostaphin (Sigma Chemicals, St. Louis, U.S.A.) were allowed to solidify at 35°C for 18-24 h. The strains were then spot-inoculated on the

agar plates and reincubated at 35°C for 2 days. Sensitivity to lysostaphin was shown by inhibition of growth.

Production of bacteriocin-like substance: Each of the strains examined in this study (Table 3.1a) was replicated onto a semisolid agar plate without disturbing the colonies. The plates were then incubated at 35°C for 18-24 h. Three drops of overnight broth culture of an indicator strain (wild strain of Staphylococcus carnosus, T.U.M. Munchen, W. Germany) were added to 30 ml of a molten medium cooled to 45°C. This was then used as an overlay on top of the primary medium and was allowed to solidify after which it was incubated at 35°C for 18-24 h. Inhibition of the indicator strain was taken as evidence of the production of a bacteriocin-like substance.

Production of haemolysins: The production of alpha, beta and delta haemolysins was detected by using blood agar base (Difco, Detroit, U.S.A.) to which 4.0 per cent of washed sheep, rabbit and human red blood cells were added. Filter paper strips soaked in anti-alpha haemolysin serum (Burrough Wellcome, Co., U.K.) were used for the

differentiation between alpha and delta lysins. The plates were incubated in a 30 per cent carbon dioxide (CO_2) atmosphere at 37°C and later examined. The plates were left on the bench at room temperature (22-28°C) in contact with air for 6 h and then re-examined. Staphylococcus caprae (CCM, 3573) was used as a control.

Coagulase (tube test). This was carried out as described in Chapter 4.

RESULTS

Identification: Generally, the staphylococcal strains examined were Gram-positive cocci in clusters and some appeared in singles and were all both active physiologically. Of the 60 strains isolated from man, 41 were found to be coagulase-negative. These were identified as Staphylococcus sciuri, Staphylococcus xylosus and Staphylococcus cohnii (Table 3.1b). Only eight Staphylococcus aureus strains and one strain of Staphylococcus intermedius were isolated from the clinical samples examined. Sixty-two of the staphylococcal strains isolated from sheep and 91 from goats were coagulase-negative. Fourteen strains of coagulase-

positive staphylococci were isolated from the goats examined. The coagulase-negative staphylococcal strains isolated from goats were identified as Staphylococcus sciuri (38.2 per cent), Staphylococcus lentus (13.6 per cent), Staphylococcus xylosus (13.6 per cent), Staphylococcus gallinarum (7.3 per cent), Staphylococcus cohnii (5.5 per cent), Staphylococcus saprophyticus (4.5 per cent), Staphylococcus hyicus subsp. hyicus (2.7 per cent) and Staphylococcus hyicus subsp. chromogenes (1.8 per cent). Of the 6 coagulase-positive staphylococci isolated from the dogs examined, 5 were identified as Staphylococcus intermedius. Also one strain of Staphylococcus intermedius was isolated from a dog owner (Table 3.1b). The coagulase-negative staphylococci isolated from the poultry under study were identified as Staphylococcus lentus, Staphylococcus xylosus, Staphylococcus cohnii, Staphylococcus gallinarum and Staphylococcus hyicus subsp. hyicus. The scheme used for the identification of these strains are shown in Appendix 1.b,c.

Novobiocin Sensitivity: Of the 281 strains isolated from both man and animals, 214 (76.2 per cent) were found to be novobocin-resistant. All the strains isolated from

sheep were novobiocin-resistant as well as the only strain isolated from an antelope.

Anaerobic growth in Brewer's thioglycollate medium: All the strains of coagulase-positive staphylococci grew in the anaerobic portion of the thioglycollate medium. Variable growth patterns were found amongst the coagulase-negative strains examined (Table 3.2). However, 6 strains of Staphylococcus lentus isolated from goats grew anaerobically producing colonies which were light scattered or of uniform density in the anaerobic portion of Brewer's thioglycollate medium. Kloos et al. (1976) however, found that mainly Staphylococcus sciuri strains showed some growth in the anaerobic portion of this medium.

Growth on P agar Medium: The Staphylococcus aureus strains isolated from the hosts examined had relatively similar cultural characteristics. However, differences as regards size, colour and consistency were noticeable amongst the coagulase-negative strains examined in this present investigation. Morphologically the colonies of Staphylococcus lentus isolated from goats resembled those

of Staphylococcus sciuri (Fig. 3. a,b). They had an average diameter of 6 mm after two days' incubation (Fig. 3c) increasing to 7-8mm after 5 days (Fig. 3.d). Pigmentation and serration of colonies were more obvious in strains of Staphylococcus sciuri than those of Staphylococcus lentus. Although the average colony size of the Staphylococcus gallinarum strains isolated from the goats under study was found to be 6-8 mm (Fig. 3.e) however, one strain (UI 158) had outstretched colony which exceeded 19 mm (Fig. 3.f). The growth characteristics of other strains of staphylococci isolated from man and animals are shown in Appendix 1.d,e.

Lysostaphin Sensitivity: Variable results were obtained in the sensitivity of both coagulase-positive and coagulase-negative staphylococci to lysostaphin (10 mcg/ml). Of the 281 strains tested, only 53 (18.9 per cent) were found susceptible (Table 3.2b).

Bacteriocin-like substance production: Generally production of a bacteriocin-like substance was not commonly found amongst the strains tested. However, 5.8 per cent

of the strains isolated from animals were found to possess some inhibitory activities against Staphylococcus carnosus (Table 3.2a).

Haemolysin production: Variable results were obtained with the strains of S. aureus of human and animal origins with regards to the production of alpha beta and delta haemolysins. Whilst 22.2% of the strains of human origin produced alpha haemolysin, 84.6% of the caprine strains of S. aureus examined produced it. Production of delta haemolysin was common amongst caprine strains of S.aureus (Table 3.2a).

DISCUSSION

Epizootics due to staphylococci (Oeding et al., 1973) have been occasionally observed in wild animals but few attempts have been made to study staphylococci in wild animals in their natural habitats when compared with the characterization of staphylococci from man, cow and poultry. However, in this present investigation, attempts have been made to characterize the staphylococcal strains isolated from a monkey, an antelope and African giant rats

(Cricetomys gambianus). It is thus of interest to note that several of the newly described species of staphylococci were also isolated in this present study. Whilst Staphylococcus intermedius occurs in dogs, horses, mink and pigeons (Hajek, 1976), Staphylococcus hyicus strains frequently occur on the skins of pigs, cattle and poultry (Devriese and Oeding, 1975; Devriese, 1977; Amtsberg, 1979; Devriese and Derijcke, 1979). Staphylococcus hyicus can also be found in milk samples from cows with subclinical mastitis (Devriese et al., 1978). The strains presently referred to as Staphylococcus intermedius were formerly named Staphylococcus aureus var. canis (Meyer, 1966a) or as Staphylococcus aureus biotypes E and F (Hajek and Marsalek, 1971).

Staphylococcus intermedius and Staphylococcus hyicus do not frequently occur in man (Devriese and Hajek, 1980). In this present study, however, in addition, to the strains of Staphylococcus intermedius isolated from the dogs examined, one strain was isolated from the nares of one of the dogs' owner. Thus the close association

of pet animals may enhance cross contamination by staphylococci (Live, 1972; Adegoke and Ojo, 1981). In addition, Staphylococcus intermedius are known to share with Staphylococcus aureus the diagnostically important tube coagulase reaction and in many cases also the heat-stable deoxyribonuclease reaction and beta haemolysin production.

In lambs, tick pyaemia is usually caused by staphylococci. Staphylococcus aureus appears to reside on the skin of lambs. The animals become susceptible to infection when both ticks (Ixodes ricinus) and Staphylococcus aureus are present. Thus the inter-relationship of staphylococcus, ticks, virus and lambs is complex. Cross-colonization of lambs by a virulent strain of staphylococcus renders the animal susceptible to staphylococcal disease should ticks be present. Ticks do not really initiate the disease, but provide entry by their bites for staphylococci. However, virulent strains of staphylococci with or without concurrent infection with the virus of tick-borne fever (Foggie, 1954; Watson, Brown and Wood, 1966) may occur. In this present study, the

presumably virulent strain of Staphylococcus aureus isolated from a sheep was from an apparently clinically tick-borne-free herd.

In the study of staphylococci isolated from the udders of goats in India, Rao and Seetharam (1967) found that of the 105 strains examined, 88 per cent were coagulase-negative strains. The authors did not however identify the strains to species level. In this present investigation, coagulase-negative staphylococci accounted for most of the strains studied (Table 3.1b). Hitherto, coagulase-negative staphylococci isolated from various clinical samples are often discountenanced. However, Krzeminski (1980) has even suggested that some staphylococci may take part in the process leading to peridental tissue injury, Thus the carriage of some coagulase-negative staphylococci by the goats examined needs further epidemiological studies as these pathogenic strains notably Staphylococcus saprophyticus, Staphylococcus epidermidis and Staphylococcus hyicus subsp. hyicus are of clinical importance in man or animals (Holt, 1972; Devriese and De Keyser, 1980). Staphylococcus epidermidis,

though a common inhabitant of the skin of man (Kloos and Musselwhite, 1975) has also been found associated with various human infections in Sweden (Nord, Holta-Oie, Ljungh and Wadstrom, 1976). Of the 97 strains of coagulase-negative staphylococci isolated from the goats under study, Staphylococcus sciuri, Staphylococcus lentus and Staphylococcus xylosus were very common. Staphylococcus sciuri however differs from other strains of staphylococci in its peculiar possession of a c-type cytochrome and in contrast to the micrococci, Staphylococcus sciuri, like other staphylococci contains no cytochrome - d (Faller, Gotz and Schleifer, 1980). In addition to this, Staphylococcus sciuri possesses tetraglycl-L-alanine in the peptidoglycan, a feature which is absent in other staphylococci. Staphylococcus sciuri was originally described with two subspecies; Staphylococcus lentus and Staphylococcus sciuri (Kloos et al., 1976). Later, Schleifer, Geyer, Kilpper-Balz and Devriese (1983) found that genetic studies have shown that the two subspecies should be elevated to species rank. Although goats carry relatively few population of Staphylococcus

hyicus (Table 3.1b), it should be noted that Staphylococcus hyicus is however not exclusively a skin pathogen of pigs as it has been isolated from arthritis and periarthritis in piglets (Devriese, 1977; Amtsberg, 1978a). Staphylococcus hyicus produces epidermolytic toxin (Amtsberg, 1979) which is responsible for most of its pathogenic effects. The pathogenic significance of Staphylococcus hyicus in poultry is unknown (Devriese et al., 1978). In cattle, it causes exfoliation of the epidermis and exudation (Amtsberg, 1978b; Devriese and Derijcke, 1979). Staphylococcus hyicus is most frequently found in cattle as a complicating factor in mange lesions (Devriese and Derijcke, 1979). Staphylococcus hyicus has also been associated with subclinical mastitis in some herds (Devriese et al., 1978).

The species distribution of the ovine strains examined showed that all the coagulase-negative staphylococci were novobiocin-resistant (Table 3.1b). This differs from the distribution pattern observed by Guittierrez, Menes, Garcia, Moreno and Bergdoll (1982). These authors identified Staphylococcus simulans, Staphylococcus epidermidis and Staphylococcus haemolyticus

in their samples of ovine mastitic milk. In nature, novobiocin-resistant species of staphylococci are widely distributed (Kloos, Musselwhite and Zinmmerman, 1975). Although Pelzer, Pulverer, Jeljaszewich and Pillich (1973) in their study of some coagulase-negative staphylococci found that 4 strains of Micrococcus behaved as novobiocin-sensitive and also many strains of Staphylococcus albus exhibited a distinct resistance to novobiocin and therefore suggested that novobiocin susceptibility testing was of no significance, however, in the descriptions of several novel species of staphylococci from man and animals (Schleifer and Kloos, 1975; Hajek, 1976; Devriese et al., 1978), novobiocin sensitivity has been found to be a useful differential test for staphylococci. Thus Devriese and De Keyser (1980) noted that in cattle, novobiocin-sensitive Staphylococcus epidermidis, Staphylococcus hyicus subsp. chromogenes, Staphylococcus simulans and Staphylococcus warneri predominate in teat canals and freshly drawn milk whilst Staphylococcus haemolyticus and the novobiocin-resistant Staphylococcus xylosus, Staphylococcus lentus and Staphylococcus sciuri prevail on the skin of the teats.

Invariably, the use of novobiocin paper disks used by these authors as well as in this present investigation appeared simple and convenient as they facilitated identification of the various staphylococcal population heterogenecity with respect to their sensitivity.

In the novel descriptions of Staphylococcus gallinarum, Devriese, Poutrel, Kilpper-Balz and Schleifer (1983) found that most well separated colonies of this staphylococcal species measured up to 15 mm diameter on brain heart infusion agar (Oxoid) with outgrowths reaching up to 40 mm at times. This is in agreement with the characteristics of some of the strains of Staphylococcus gallinarum isolated from goats in this investigation. The colonial characteristics of several other strains of staphylococci become more readily distinguishable with age that by 4-5 days of incubation the colonies are usually distinctive on some suitable media such as P agar. The identification of any species on primary isolation plates on the basis of colony morphology and pigment however demands special care. Generally, cultural characteristics can be subdivided into the parameters of profile (elevation), size, edge and

some internal structure including texture and consistency. These parameters were employed by Kloos and Schleifer (1975a) in the initial identification and characterization of several new species of staphylococci. Furthermore, Kloos et al. (1975a) found that the cell arrangements of diluted cultures grown on P agar for 24 to 48 h can be used as a secondary character for resolving some species of staphylococci. The authors noted that the cells of Staphylococcus xylosus, Staphylococcus cohnii and Staphylococcus saprophyticus were usually arranged outside of clusters as single cells or pairs whilst many strains of Staphylococcus hominis had cells that were arranged as tetrads. Pigmentation though not commonly used for taxonomic purposes has been found associated with some staphylococcal species. Abo-Elnaga and Kandler (1965) found that many coagulase-positive staphylococci isolated from cattle produced orange or cream-coloured pigment whilst 2 of the 69 coagulase-negative strains examined produced whitish colonies. Reid and Wilson (1959) isolated some staphylococci from acute bovine mastitis and found that they produced orange to yellow pigment. Cowan (1938),

Smith (1947), Shimizu, Takizawa and Shibata (1965) in their studies of coagulase-positive staphylococci of canine origin found that many strains formed white colonies. Thus Hajek and Marsalek (1975) regarded pigmentation as a classical means of differentiating staphylococci. The authors further reported that strains of Staphylococcus aureus belonging to the biochemically-active biotypes A, B and C have high incidence of pigmentation. Both coagulase-positive and coagulase-negative staphylococci are known to be pigmented. Thus of the 79 strains of Staphylococcus sciuri isolated from goats and sheep in this study, 20 (25.3 per cent) were pigmented. Whilst pigmentation was absent in strains of Staphylococcus gallinarum, Staphylococcus cohnii and Staphylococcus xylosus, two of the 31 strains of Staphylococcus lentus examined were pigmented (Table 3.4).

Lysostaphin, an investigational chemotherapeutic agent, is an enzyme with a molecular weight of about 30,000 and is produced by Staphylococcus staphylolyticus (Schindler and Schuhardt, 1960, 1965). Lysostaphin is useful as an antistaphylococcal therapeutic agent as well

as in the chemotaxonomic classification of the Micrococcaeceae. However, whilst direct evidence of the efficacy of lysostaphin in the treatment of serious staphylococcal infection in man is yet to be reported (Eickhoff, 1972), its topical administration has been found effective as other available antibiotics in suppressing nasal carriage of staphylococci during treatment and the acquisition rate was found to be considerably slower than with other forms of therapy (Martin and White, 1967). In the taxonomic study of several strains of staphylococci, Kloos and Schleifer (1975a), Schleifer and Kloos (1975a) used the spot test for lysostaphin susceptibility and found that Staphylococcus saprophyticus, Staphylococcus haemolyticus, Staphylococcus warneri, Staphylococcus hominis, Staphylococcus epidermidis having peptidoglycan type L-Lys-$Gly_{3.5-5.1}$ $L\text{-}Ser_{0.6-1.5}$ were only slightly inhibited by 200 mcg/ml of lysostaphin. The authors also noted that Staphylococcus aureus and Staphylococcus simulans possessing peptidoglycan type L-Lys-$Gly_{4.7-6.0}$ were most susceptible to lysostaphin. The principle of the activity of lysostaphin is due to an

endopeptidase with a high activity for the pentaglycine interpeptide bridge of the peptidoglycan (Kloos et al., 1975). The apparent resistance of the staphylococcal strains examined here to lysostaphin may be due to their peculiarity as has been found also by Kloos and Schleifer (1975a); Heddaueus, Heczko and Pulverer (1979) who despite the usage of lysostaphin at different levels which ranged from 50-200 mcg/ml, observed considerable strain-to-strain variation in susceptibility to lysostaphin.

Whilst the characteristics of the bacteriocin-like substance produced by some strains of staphylococci examined herein have not beeen analysed, nonetheless they showed some inhibitory activities against another staphylococcal strain (Table 3.2a). Morris, Lanton and Rogolsky (1978) found that the bacteriocins produced by a staphylococcal strain inhibited the growth of Neiserria gonorrhoeae. Antineisserial activities produced by some strains of staphylococci have also been found (Bisaillon, Beaudet, Lafond, Saheb and Sylvestre, 1981). These authors found that the inhibitory activities of the staphylococci they examined were different from the previously described

staphylococcins (Dajani and Wannamaker, 1969; Hale and Hinsdill, 1973) which were shown to be active against a wide spectrum of gram-positive bacteria.

Generally, staphylococci from a variety of hosts are known to produce haemolysins. The lethal and invariably haemolytic effects of cultures on rabbit cells established long before the multiplicity of staphylococcal haemolysins was appreciated, were however associated with alpha-haemolysin. However, the relationship of alpha haemolysin to pathogenicity in animals has not been fully established. Several views have been adduced on the mode of action of alpha haemolysin. Some authors (Marucci, 1963; Wiseman and Caird, 1970; Cassidy and Harshman, 1973) suggested that alpha haemolysin is bound to erythrocyte membranes. Bernheimer, Kim, Remsen, Antanavage and Watson (1972) studied the action of haemolysin on erythrocyte membranes by scanning and transmission electron microscopy and found that segments of membrane separated from the cell surface. Hajek and Marsalek (1975) in evaluating some classificatory criteria for staphylococci noted that alpha haemolysin production is typical for

human and to a great extent variable or absolutely negative as in cases of strains from sheep, hare, dog and horse. However, in this present study, whilst caprine strains of Staphylococcus aureus which belong to biotype C (Adegoke et al., 1983a) to which cattle and sheep belong (Hajek and Marsalek, 1971), the production of alpha haemolysin by Staphylococcus aureus of caprine origin (Table 3.7a) appeared more obvious than other members of biotype C. Of the 13 caprine strains examined herein, 11 (84.6 per cent) produced alpha haemolysin whilst only 59.8 per cent of the 82 strains of Staphylococcus aureus of bovine origin and less than 5 per cent of Staphylococcus aureus of ovine origin were found to have produced alpha haemolysin by Hajek and Marsalek (1975). In the examination of the characteristics of Staphylococcus aureus strains isolated from slaughtered fowls in Bulgaria, Bajljsov, Sachariev and Georgiev (1974) found that they produced mainly alpha and delta haemolysins rarely beta haemolysin. The mode of action of beta haemolysin has been eruditely reviewed by Jeljaszewich (1972) and Bernheimer (1974). In the pioneering description of beta

haemolysin, Doery, Magnusson, Cheyne and Gulasekharam (1963) noted that beta haemolysin released acid soluble phospholipase from erythrocytes, the source of which was sphingomyelin, a phospholipid widely distributed in mammalian cell membranes. This finding has since been confirmed by several authors (Wiseman and Caird, 1966; Maheswaran and Lindorfer, 1967; Fritsche, 1970). The phospholipase C activity of beta haemolysin was established independently by Wiseman (1967) and Maheswaran and Lindorfer (1967). According to Wiseman and Caird (1967), beta toxin hydrolyses sphingomyelin to phosphorycholine and N-acylsphingosine. These authors also established that a relationship existed between sphingomyelin concentration and erythrocyte susceptibility to beta haemolysin. Most poultry strains of Staphylococcus aureus are beta haemolysin negative (Devriese and Hajek, 1980). This is also true of the strains of Staphylococcus aureus of poultry origin examined in this present study. However, Jonsson and Holmberg (1981) in a survey of Staphylococcus aureus from bovine mastitis found that of the 50 strains examined, 46 (88 per cent) produced alpha

haemolysin and 41 (81 per cent) produced beta haemolysin. Alpha haemolysin production by the bovine strains of Staphylococcus aureus examined by these authors are close to those obtained for caprine strains of Staphylococcus aureus (Table 3.2a).

Whilst some strains of Staphylococcus aureus have been isolated from both healthy and sick hosts in this present study, it must be noted that virulent and avirulent strains of Staphylococcus aureus cannot be defined in the same way as rough and smooth pneumococci, or tox^+ and tox^- strains of Corynebacterium diptheriae because even epidemic strains, for example, the 80/81 complex demonstrate no qualitative or quantitative difference in presumed virulence factors and indeed may be less virulent for animals than conventional strains. Therefore, it is likely that a number of bacterial factors interact with human fluids and cells in a complex, semistable equilibrium which is shifted in favour of the organism by a variety of local or systematic factors (Morse, 1980). Thus, the susceptibility patterns and resistance to staphylococcal infections play an important

role in determining which individuals become ill and whether the infection remains localized or becomes generalized.

Fig: 3.a S. sciuri on P agar (2 days). Average diameter 6mm. Fairly round edges and non-pigmented colonies.

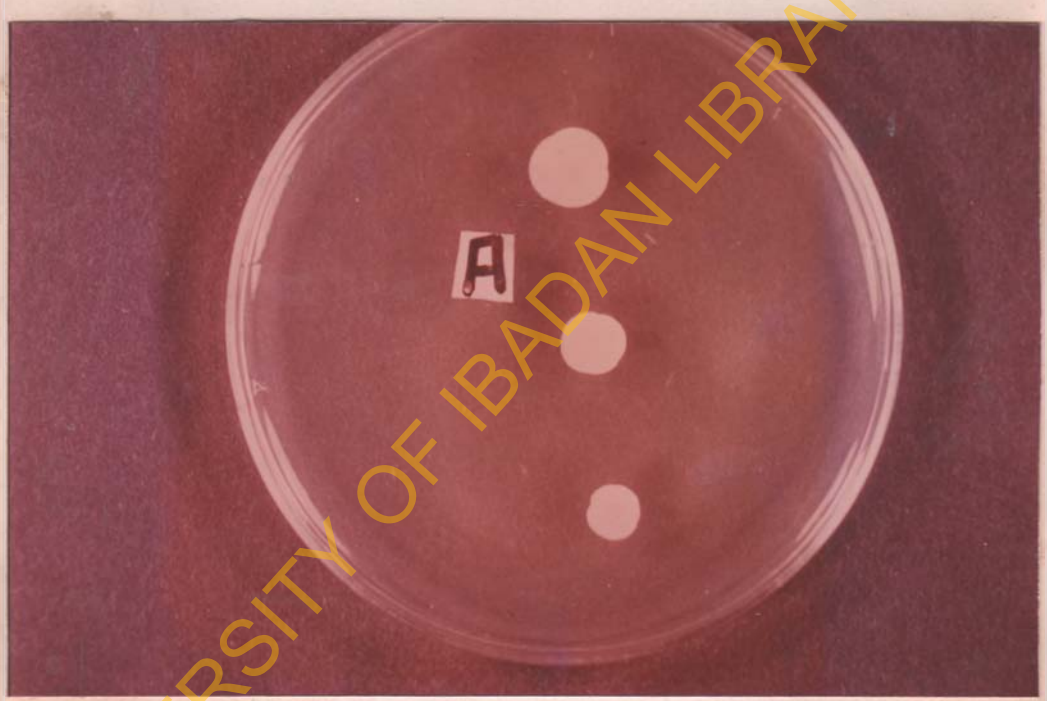


Fig. 3.b: S. sciuri on P agar (5 days). Average diameter 7-8mm. Bottom colony: fairly serrated. All colonies appeared non-pigmented.

Fig. 3.c: S. lentus on P agar (2 days). Average diameter 6mm. Serrations obvious in central colony. Topmost colony: fairly pigmented.

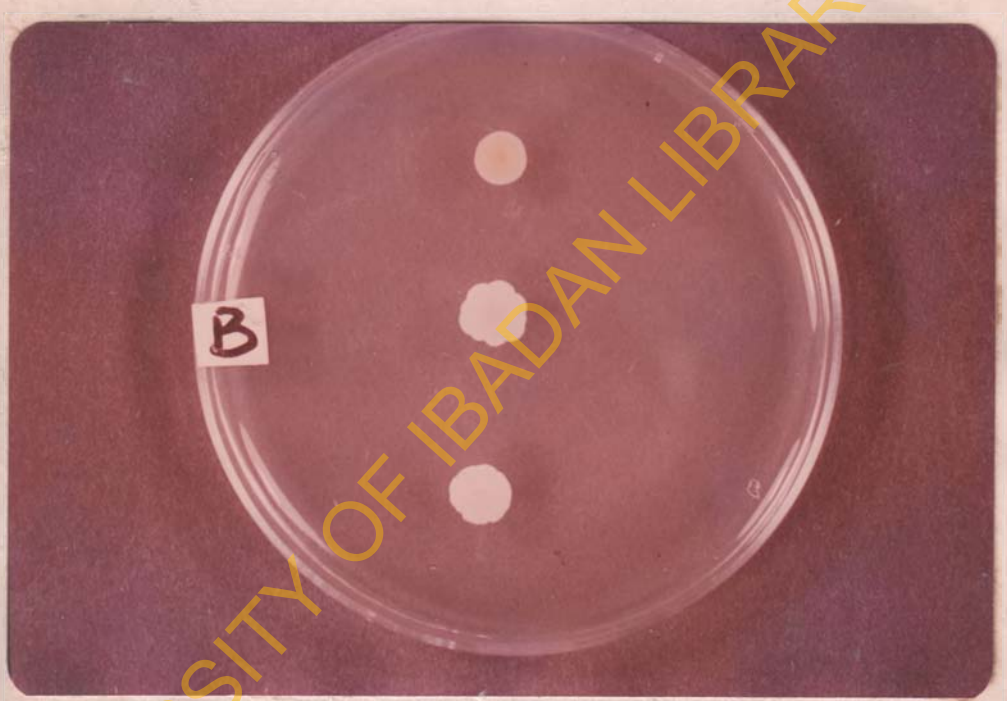


Fig. 3.d: S. lentus on P agar (5 days). Average diameter, 7-8mm. Bottom and central colonies: Serrated and non-pigmented. Topmost colony: round and yellowish.

Fig. 3.e: S. gallinarum on P agar (2 days). Colony diameter, 6-8mm. Extensively serrated, whitish colony.

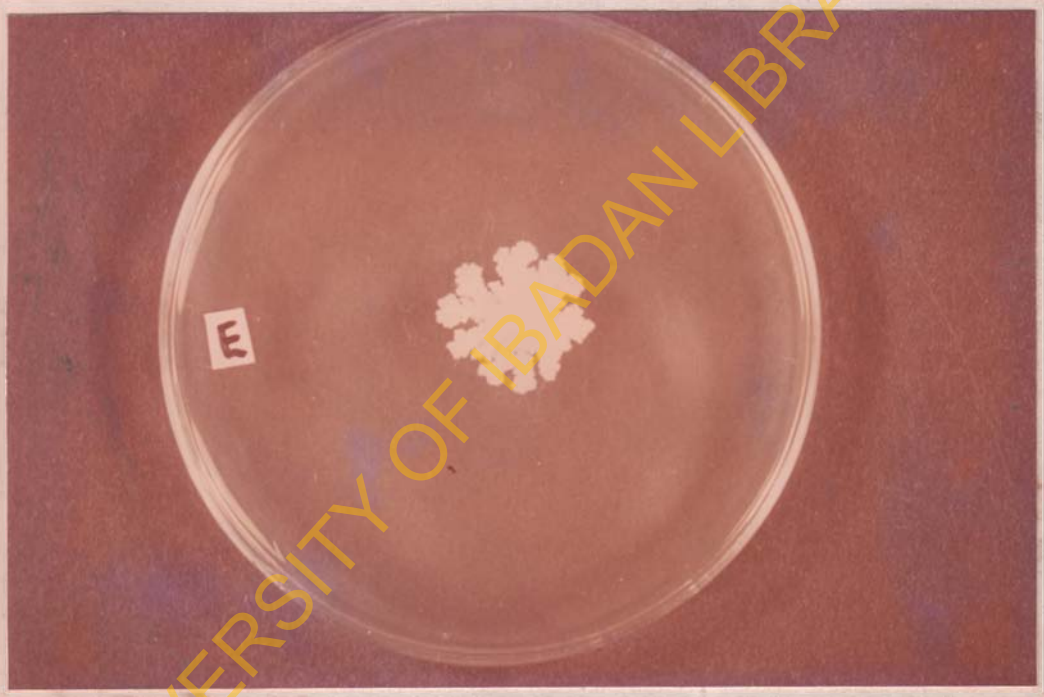


Fig. 3.f: S. gallinarum on P agar (5 days). Outstretched serrated colony which exceeded 19mm in diameter.

Table 3.1a. Sources and numbers of strains of staphylococci tested

Origin	Source of strain	Number that are:		Number of strains examined for:				
		healthy	sick	Physiological tests	Biochemical tests	Phage typing	Serotyping	Antibiogram
Humans	Boil, wound	-	60	60	60	8	9	9
Goats	Nares, eye and faeces	90	20	110	110	14	14	14
Sheep	Nares	69	1	70	70	1	1	1
Rabbits	Wound	-	11	1	1	1	1	1
Bovine mastitis cases	Milk	-	3	3	3	-	-	-
Monkey	Wound	-	1	1	1	-	1	1
Giant rats	Wound	-	3	3	3	3	3	3
Poultry	Trachea	11	12	23	23	2	2	2
Pig	Abscess	-	1	1	1	-	-	-
Dogs	Nares	-	8	8	8	1	5	5
Antelope	Wound	-	1	1	1	-	-	-
"Unclassifiables"	Goat and sheep (nares)	12	5	17	17	-	-	-

Table 3.1b: Distribution of species and strains identified

Species	Man	Goats	Sheep	Poultry	Dogs	Rabbit	Giant rat	Monkey	Antelope	Pig	Cow
Novobiocin-resistant											
S. sciuri	5	42	37	1	2	0	0	0	1	0	0
S. lentus	0	15	16	7	0	0	0	0	0	0	0
S. xylosus	20	15	6	5	0	0	0	0	0	0	0
S. saprophyticus	0	5	3	0	0	0	0	0	0	0	0
S. cohnii	16	6	0	2	0	0	0	0	0	0	0
S. gallinarum	0	8	0	2	0	0	0	0	0	0	0
Novobiocin-sensitive											
S. aureus	8	13	1	2	1	1	3	1	0	0	0
S. intermedius	1	0	0	0	5	0	0	0	0	0	0
S. epidermidis	0	1	0	0	0	0	0	0	0	0	1
S. hyicus subsp. *hyicus*	0	3	0	1	0	0	0	0	0	1	0
S. hyicus subsp. *chromogenes*	0	2	0	0	0	0	0	0	0	0	2
S. warneri	10	0	0	1	0	0	0	0	0	0	0
Total	60	110	63	21	8	1	3	1	1	1	3

Table 3.2a. Physiological characteristics of staphylococci isolated from man and animals

Hosts	Catalase test		Clumping factor test		Novobiocin sensitivity 30 mcg		Growth in thioglycollate		Haemolysins (S. aureus)				Production of bacteriocin-like substance	
	Positive	Negative	Positive	Negative	Resistant	Sensitive	Absent	Light/ scattered/ Uniformly dense	α	β	δ	Absent	Positive	Negative
Humans	100*	0	13.3	86.7	83.3	16.7	83.3	16.7	22.2	11.1	22.2	44.5	3.3	96.7
Goats	100	0	10.2	89.8	84.3	15.7	58.3	41.7	84.6	61.5	69.2	15.4	0	100
Other animals	100	0	11.6	88.4	66.1	33.9	75.2	24.8	31.3	50.0	31.3	25.0	5.8	94.2

*Percentage (%) ; : Table 3:1.b shows total number examined

Table 3.2b Lysostaphin susceptibility of coagulase-positive and coagulase-negative staphylococci isolated from man and animals

	HOSTS				
	Man	Goats	Other animals	Total number of strains tested	Number susceptible to lysostaphin (10 mcg/ml)
Number of coagulase-positive strains tested	9	13	16	38	2
Number of coagulase-negative strains tested	51	97	95	243	51
Total number of strains tested	60	110	111	281	-
Total number of strains susceptible to lysostaphin (10 mcg/ml)					53

Table 3.3 Growth characteristics of coagulase-positive staphylococci isolated from goats

COLONY		UI27	UI96	UI118	UI145	UI150	UI153	UI154	UI160	UI161	UI166	UI327	UI331	UI334
Size (mm)	2 days	4.0	5.0	5.5	5.5	6.0	5.0	4.0	5.0	5.0	5.5	3.0	5.0	5.0
	5 days	5.0	6.0	6.5	6.5	7.0	7.0	5.6	7.0	6.0	6.0	4.0	5.5	5.5
Colour	Yellow		+											
	Cream/White	+*		+	+	+	+	+	+	+	+	+	+	+
Consistency	Moist	+	+	+	+	+	+	+	+	+	+	+	+	+
Edge	Irregular							+		+		+		+
	Round	+	+	+	+	+	+		+		+		+	

*Positive results

Table 3.4 Growth characteristics of coagulase-negative staphylococci isolated from goats

	COLONY Size (mm) 2 days	5 days	Colour Yellow	Cream/White	Consis- Moist tency	Dry	Edge Irregular	Round	Number of strains tested
S. sciuri	6-7	7-9	12	30	42	0	16	26	42
S. lentus	6	7-8	2	13	15	0	2	13	15
S. xylosus	5-6	5-8	0	15	14	1	14	1	15
S. cohnii	5	6-7	0	6	6	0	1	5	6
S. saprophyticus	6	7	0	5	5	0	3	2	5
S. epidermidis	2-3	3-4	0	1	1	0	0	1	1
S. hyicus subsp. hyicus	4-6	6	0	2	2	0	2	0	2
S. hyicus subsp. chromogenes	4.5	5.5	0	1	1	0	1	0	1
S. gallinarum	5-6	6-8	0	8	7	1	3	5	8
S. aureus ATCC 25923	6-9	7-10	-	+	+	-	+	-	
S. hyicus subsp. hyicus NCTC 10350	7-9	9-10	-	+	+	-	+	-	

+ : Positive
- : Negative

6-aminopenicillanic acid or 7-aminocephalosporanic acid/ or their N-acyl derivatives. Thus penicillin-resistant strains of Staphylococcus aureus have often been linked with penicillinase production. Other physiological differences have however been found to exist between penicillin-sensitive and penicillin-resistant strains of Staphylococcus aureus. Miki (1968) who studied the electron micrographs of ultrathin sections of penicillin-sensitive and penicillin-resistant strains of Staphylococcus aureus found some differences in the thickness of the outer dense layer of the cell wall of resistant and sensitive strains. Thus, following the discovery of this extracellular product (Abraham and Chain,1940) and the subsequent evaluation of its role in the penicillin resistance of Staphylococcus aureus (Kirby, 1944), several methods were proposed for the assay of penicillinase (Wolff and Hamburger, 1962; Lucas, 1979). Sng, Yeo and Rajan (1981) used the filter paper acidometric test with bromocresol purple as the pH indicator and on account of the simplicity and cheapness of the test, the authors suggested that it could be used to screen for penicillinase

producing strains of Neisseria gonorrhoeae and Staphylococcus aureus.

Meyer (1965) suggested the use of plasma from different animal species for differentiating strains of Staphylococcus aureus. The rabbit plasma tube coagulase test recommended as the reference method for the identification of Staphylococcus aureus (Anon, 1965) because of its simplicity is widely employed in routine testing of staphylococci. The importance of coagulase test cannot be overemphasised as in food microbiology only the tube coagulase and thermostable deoxyribonuclease (DNAse) tests are recommended for the identification of Staphylococcus aureus (Anon, 1978).

Prior to the introduction of molecular systematics to the study of the genus Staphylococcus, the biotyping schemes of Baird-Parker (1963, 1965, 1972) were used for species identification. However, several authors (Forbes, 1968; Holt, 1969; Pelzer, Pulverer, Jeljaszewich and Pillich, 1973) could not classify their strains of staphylococci particularly the coagulase-negative species by using the schemes of Baird-Parker. Thus in an attempt

to apply more effective and workable criteria to the taxonomy of staphylococci, Schleifer and Kocur (1973) proposed that chemical cell wall composition and other biochemical features could be used for the characterization of staphylococci as was done in this study.

MATERIALS AND METHODS

Characterization Procedures:

Catalase activity: A loopful of the bacterial growth on nutrient agar or trypticase soya agar was placed on a clean, grease-free glass slide and two drops of sterile 3 per cent hydrogen peroxide added. Bubbles of gas within 2-5 min. was taken as a positive reaction. Staphylococcus aureus (ATCC 25923) and Streptococcus faecalis (NCTC 8213) were used as positive and negative controls respectively.

Clumping Factor reaction: A loopful of each strain from Brain Heart Infusion (BHI) agar was emulsified in a drop of phosphate buffered saline (pH 7.2) on a clean glass slide. To another loopful of the organism was added rabbit plasma. The emulsions were mixed gently with minimum

spreading. A positive result was indicated by macroscopical coagulation of the plasma within 3-5 min. Staphylococcus aureus (ATCC 25923) and Staphylococcus xylosus (CCM 2738) were employed as positive and negative controls respectively.

Coagulase (tube test): 0.1 millilitre of overnight Brain Heart Infusion broth (BHI, Oxoid CCM 225) culture of each strain was added to tubes of 10 mm inner diameter containing 0.5 ml of 1 in 3 diluted plasma. Human, rabbit (Institut Pasteur, Paris), bovine, equine, porcine and poultry plasmas were used. The reactions were read after 1,4, 6 and 24 h incubation at 37°C. Only 3^+(loose, voluminous clots) or 4^+ (firm clots attaching to the sides of the tubes) appearing within 6h were considered positive for biotyping purposes. The same controls used for clumping factor reaction were employed (Devriese, In press).

Production of Staphylokinase: 60 mg of bovine fibrinogen (Behring, Marburg, W. Germany) was added to 6.0 ml of phosphate buffered saline (PBS) and later transferred to 37°C water bath for 10 min. A further four millilitres of PBS were

added. The suspension was then added to 100 ml of molten nutrient agar (Oxoid) and later transferred to 56°C Water bath for 10-15 min after which 0.2 ml of dog's plasma was added. The medium was then spot-inoculated lightly with the strains of staphylococci and later incubated at 37°C for 18-24 h. Strongly and weakly positive as well as negative controls were included in the test. Control fibrin plates were also prepared without dog plasma and also incubated as previously described. Strains producing zones of complete clearing on plates supplemented with dog plasminogen and not on control plates were regarded as positive.

Proteinase test: Twenty-nine grammes of calcium-caseinate agar (Merck, Darmstadt, W. Germany) were suspended in a litre of cold, freshly distilled water and was allowed to soak for 30 min. Afterwards, the flask was placed in a cold water bath and heated slowly to boiling for 10 min with frequent shaking until the medium was completely dissolved. Coarse particles were subsequently removed by filtration through several layers of gauze. The medium was later sterilized by autoclaving at 121°C for 15 min

after which plates were poured and inoculated as described for the production of staphylokinase.

Production of deoxyribonuclease: Narrow streaks of about 2 to 3 mm in diameter were made with sterile straight wire on 90 mm Petri dishes containing 20 ml of deoxyribonuclease (DNAse) agar (Difco, Detroit, U.S.A.). The results were read after 24 hours of incubation at 37°C with subsequent flooding with 1 N hydrochloric acid (HCl). The results were recorded as strongly positive when the diameters of the reaction zones were at least 4 times as large as the diameters of the growth streaks. Narrower zones were interpreted as weakly positive. Controls were also included in the test.

Thermostable nuclease: A modified microslide method of Lachica et al. (1971) was used for the detection of thermonuclease activity. Wells measuring 2 mm in diameter were cut into a toluidine blue-deoxyribonucleic acid agar plate and filled with 5 microlitre (ul) of brain heart infusion broth cultures previously heated to boiling for 15 min. A prediffusion time of about 3 min was allowed prior to incubating the plates at 37°C for 4 to 6 h.

Positive and negative controls were also set up.
A positive reaction was indicated by a bright pink zone around the wells within 4 h.

Phosphatase: Two and half millilitres of a filter-sterilized solution containing 20 mg/ml disodium 4-Nitrophenyl phosphate dissolved in distilled water were added to 100 ml of melted Trypticase soya agar (Difco). Templates were inoculated with bacterial growths and incubated at 37°C for 18 - 24 h. Positive strains produced a yellow discolouration of the medium around the growths whilst the medium remained unchanged when inoculated with negative strains. Templates were reincubated for two days to facilitate recognition of weakly-positive strains.

Penicillinase: (a) One drop of sterile Nitrocelin was added to each of the antibiotic sensitivity plates used in Table 5.3. Reddish colouration within 30-60 min was indicative of penicillinase production.

(b) The method described by Hodge, Ciak and Tramont (1978) was employed. Suspended organisms in phosphate buffered saline (McCrady's Tube No.1) were smeared onto the surface of Isosensitest medium (Oxoid) with the

aid of a sterile cotton wool-swab stick. Penicillin low disks (NCo-sensitabs A/S Rosco, Denmark) were placed at the centre of the plates. Suspension of Micrococcus luteus was also thoroughly smeared on another Isosensitest agar plate as above after which each strain to be tested was inoculated as narrow streaks. Penicillin discs were also applied and the plates were then incubated at 37°C for 18 - 24 h. Penicillin-sensitive strains were recognized on the first plate by their zone of inhibition (measured in mm) as compared with the manufacturer's specifications. Penicillin-resistant strains were detected by the absence of inhibition. On the second plate, penicillinase-producing strains were identified by their growths which extended to the penicillin disk at the centre of the plate. Non-penicillinase producing strains were sensitive to the antibiotic. Controls were also included in the testing procedure.

Hyaluronidase: A light suspension of capsulated strain of Pasteurella multocida type A was swab-inoculated onto tryptose agar containing 5 per cent inactivated sterile horse serum. Three to five minutes were allowed for

prediffusion after which bacterial strains of up to 6 per plate were spot-inoculated onto the tryptose agar smeared with Pasteurella multocida type A. Control strains were included and the plates were then incubated at 37°C for 18-24 h. Hyaluronidase-producing strains showed clearing around the spotted inocula. Staphylococcus aureus (ATCC 25923) and Staphylococcus xylosus (CCM 2378) were employed as positive and negative controls respectively.

Oxidase: The modified oxidase test described by Faller and Schleifer (1981) was employed. One drop of 6 per cent solution of N.N.N.-tetramethyl-1,4 - phenylendiamin (Merck, W. Germany) in dimethyl sulfoxide (DMSO) was added onto a filter paper and allowed to diffuse. Bacterial strains were then smeared onto the paper using a sterile platinum wire. Oxidase-positive strains turned dark-blue within 30 - 60 secs. Staphylococcus aureus (ATCC 25923) and Staphylococcus sciuri (ATCC 29062) were included as negative and positive controls respectively.

Nitrate reduction: The medium recommended by Kloos and Schleifer (1975a) was used. Five millilitre amounts of the nitrate medium were distributed into test-tubes (150 by 13 mm) and sterilized by autoclaving at 121°C for 15 min after which they were inoculated with the bacterial growths (approximately 10^8 cfu/ml). They were later shaken vigorously in a 35°C water-bath for 48 h, 0.5 ml. of sulphanilic acid (0.8 g/100 ml of 5N acetic acid) and 0.5 ml of α-naphytylamine solution (0.5 g in 100 ml 5N acetic acid) were added then to each of the broth cultures. Red colouration which appeared within 2 min was taken as a positive reaction. Weakly-positive results showed pinkish colouration. Zinc powder was added to each of the broth that gave no colour change (negative reactions) and observed as described above. A persistent colourless reaction was taken as a positive result whilst a red colouration was indicative of a negative nitrate test. Positive and negative controls were also set up.

Urease: Christensen's urea medium was seeded with bacterial strains and incubated at 37°C for 1 to 7 days. A reddish discolouration of the medium was taken as evidence of urease production.

Aesculin hydrolysis: Bile aesculin agar (Difco, Detroit, U.S.A.) slopes were seeded with bacterial strains and incubated at 37°C for 1 to 2 days. Complete blackening of the medium was taken as a positive reaction.

Tween hydrolysis: Bacterial strains maintained on blood agar plates were seeded onto Sierra's medium - with up to seven strains per plate. The plates were later incubated at 37°C for 24 to 48 h. Clearing around the spot-inocula was regarded as a positive reaction.

Egg-yolk reaction: Five millilitres of egg yolk suspension (1 egg yolk in 100 ml of distilled water) was added to 100 ml of molten Baird-Parker medium (Oxoid CM 275). The plates were then inoculated as described previously for staphylokinase after which they were incubated at 37°C for 1 to 2 days. Clearing with precipitation or clearing without precipitation showed egg yolk reaction. Controls were included in the test.

Carbohydrate fermentation

(a) Purple agar plate method: Ninety five millilitres of purple agar medium was sterilized by autoclaving at 121°C

for 15 min after which 5.0 ml of a 10 per cent sterile solution of the specific carbohydrate was added. Up to ten isolates were spot-inoculated onto one plate which was later incubated at 37°C in air for 24 to 48 h. Yellow colouration of spot-inocula indicated acid production from the carbohydrate used for test. Positive and negative controls were included for each carbohydrate used.

(b) Phenol red broth method: One and half millilitres of sterile solution of 20 per cent stock solution of each sugar was added to 30 ml of sterile phenol red broth (Gibco, Pairley, Scotland). About 1.0 to 1.2 ml of this sugar base broth was added to wells of template petri dishes (Sterilin, Teddington, U.K.). The plates were covered properly after which they were inoculated dropwise with overnight brain heart infusion broth cultures (adjusted to MacFarland Tube No. 1) of each strain examined. The plates were incubated at 37°C for 1 to 3 days with daily examination for evidence of yellowish discolouration (fermentation) of the sugars used. A template of phenol red broth and all organisms tested was set up without the addition of

any sugar. A control well in which equal amounts of phenol red broth and the respective sugar used in the template as described above was also set up. Reference staphylococcal strains known to give positive and negative results with the respective sugars used in the test were also included in the procedures.

Acetoin: A modification of the method of Davis and Hoyling (1973) was employed. Five millilitres of sterile 20 per cent glucose was added to 100 ml of molten brain heart infusion agar. Up to 6 strains were patch - inoculated onto each of the poured plates. After 24 h of incubation at 37°C, an antibiogram-type paper disc (Whatman's Antibiotic Assay discs of 6.0 mm diameter) freshly soaked in a 10 per cent sodium pyruvate solution was then placed on each growth patch. The plates were reincubated for a further 3 h after which one drop of 40 per cent potassium hydroxide, 1 drop of creatine and 1 drop of an alcoholic solution of 1 per cent alpha-naphthol were spotted successively on each disc. The development of a pink or red coolour within 1 h was indicative of acetoin production.

Controls were also set up.

Crystal violet test: 0.3 millilitre of crystal violet (Merck, Darmstadt, W. Germany) from a stock solution (2 g/l.) was added to 100 ml of molten tryptose agar (Difco, Detroit, U.S.A.). The plates were heavily inoculated with fresh bacterial strains after which they were incubated at 37°C for 1 to 2 days. Controls were also set up. Yellow to yellowish-blue macrocolonies and violet to blue growths were designated crystal violet (CV) type A and CV type C respectively.

RESULTS

Catalase activity: All the strains examined herein were catalase-positive. → How many?

Clumping factor reaction: Of the 60 strains isolated from humans, 8 (13.3 per cent) gave positive clumping factor whilst only 11.8 per cent produced clumping factor out of the 110 staphylococcal strains isolated from goats. Variable results were obtained with other animals (Table 3.2a; Appendix 2. B,C,D and E).

Coagulase: All the eight Staphylococcus aureus and 1 Staphylococcus intermedius strains isolated from humans clotted rabbit plasma after 24 h. Coagulase-positive staphylococci obtained from goats showed some variations in the coagulation of different plasmas. Of the 14 caprine strains of S. aureus examined, 3, 12 and 9 strains clotted rabbit, human and bovine plasmas respectively after 1 h incubation at 37°C. After 4 h, more strains coagulated human and bovine plasmas more than other plasmas whilst after 6 h, most strains coagulated human plasma. Most caprine strains of coagulase-positive staphylococci that coagulated human plasma also coagulated rabbit and/or horse plasma. None of the strains coagulated turkey and pig plasmas but 3 strains coagulated sheep plasma (Tables 4.1, 7b). All the 3 strains of Staphylococcus aureus isolated from giant rats (Cricetomys gambianus) produced coagulase after 24 h incubation at 37°C.

Staphylokinase: Whilst the Staphylococcus intermedius strain isolated from humans did not produce kinase, all the Staphylococcus aureus strains did produce it (Table 4.2).

Only 2 caprine strains of Staphylococcus aureus (UI 166 and UI 118) produced staphylokinase. However, whilst none of the coagulase-negative staphylococci isolated from humans produced staphylokinase, one strain of Staphylococcus sciuri and a strain of Staphylococcus lentus isolated from goats had strong kinase activities (Table 4.3b). The Staphylococcus aureus strains isolated from dogs had strong kinase activities whilst none of the Staphylococcus intermedius strains produced kinase (Table 4.4).

Proteinase: Whilst 11 of the 13 coagulase-positive staphylococci strains of caprine origin produced strong proteinase activities after 48 h incubation, only 4 of the 8 coagulase-positive staphylococci isolated from humans did so. Three of the 4 Staphylococcus intermedius strains of canine origin also produced proteinase (Table 4.4). However variable results were obtained with coagulase-negative staphylococcal strains isolated from humans and animals (Table 4.3b, Appendix 2.D).

Deoxyribonuclease production: Coagulase-positive staphylococci isolated from both humans and goats had

strong deoxyribonuclease activities. All the Staphylococcus aureus and Staphylococcus intermedius strains of canine origin also produced deoxyribonuclease (Table 4.4). Weak deoxyribonuclease activities were however found amongst some coagulase-negative staphylococci. Of the 79 Staphylococcus sciuri strains isolated from goats and sheep, 38 (48.1 per cent) produced weak deoxyribonuclease reactions. However, the 2 strains of Staphylococcus hyicus subsp. hyicus isolated from goats had strong deoxyribonuclease activities (Table 4.3b).

Thermostable nuclease: All the Staphylococcus intermedius and Staphylococcus aureus strains isolated from humans produced very strong thermostable nuclease. Identical strong reactions were obtained with caprine strains of Staphylococcus aureus (Fig. 4.1). Of the 31 Staphylococcus lentus strains isolated from sheep and goats, 2 also produced thermostable nuclease. The Staphylococcus hyicus subsp. hyicus strain of caprine origin also produced thermostable nuclease.

Phosphatase: Staphylococcus aureus, Staphylococcus intermedius strains isolated from humans and dogs produced

phosphatase within 48 h. The same reactions were noticed amongst caprine strains of Staphylococcus aureus. Varied results were however obtained with coagulase-negative staphylococcal strains isolated from goats and sheep (Table 4.3b).

Penicillinase: The two methods employed produced almost similar results. Penicillinase production was common amongst most of the strains resistant to penicillin although some strains gave relatively delayed results with the use of chromogenic cephalosporin. Three of the 4 strains of Staphylococcus intermedius of canine origin produced penicillinase (Table 4.4). Figure 4.2 shows penicillinase production by some caprine strains of Staphylococcus aureus.

Hyaluronidase: All the strains of Staphylococcus aureus isolated from humans and animals produced hyaluronidase. None of the strains of Staphylococcus intermedius or coagulase-negative staphylococci of human and animal origins produced hyaluronidase.

Oxidase: Whilst all coagulase-positive staphylococci isolated from man and animals gave oxidase-negative

reactions, all the Staphylococcus sciuri and Staphylococcus lentus strains isolated from humans, sheep, goats and other animals were found to be oxidase positive. The degree of positivity however varied from strain to strain (Table 4.3b).

Nitrate reduction: Most of the coagulase-positive and coagulase-negative staphylococcal strains isolated from humans and animals reduced nitrates. However only 2 of the 8 strains of Staphylococcus saprophyticus isolated from goats and sheep reduced nitrates but all the 2 Staphylococcus hyicus strains isolated from mastitic bovine milk produced nitratase (Appendix 2.E).

Urease: Staphylococcus aureus and Staphylococcus intermedius strains isolated from man and animals produced strong urease reactions which were noticeable between 1 to 7 days of incubation at 37°C. However, of the 79 strains of Staphylococcus sciuri isolated from goats and sheep, only 4 (5.1 per cent) produced urease within 7 days whilst none of the 31 Staphylococcus lentus strains produced the enzyme (Table 4.3b). The Staphylococcus sciuri strain isolated from an antelope however

produced urease (Appendix 2.E).

Aesculin hydrolysis: Variable results were found amongst the coagulase-positive staphylococci isolated from humans and animals. Six of the 8 Staphylococcus aureus strains isolated from humans gave weakly positive reactions after 24 h and only 5 of the 13 caprine strains of Staphylococcus aureus produced strong reactions after 48 h. All the 31 Staphylococcus lentus and 76 of the 79 Staphylococcus sciuri strains of caprine and ovine origins produced intense aesculin activities within 48 h.

Tween 80 hydrolysis: Whilst 6 of the 8 strains of Staphylococcus aureus of human origin hydrolysed Tween 80 after 2 days, only 6 of the 13 caprine strains of Staphylococcus aureus possessed this property. Variable results were seen amongst Staphylococcus intermedius strains isolated from dogs. The Staphylococcus intermedius strain isolated from humans failed to hydrolyse Tween 80. However very strong reactions were found amongst Staphylococcus aureus strains isolated from poultry. All the 3 strains of Staphylococcus aureus isolated from giant rats (Cricetomys gambianus) also hydrolysed Tween 80

(Appendix 2.B). Most of the coagulase-negative staphylococcal strains isolated from humans and animals failed to hydrolyse Tween 80 (Table 4.3b).

Egg Yolk reaction: Of the 7 strains of Staphylococcus aureus of human origin, 6 produced intense clearing with precipitate on Baird-Parker's medium (Fig. 4.3). The Staphylococcus intermedius strain isolated from humans and dogs also produced strong clearing with precipitate after 48 h. None of the Staphylococcus sciuri and Staphylococcus lentus stains from goats and sheep produced any visible reaction on Baird-Parker's medium. However, all the 6 Staphylococcus cohnii strains isolated from goats produced clearing on Baird-Parker's medium (Table 4.3b).

Carbohydrate fermentation: All the Staphylococcus aureus and Staphylococcus intermedius strains isolated from humans and animals produced acid from a variety of carbohydrates (Fig. 4.4). Staphylococcus aureus fermented maltose, D-(+)-trehalose, D-(+)-mannose, D-(+)-turanose, D-(+)-mannitol and sucrose. The Staphylococcus intermedius strain isolated from humans did not produce acid from

D-(+)-mannitol and D-(+)-turanose but all the strains isolated from dogs produced acid from D-(+)-mannitol but gave variable reactions with D-(+)-turanose. Similar results were obtained in the purple agar plate and the phenol red broth methods. Acid production from raffinose, melibiose and D-(+)-xylose was not common amongst coagulase-positive staphylococcal strains from humans and animals (Table 4.5). However, a variety of coagulase-negative staphylococci produced acid from these carbohydrates particularly Staphylococcus lentus and Staphylococcus gallinarum (Table 4.6b). Staphylococcus lentus strains of caprine origin, appeared more biochemically active than Staphylococcus sciuri. Almost all the coagulase-negative staphylococci examined produced acid aerobically from maltose, fructose and D-(-)-ribose. All the strains of Staphylococcus sciuri isolated from various hosts produced acid from D-(+)-cellibiose, D-(+)-mannose and D-(-)-ribose whilst the strains of Staphylococcus lentus produced acid from fructose, maltose, raffinose, D-(+)-trehalose, D-(+)-turanose and D-(+)-xylose. Acid production from fructose, maltose, D-(+)-mannose, D-(-)-ribose, sucrose and D-(+)-trehalose were commonly found

amongst all the strains of Staphylococcus gallinarum examined. These biochemical reactions are in agreement with the novel descriptions of the organism (Devriese et al., 1983). The carbohydrate reactions of coagulase-negative staphylococci isolated from goats and sheep had little or no differences (Table 4.6b). However, one caprine strain of coagulase-positive staphylococci produced acid from raffinose and xylose (Table 4.6a).

Acetymethylcarbinol (acetoin): Only 3 of the 9 coagulase-positive staphylococcal strains isolated from humans demonstrated intense acetylmethylcarbinol production (Table 4.5). Of the 13 caprine strains of coagulase-positive staphylococci examined, 10 strains showed strongly positive reactions whilst others were either negative or produced weakly-positive reactions (Table 4.6a). All the Staphylococcus intermedius strains of canine origin failed to produce acetylmethylcarbinol (Appendix 2.C). Of the 79 Staphylococcus sciuri strains of caprine and ovine origins only 20 (25.3 per cent) produced acetylmethylcarbinol whilst none of the Staphylococcus xylosus and Staphylococcus cohnii produced

it. However the strain of Staphylococcus hyicus subsp. hyicus of caprine origin produced acetylmethylcarbinol (Table 4.6b).

Crystal violet test: Whilst the strains of Staphylococcus aureus of human origin were of human biotype (Table 4.7a), caprine strains of coagulase-positive staphylococci belonged to the ovine biotype (Table 4.7b). Crystal violet type C was however common to both coagulase-positive staphylococci of human and ovine biotypes. Biotype A was prevalent amongst Staphylococcus aureus of human origin and biotype C amongst caprine strains of coagulase-positive staphylococci (Table 4.7b).

DISCUSSION

Until, recently, staphylococci isolated from man and animal sources have been subdivided taxonomically into two species: Staphylococcus aureus and Staphylococcus epidermidis. Staphylococcus aureus was further subdivided into serotypes or phage types and Staphylococcus epidermidis into biotypes (Baird-Parker, 1963) or occasionally phage types (Verhoef, Boven and Winkler, 1971).

Furthermore, coagulase-positive staphylococci were previously regarded as a homogenous group hence the terms human and animal S. aureus have been used to indicate the host from which a culture was isolated. However, based on some phenotypic and genetic characteristics, S. intermedius (Hajek, 1976), S. hyicus subsp. hyicus (Devriese et al., 1978) are now regarded as distinct from S. aureus. Generally, the differentiation of S. aureus from other species of staphylococci is based on the presence of free or bound coagulase (Kloos and Smith, 1980). Isolates that fail to give a positive slide test for clumping factor require testing for free coagulase by the tube test. Although this procedure takes up to 4h and is subject to significant variations in performance and interpretation (Devriese, In press), it is often used in most laboratories for the presumptive identification of pathogenic S. aureus. Testing for free coagulase is also often subject to uncertainly as false-positive results may be associated with some plasmas. False-negative or false-positive reactions may also occur if the tests are incubated for 24 h (Sperber and Tatini,

1975). Thus Rayman, Park, Philpott and Todd (1975), Zarzour and Belle (1978) suggested that thermostable nuclease test be performed on all cultures with doubtful coagulase reactions before classifying them as S. aureus. In view of the aforementioned inherent faults in coagulase test, Essers and Radebold (1980), Myrick and Ellner (1982) suggested latex slide agglutination test to replace the slide and tube coagulase tests for identifying S. aureus strains met in clinical practice. Various methods for performing coagulase test have been proposed. Alami and Kelly (1959) suggested the use of an albumin-plasma-fibrinogen soft agar for the simultaneous detection of staphylococcal clumping factor and free coagulase activities. The authors found that compact colonies in the fibrinogen soft agar indicated clumping factor whilst diffuse colonies in the albumin-plasma-fibrinogen soft agar showed the presence of free coagulase. Later the soft agar method was modified (Alami and Kelly, 1968) by the incorporation of phenol red-mannitol into the fibrinogen-plasma soft agar and the authors were able to demonstrate visually, mannitol fermentation, free

coagulase and clumping factor production. With the soft agar method not widely accepted, Orth, Anderson and Montgomery (1969) suggested the addition of a trypsin inhibitor to the coagulase test system thereby making the tube test more sensitive as the plasmin in the blood serum becomes inactivated on the addition of trypsin and thus in turn increases the rate of clotting. However, since these aforementioned techniques have not gained world-wide acceptance, the conventional methods for coagulase test were adopted in this study. Despite the convenience of coagulase test for routine diagnostic purposes, Heczko, Wegrzynowicz, Bulanda, Jeljaszewich and Pulverer (1981), doubted the usefullness of the procedure for the classification of staphylococci. However, coagulation of human and bovine plasma continues to be used for biotyping S. aureus strains (Hajek and Marsalek, 1971; Witte, Hummel, Meyer, Exner and Wundreck, 1977; Devriese, In press). Meyer (1966a) even suggested that ability to coagulate bovine plasma within a short period of time was characteristic of bovine strains of S. aureus. The coagulation of some plasmas were employed

along with other tests in the classification of S.aureus into biotypes (Table 4.7b). Furthermore, coagulation of rabbit, human, bovine and horse plasmas at different times was found helpful in the biotyping procedures (Table 4.1). In the study of the characteristics of 360 strains of S. aureus isolated from slaughtered fowls in Bulgaria, Bajljsov, Sachariev and Georgiev (1974) found that although the strains did not coagulate sheep or bovine plasma, they showed some species specificity as the strains coagulated rabbit plasma. This is also true of some caprine strains of S. aureus examined herein (Table 4.1,7b). The strains did not coagulate ovine plasma but coagulated rabbit or human plasma. The negative reaction in rabbit plasma coagulase test produced by the caprine strain of S. hyicus subsp. hyicus after 4 h (Table 4.1) is in agreement with the findings of Devriese (1977) who noted that S. hyicus strains were negative after 4 h in rabbit plasma and some only produced firm clots after 24 h. However, the S. hyicus strain (UI 151) examined herein failed to coagulate pig plasma. This is in contracdiction to the suggestion of Amtsberg (1979) who noted that

S. hyicus subsp. hyicus strains were usually coagulase-positive in pig plasma.

Whilst no single test could at present be regarded as a distinctive criterion for the recognition of a potentially pathogenic staphylococcus, all the coagulase-positive staphylococci isolated from humans produced deoxyribonuclease (DNAse) (Table 4.2). However, such a good correlation was not found amongst caprine strains of S. aureus and the canine strains of S. intermedius studied (Tables 4.3a, 4). S. aureus biotype B strains from poultry are generally associated with weak DNase positive results (Devriese and Van de Kerckhove, 1979). Takeuchi and Suto (1973) noted that weakly-reacting or deoxyribonuclease-negative S. aureus strains of poultry do occur frequently. However, the two S. aureus strains of poultry origin examined herein were found to have produced strong reactions in heat-labile and heat-stable deoxyribonuclease tests. Thus there are conflicting results about the DNase activity of poultry strains of S. aureus. Whilst Devriese, Devos, Beumer and Maes (1972) found the strains of S. aureus tested positive for DNase

after 3 days incubation, Takeuchi and Seto (1973) reported negative results in the conventional 24 h test. However, when Devriese and Oeding (1976) used the technique of Lachica, Hoeprich and Genigeorgis (1971), they also obtained negative results in some of their strains. Gibbs, Patterson and Harvey (1978a) found that their poultry strains of S. aureus gave weak or poor reactions in the plate test but the strains were positive when tested for heat-stable DNase activity using supernatants from dialysis sac cultures. Some other staphylococcal strains are also known to give weakly-positive or negative DNase reactions. Hajek (1976) found that S. intermedius strains isolated from mink and hares were weakly DNase positive. However, the S. intermedius strains of canine origin examined herein produced strongly positive $\frac{DNase}{+}$ results in the plate test (Table 4.4). S. hyicus subsp. chromogenes (Devriese and Van de Kerchkhove, 1979) are also known to give weakly positive results in the plate culture test and the heat-stable DNse test. Ideally the plate culture method for DNase cannot differentiate between the effects of weakly-

positive heat-stable DNase-producing strains of staphylococci and relatively poorly visible zones which may appear when a variety of coagulase-negative strains are examined. However, since Devriese and Van de Kerckhove (1979) obtained paralleled results with staphylococcal strains that gave very large zones in the heat-resistant DNase tests and also very strong reactions in the plate culture tests, the authors therefore suggested that the reactions in the plate culture tests of the strains examined were due to the same enzymes as in the heat-resistant DNase tests. Hajek and Marsalek (1975) in their evaluation of classifactory criteria for staphylococci noted that ovine S. aureus of biotype C were commonly associated with the production of both deoxyribonuclease and thermostable nuclease. The authors also noted that human strains of S. aureus produced deoxyribonuclease and thermostable nuclease. These findings are in agreement with the results obtained for both ovine and human strains of S. aureus (Table 4.2).

In the descriptions of new coagulase-negative staphylococci, S. simulans, S. capitis and S. sciuri

alongst with some members of S. xylosus were found to possess weak or moderate deoxyribonuclease activity (Kloos and Schleifer, 1975a; Schleifer and Kloos, 1975a, Kloos et al., 1976). However, these authors did not report on the thermostable nuclease activity of the aforementioned strains. In this present study, 2 S. lentus strains of caprine origin and one S. sciuri strain of ovine origin were found to have produced thermostable nuclease (Table 4.3b). These findings are thus in agreement with the suggestions of Gramoli and Wilkinson (1978), Devriese and Hajek (1980) on the production of thermostable nuclease by staphylococcal strains other than S. aureus.

The ability of pathogenic staphylococci to form hyaluronidase correlates with coagulase production (Schmidt, 1965). This is true of the caprine strains of S. aureus examined herein. Of the 13 strains studied, 12 produced hyaluronidase. All the 8 S. aureus strains of human origin also produced both coagulase and hyaluronidase. However, none of the S. intermedius strains isolated from humans and animals produced hyaluronidase although they reacted in rabbit plasma. Production of

hyaluronidase can therefore be assumed to be a reasonably useful index for differentiating S. aureus and S. intermedius. Rajchert-Trzpil (1961) correlated hyaluronidase production with that of phosphatase and coagulase. The author found that coagulase-negative, phosphatase-positive strains produced hyaluronidase but coagulase-negative, phosphatase-negative strains did not show this peculiarity. However, in this present study, none of the coagulase-negative staphylococci isolated from humans and animals produced hyaluronidase but production of phosphatase was found amongst coagulase-positive and coagulase-negative staphylococcal strains examined.

In the study of the production of opacity in egg-yolk broth by staphylococci from various sources, Alder, Gillespie and Herdan (1953) found that egg-yolk activity occurred more frequently with pathogenic strains of S. aureus. Koshitalo and Milling (1968) however noted that the egg-yolk reaction did not differentiate between coagulase-positive and coagulase-negative staphylococci as they found that about 90 per cent of 150 egg-yolk negative isolates they examined were coagulase-positive staphylococci.

In this present study, differentiation between coagulase-positive and coagulase-negative staphylococci isolated from humans and animals on the basis of egg-yolk activities was impracticable as strong egg-yolk activities were noticeable amongst some coagulase-negative staphylococci particularly S. xylosus and S. hyicus subsp. hyicus (Table 4.3b; Appendix 2.E). The egg-yolk characteristics of S. hyicus subsp. hyicus on Baird-Parker's medium examined herein are similar to the findings of Devriese and Hajek (1980). These authors noted that colonies of S. hyicus subsp. hyicus on egg-yolk agar are characterized by zones of incomplete clearing without surface-or subsurface-precipitation in the areas immediately surrounding the colonies. Hajek and Marsalek (1969) in their study of the characteristics of bovine strains of S. aureus found that 77.2 per cent of the strains isolated from bovine mastitis were egg-yolk positive whilst only 21.3 per cent of the strains from bovine nasal carriers produced opacity in egg-yolk. These findings were in agreement with those of Reid and Wilson (1959) who earlier on noted that 73.3 per cent of the strains of staphylococci isolated

from cases of bovine mastitis were lipolytic. Jonsson and Holmberg (1981) found that of the 52 strains from chronic mastitis, 31 (60 per cent) and 9 (17 per cent) produced positive egg-yolk reaction and beta-lactamase (penicillinase) respectively. Although penicillinase can be assayed by several methods (Citri and Pollock, 1966), some factors which border on contaminating enzymes, location of the enzyme, loss of activity by solution, inactivation by reagents used in the assay, proper controls and choice of appropriate substrates may affect the results of assays for penicillinase production. Thus Cooper, Brown and Versey (1966) proposed an iodometric method for the detection of staphylococcal penicillinase. Workman and Farrar (1970) modified the iodometric assay by streaking pure cultures of S. aureus onto nutrient agar plates containing 0.2 per cent soluble starch. After overnight incubation, the plates were flooded with 3 ml of freshly prepared phosphate-buffered saline (pH 6.4) containing iodine (3 mg/ml), potassium iodide (15 mg/ml) and aqueous penicillin G (50 mg/ µl). Penicillinase-positive colonies produced a characteristic

decolourization within 30 min. Although the latter method was not employed in this present study, the use of chromogenic cephalosporin and the method recommended by Hodge, Ciak and Tramont (1978) were found adequate. However, the production of penicillinase by 3 of the 4 S. intermedius strains of canine origin (Table 4.4) may not be unconnected with previous exposure to penicillin. On the other hand, the indiscriminate use of penicillins may however be responsible for the high rate of penicillinase production by the strains of S. aureus isolated from humans (Table 4.2).

In the estimation of character parameters for differentiating coagulase-negative Staphylococcus species, Kloos, Schleifer and Noble (1975) found that nitrate test was useful in separating nitrate-negative species S. cohnii and S. saprophyticus and most strains of S. warneri from other staphylococci. However, in this present study, 4 of the 6 S. cohnii and 2 of the 8 S. saprophyticus strains isolated from goats and sheep reduced nitrates. Also, in the characterization of S. aureus strains isolated from man and animals, Hajek

and Marsalek (1975) found that all of the 50 S. aureus strains isolated from humans produced acetylmethy-carbinol. This is in agreement with the characteristics of the S. aureus strains of human origin examined herein although weakly-positive reactions were found amongst the strains. However, intense acetylmethylcarbinol production was commonly associated with some of the 20 strains of S. sciuri isolated from goats in this present study.

At present, only S. aureus strains are generally biotyped (Hajek and Marsalek, 1971; Witte, Hummel, Meyer, Exner and Wundrach, 1977; Devriese, In press). Thus after extensive studies of the characteristics of S.aureus from various hosts, Oeding, Marandon, Hajek and Marsalek (1970), Oeding et al. (1976) found that one biotype of S. aureus was often predominant on each different host species. In this present study, whilst some caprine strains of S. aureus were found to belong to the human biotype, two strains of S. aureus isolated from humans appeared to belong to biotype bf of Hajek and Marsalek (1976). Adekeye (1981) in identifying S. aureus of human biotype of animal origin chose agglutination in serum 17H,

fibrinolysin production, negative crystal violet test, phage typing and coagulation of human plasma. A relatively more comprehensive biotyping scheme has now been proposed. Devriese (In press) in his simplified biotyping scheme suggested few phenotypic markers such as beta-hemolysin, staphylokinase, coagulation of bovine plasma and crystal violet reaction. The scheme which excludes the application of phage typing recognises only four biotypes and a non-host-specific biotype. Devriese (In press) has also concluded that the coagulation of bovine plasma within 6 h was characteristic of only S. aureus strains of bovine, ovine or caprine origins and incidentally cow, sheep and goats' strains of S. aureus belong to biotype C (Hajek and Marsalek, 1971; Adegoke et al., 1983a). Hajek and Marsalek (1973) in the study of the characteristics of 655 animal S. aureus strains isolated in Czechoslovakia, found that only 8.6 per cent were of human biotype. This is in agreement with the findings of Adegoke et al. (1983a) who found that only one of the 11 caprine S.aureus strains showed the typical biotype characteristics of human strain of S. aureus. Thus it is apparent that

human biotype S. aureus strains occur in many animals hosts. In the United States of America, a human epidemic strain of S. aureus produced persistent furunculosis in a herd of dairy cows attended by persons harbouring such staphylococci in their anterior nares (Zinn, Anderson and Skaggs, 1961). Furthermore, Devriese (1980), in a comparative study of 53 strains of S. aureus of porcine origin isolated in various farms in Belgium between 1979 and 1980 found that 6 of the strains belonged to the human biotype and 2 belonged to bovine biotype. In dairy industry, economic loss due to mastitis is usually enormous. Mastitis caused predominantly by S. aureus in cattle, goats and sheep can either be chronic or acute. Staphylococcal mastitis may vary in intensity from inapparent infection which is only detected by culturing formilk to fulminating infection which destroys the infected quarter.

Whilst animal strains of S. aureus may differ from those of human strains by their ability to produce beta-haemolysin, lack of fibrinolysin, their antigenic structure and sensitivity to bacteriophages, some

differences with regards to the crystal-violet type and the specificity of phage reactions have been found to exist between S. aureus of bovine and ovine origins which both belong to biotype C (Witte, Grigorova, Bajljosov, Hummel and Korukov, 1978). Generally, ovine strains of S. aureus are known to form a group of staphylococci characterised by their belonging to biotype C, sensitivity to phage 78 and ability to produce staphylococcal enterotoxin C (Hajek, 1978; Guittierrez, Menes, Garcia, Moreno and Bergdoll, 1982). Occasionally, S. aureus of human biotype A and canine biotype E have been isolated from sheep. In this present study, the strains of S. aureus of ovine origin examined was found to be of human biotype A thus suggesting possible transfer of staphylococci between the various hosts coming in contact with the animal. Thus the suggestions of Blackmore and Francis (1970) that animal staphylococcal infections could originate from human carriers may be true of this ovine strain of human S. aureus biotype.

Hajsig (1973), Devriese, Godard, Okerman and Renault (1981) in their studies of S. aureus strains isolated

from rabbits found that they possessed some characteristics similar to those of human biotypes whilst some strains could not be associated with a specific host species. The S. aureus strain isolated from a rabbit in this study, could not be assigned to any biotype but it gave a type C crystal violet reaction. However, all the 3 S. aureus strains isolated from giant rats (Cricetomys gambianus) were found to belong to the ovine biotype (Appendix 2.B).

In the old classification system of staphylococci, mannitol fermentation was considered an important test (Blair, 1938; Allen and Fallows, 1940; Elek, 1959). It is now known that several coagulase-negative staphylococci isolated from man and animals do produce acid from mannitol. Choudhuri and Aikat (1968) in the study of 211 strains of coagulase-negative staphylococci found that 74 per cent fermented mannitol. On the other hand, Panda, Mohanty, Nanda and Niak (1969) found that 16 per cent of coagulase-negative staphylococci isolated from post-operative wounds and urinary tract infections fermented mannitol. Also, in the examination of 193

strains of coagulase-negative staphylococci isolated from staff and patients in a hospital environment, Reiss, Lachowicz and Lacki (1969) found that 28 (14.5 per cent) of the strains fermented mannitol. In this present study, mannitol fermentation was common amongst several strains of coagulase-negative staphylococci particularly S. sciuri, S. lentus, S. xylosus and S. gallinarum isolated from various hosts (Tables 4.3a, 3b, 5).

how many?

What is the clinical significance of this finding?

Are they pathogens?

Fig. 41: **Thermostable nuclease production by some staphylococcal strains.** Pinkish colouration: positive reaction.

Fig. 4.2: Penicillinase production by some strains of S. aureus. Growths reaching or close to penicillin disc: penicillinase producers.

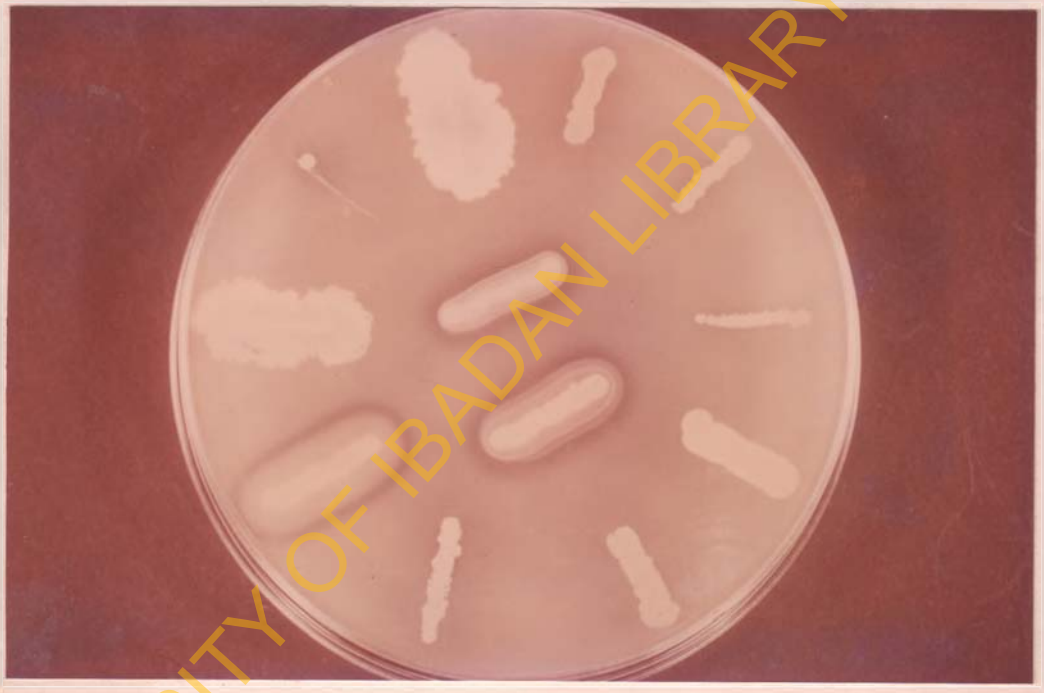


Fig. 4.3: Egg-yolk reactions of some staphylococcal strains. Central strains: clearing and precipitation.

Fig. 4.4: Carbohydrate fermentation by some staphylococci.

YELLOW COLOURATION: Positive)
RED COLOURATION : Negative) reactions.

Table 4.1 Coagulation of plasmas (tube method) at different times by coagulase-positive staphylococci isolated from goats

STRAIN NO.	PLASMA	COAGULATION																							
		RABBIT				HUMAN				BOVINE				TURKEY				HORSE				PIG			
		1*	4	6	24	1	4	6	24	1	4	6	24	1	4	6	24	1	4	6	24	1	4	6	24
UI 27		+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-
UI 96		+	+	+	+	NT				+	+	+	+			NT				NT				NT	
UI 118		-	-	-	-	-	+	+	+	-	-	-	+	-	-	-	-	-	-	-	+			-	
UI 145		+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
UI 150		-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	±	+	+	-	-	-	-
UI 151		-	-	-	+	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-
UI 153		-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-
UI 154		-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	-	-	-	-
UI 160		-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
UI 161		-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-
UI 166		+	+	+	+	+	+	+	+	-	-	-				NT				NT				NT	
UI 327		-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-
UI 331		-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-
UI 334		-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	-	-	-	-
S. aureus ATCC 25923		+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-

*hour(s); NT - not tested; ± : Weak Reactions,
+: Strong reactions; - : Negative reactions.

Table 4.2 Enzymatic activities of coagulase-positive staphylococci isolated from humans

Strain No.	1 4 24h	Coagulase	Staphylokinase	Proteinase 24h	48h	Fibrin protease	Desoxyribonuclease	Thermostable nuclease	Phosphatase 24h	48h	Hyaluronidase	Oxidase	Nitrate reduction	Strong Urease activity Aesculin hydrolysis 24h	48h	Tween 80 hydrolysis 24h	48h	Penicillinase production	Egg yolk reaction: clearing with precipitate	STRAIN IDENTIFICATION
M221	+ + +	+	+	−	−	−	+	+	+	+	+	−	+	+	+	−	+	+	+	S. aureus
M222	+ + +	+	+	±	+	−	+	+	+	+	+	−	+	+	+	−	+	+	+	S. aureus
M223	− + +	+	+	+	+	−	+	+	+	+	+	−	+	±	+	+	+	+	+	S. aureus
M224	+ + +	+	+	−	−	−	+	+	+	+	+	−	+	±	+	−	+	+	+	S. aureus
M225	+ + +	+	+	−	−	−	+	+	+	+	+	−	+	±	+	−	+	−	+	S. aureus
M226	+ + +	+	+	−	−	+	+	+	+	+	+	−	+	±	+	−	+	+	+	S. aureus
M232	− + +	+	+	+	+	−	+	+	+	+	+	−	+	±	+	−	+	+	+	S. aureus
M253	+ + +	+	−	+	+	−	+	+	+	+	−	−	+	−	−	−	−	−	+	S. intermedius
M337	+ + +	+	+	−	−	−	+	+	+	+	+	−	+	±	+	−	+	+	+	S. aureus
S. aureus (ATCC 25923)	+ + +	+	+	±	±	−	+	+	+	+	+	−	+	−	−	+	+	NT	+	

NT - Not tested; ± = weak reaction; + = Strong reactions

Table 4.3a. Enzymatic activities of caprine strains of coagulase-positive staphylococci

	UI27	UI96	UI118	UI145	UI150	UI153	UI154	UI160	UI161	UI166	UI327	UI331	UI334	S. aureus ATCC 25923	S. hyicus subsp. hyicus NCTC 10350
Coagulase (Rabbit plasma)	+	+	-	+	+	+	+	+	+	+	+	+	+	+	NT
Clumping factor	+	+	+	+	-	+	+	+	+	+	+	-	+	+	NT
Deoxyribonuclease	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thermostable nuclease	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phosphatase (48h)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Staphylokinase (48h)	-	-	+	-	-	-	-	-	-	+	-	-	-	-	+
Fibrin protease (48h)	-	-	-	±	-	-	-	-	-	-	-	-	-	-	-
Strong hyaluronidase activity	+	+	+	+	+	+	+	±	±	+	+	+	+	+	±
Proteinase (48h)	+	-	±	+	+	+	+	+	+	+	+	+	±	±	+
Tween 80 hydrolysis (48h)	-	+	+	+	+	+	+	-	-	+	-	-	+	+	+
Strong urease production (24h)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aesculin hydrolysis (48h)	+	±	±	+	-	+	+	±	±	±	+	±	-	-	-
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Egg yolk reaction	+	+	+	+	+	+	±	±	±	+	+	-	+	+	-
Penicillinase production	-	+	+	-	-	-	-	-	-	+	+	-	-	NT	NT

NT: Not tested; ± : weak reactions; + : strong reactions; - : negative reactions.

Table 4.3b. Enzymatic activities of coagulase-negative staphylococci isolated from goats and sheep

	Deoxyribonuclease	Thermostable nuclease	Phosphatase 24 h	Phosphatase 48 h	Proteinase 24h	Staphylokinase	Fibrin protease	Urease production (7 days)	Aesculin hydrolysis (48 h)	Egg yolk reaction	Oxidase test	Nitrate reduction	Tween 80 hydrolysis (48 h)	Acetylmethylcarbinol	Total number of strains tested	Number isolated from goats	Number isolated from sheep
S. sciuri	38*(14)	(1)	51[a](26)	61(32)	76(40)	0	53(19)	4(3)	76(39)	0	79(42)	79(42)	0	20(5)	79	42	37
S. lentus	13*(1)	2(0)	27(14)	29(15)	29(14)	(1)	21(8)	0	31(15)	0	31(15)	31(15)	0	5(2)	31	15	16
S. xylosus	(1)*	0	15(10)	17(12)	2*(0)	0	0	16(10)	13(10)	12(10)	17(12)	17(12)	9(7)	0	21	15	6
S. cohnii	0	0	(4)	(5)	(1)	0	0	(6)	(2)	(6)	0	(4)	(3)	0	6	6	0
S. saprophyticus	0	0	(1)	2(1)	1(0)	0	0	7(1)	(1)	4(1)	0	(2)	1(0)	3(1)	8	5	3
S. gallinarum	(1)*	0	(7)	(7)	(2)	0	(1)	(8)	(5)	(1)	0	(8)	(3)	(2)	8	8	0
S. epidermidis	0	0	(1)	(1)	(1)*	0	0	(1)	0	(1)	0	(1)	0	(1)	1	1	0
S. hyicus subsp. hyicus	(2)	(2)	(2)	(2)	(2)*	0	(2)	(2)	0	(2)	0	(2)	(2)	(2)	2	2	0
S. hyicus subsp. chromogenes	(1)*	0	(1)	(1)	(1)	0	(1)	(1)	(1)	(1)	0	(1)	(1)*	(1)	1	1	0
S. aureus ATCC 25923	+	+	+	+	+	-	-	+	-	+	-	+	+	+			
S. lentus ATCC 29070	*	-	-	-	-	+	-	-	+	-	+	+	-	-			

a : Number giving positive results
( ): Number of caprine strains.

* : weak reaction

Table 4.4 Enzymatic activities of S. intermedius and S. aureus strains of canine origin

	D238	D240	D241	D242	D243	Staphylococcus aureus ATCC 25923
Clumping factor	+	-	-	-	-	+
Coagulase	+	+	+	+	+	+
Deoxyribonuclease	+	+	+	+	+	+
Thermostable nuclease	+	+	NT	NT	:	+
Phosphatase (48h)	+	+	+	+	+	+
Staphylokinase (48h)	+	-	-	-	-	-
Fibrin protease (48h)	-	±	+	-	-	-
Hyaluronidase	+	-	-	-	-	+
Proteinase (48h)	+	+	±	+	+	±
Tween 80 hydrolysis (48h)	+	±	+	+	-	+
Urease production (48h)	+	+	+	+	+	+
Aesculin hydrolysis (48h)	±	-	±	-	-	-
Nitrate reduction	+	+	+	+	+	+
Egg yolk reaction	+	+	+	+	+	+
Penicillinase production	- (1)	- (2)	+ (2)	+ (2)	+ (2)	NT

(1) : S. aureus NT: Not tested
(2) : S. intermedius

Table 4.5 Biochemical characters of Staphylococcus aureus and Staphylococcus intermedius strains of human origin

	M_{221}	M_{222}	M_{223}	M_{224}	M_{225}	M_{226}	M_{232}	M_{253}*	M_{337}	S. aureus (ATCC 25923)
Maltose	+[a]	+	+	+	+	+	+	+	+	+ +
D-(+)-Trehalose	+	+	+	+	+	+	+	+	+	+ +
Melibiose	-	-	-	-	-	-	-	-	-	-
Raffinose	-	-	-	-	-	-	-	-	-	-
D-(+)-Cellibiose	-	-	-	-	-	-			-	-
D-(+)-Xylose	-	-	-	-	-	-	-	-	-	-
L-(+)-Arabinose	-	-	-	-	-	-	-	-	-	-
D-(+)-Mannose	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+
D-(+)-Turanose	+	+	+	+	+	+	+	-	+	+
Fructose	+	+	+	+	+	+	+	+	+	+
D-(-)-Ribose	+	+	-	+	-	+	-	+	-	+
D-(+)-Mannitol	+	+	+	+	+	+	+	-	+	+
Acetylmethylcarbinol (acetoin)	±	±	+	±	±	±	+	±	+	+

a: Aerobic acid production (phenol red broth)

• S. intermedius (Table 4.2)

± Weak reactions

Table 4.6a Variable biochemical reactions of coagulase-positive staphylococci isolated from goats

	Maltose	D-(+)-Trehalose	Melibiose	Raffinose	D-(+)-Cellobiose	D-(+)-Xylose	L-(+)-Arabinose	D-(+)-Mannose	Sucrose	D-(+)-Turanose	Fructose	D-(-)-Ribose	D-(+)-Mannitol	Acetymethylcarbinol
UI 27	+	+	-	-	-	-	-	+	+	+	+	-	+	+
UI 96	+	-	-	+	±	+	-	+	+	+	+	-	+	-
UI 118	+	+	-	-	-	-	-	+	+	+	+	-	+	+
UI 145	+	+	-	-	-	-	-	+	+	+	+	±	+	+
UI 150	-	+	-	-	-	-	-	+	+	-	+	-	-	-
UI 153	+	+	-	-	-	-	-	+	+	+	+	±	+	+
UI 154	+	+	-	-	+	-	-	+	+	+	+	-	+	+
UI 160	+	+	-	-	-	-	-	+	+	+	+	-	+	+
UI 161	+	+	-	-	-	-	-	+	+	+	+	-	+	+
UI 166	+	+	-	-	-	-	-	+	+	+	+	-	+	+
UI 327	+	+	-	-	-	-	-	+	+	+	+	-	+	+
UI 331	+	+	-	-	-	-	-	+	+	+	+	-	+	+
UI 334	+	+	-	-	-	-	-	+	+	+	+	-	+	-
S. aureus ATCC 25923	+	+	-	-	-	-	-	+	+	+	+	+	+	+
S. hyicus NTCC 10350	-	+	-	-	-	-	-	+	+	+	+	+	-	-

+: Positive reactions; ±: Weak reactions; - : Negative reactions.

Table 4.6b Carbohydrate reactions of coagulase-negative staphylococci isolated from goats and sheep*

	S. sciuri[1] (79)	S. lentus (31)	S. xylosus (21)	S. cohnii (6)	S. saprophyticus (8)	S. gallinarum (8)	S. epidermidis (7)	S. hyicus subsp. hyicus (2)	S. hyicus subsp. chromogenes (1)	S. aureus ATCC 25923	S. sciuri ATCC 29062	S. gallinarum CCM 3572	S. hyicus subsp. hyicus NCTC 10350
L-(+)-Arabinose	29	16	21	0	0	7	0	0	0	-	+[2]	+	-
D-(+)-Cellibiose	79	30	1	0	0	6	0	2	2	-	+	+	-
Fructose	77	31	21	6	8	8	1	2	1	+	+	+	+
Maltose	77	31	20	6	6	8	1	0	1	+	+	+	-
D-Mannitol	78	30	21	5	7	7	0	0	1	+	+	+	-
D-(+)-Mannose	79	30	19	6	5	8	0	2	1	+	+	+	+
Melibiose	0	27	3	0	0	5	0	0	0	-	-	+	-
Raffinose	0	31	3	0	0	7	0	0	0	-	-	+	-
D-(-)-Ribose	79	30	18	4	4	8	0	2	1	+	+	-	+
Sucrose	78	30	17	0	8	8	1	2	1	+	+	+	+
D-(+)-Trehalose	77	31	21	6	8	8	0	2	1	+	+	-	+
D-(+)-Turanose	71	31	18	2	5	7	1	0	1	+	+	+	+
D-(+)-Xylose	52	31	8	0	0	7	0	0	0	-	-	+	-

* Table 3.1b shows the distribution of species

1 Number of strains examined

2 Aerobic acid production in phenol red broth (1 - 3 days)

Table 4.7a Biotypes of coagulase-positive staphylococci of human origin

Strain No.	B-haemolysin	Clumping factor reaction	Coagulation (6h) Bovine Plasma	Kinase	Crystal violet reaction	Biotype	Ecovar
M221	-	+	-	+	C	A	Human
M223	+	+	-	+	C	A	Human B-Haem +
M224	-	+	-	+	C	A	Human
M225	-	+	-	+	A	Not-typable	NHS3
M226	-	+	-	+	C	A	Human
M232	-	+	-	+	C	A	Human
M337	-	+	-	+	A	Not-typable	NHS3

NHS: Non host specific;

B-Haem + Beta-haemolysin positive

Table 4.7b. Biotypes of coagulase-positive staphylococci isolated from goats, sheep and giant rats (Cricetomys gambianus)

Strain No.	Host	haemo-lysin	Clumping factor reaction	Coagulation (6 h) Human	Bovine Plasma	Ovine	Kinase	Crystal violet reaction	Hajek's& Marsalek's biotype	Ecovar
UI 166	Goat	+	+	+	-	-	+++	C	A	Human (B-Haem +ve)
UI 161	"	+	+	+	+	+	-	C	C	Ovine
UI 160	"	+	+	+	+	-	-	C	C	Ovine
UI 154	"	+	+	+	+	-	-	C	C	Ovine
UI 151	"	-	-	-	+	-	-	C	NT	NT
UI 153	"	+	+	+	+	-	-	C	C	Ovine
UI 150	"	-	+	+	-	-	-	C	NT	NT
UI 327	"	+	+	+	+	+	-	C	C	Ovine
UI 145	"	+	+	+	-	-	-	C	NT	Mixed C (NHS_4)
UI 331	"	+	+	+	+	-	-	C	C	Ovine
UI 118	"	+	+	+	-	-	+++	A	NT	Mixed A (NHS_4)
UI 96	"	+	+	ND	+	ND	-	A	C	Bovine
UI 27	"	+	+	+	+	-	-	C	C	Ovine
UI 334	"	+	+	+	+	+	-	C	C	Ovine
SUI 116	Sheep	-	+	+	-	ND	+++	C	A	Human
UI 167	Giant Rat	+	+	ND	-	ND	-	C	C	Ovine
UI 168	"	+	+	ND	-	ND	-	C	C	Ovine
UI 169	"	+	+	ND	-	ND	-	C	C	Ovine

1: Witte et al. (1978); +: Distinct reaction; -: No reaction;
NT: Not typable; ND: Not done; NHS: Non host specific

CHAPTER 5

ANTIBIOTIC SUSCEPTIBILITY PATTERNS

INTRODUCTION

Generally, there are difference between staphylococci from lesions and those from carrier sites in the production of toxins and other extracellular products (Williams, 1963). Invariably, staphylococci continue to present challenges to physicians, veterinarians and epidemiologists alike as the relationships between these micro-organisms and their hosts have been greatly influenced by the introduction of antibiotics into medicine.

Several new antibiotics are now available for therapeutic use. Vancomycin, ristocetin and the cephalosporins are amongst the active antibiotics against staphylococci. Marked resistance to these antibiotics are not common. Although minor, well-localized superficial infections usually respond well to local treatment alone, systemic antibiotic therapy as shown by adequate testing procedures is often necessary where

evidence of local spread or toxicity of the infection is apparent. Thus this investigation was carried out to test the efficacy of several old and newly-described antibiotics against coagulase-positive staphylococci.

MATERIALS AND METHODS

Strains: Only coagulase-positive staphylococci isolated from humans and animals were tested (Table 3.1a).

Antibiotic susceptibility test: Disc sensitivity tests were performed with all the strains of coagulase-positive staphylococci using the method of Kirby-Bauer (Bauer, Kirby, Sherris and Turck, 1966). Suitably-adjusted overnight broth culture of each strain was used to flood Mueller Hinton agar in a 140 mm Petri dish. Excess fluid was drained off and the plate was then allowed to air-dry for thirty minutes before applying the following discs: Pen_{10}, Ox_1, CF_{10}, K_{30}, GM_{10}, NN_{10}, C_{30}, TE_{30}, D_{30}, E_{15}, L_2, PR_{15}, FF_{30}, RA_{30}, VA_{30}, MA_{30}, SXT, FOX_{30}, CXM_{30} and CTX_{30} (biomerieux, France). Table 5.1 shows the symbols and concentrations of the antibiotics used in this study. The plates were later incubated at 37°C for 18-24 h after being left for 15 to 30 min at room temperature.

The zone diameters were measured with sliding calipers and the metre rule. Staphylococcus aureus (OHS, Lyon, France) was used as a control.

Minimum Inhibitory Concentration (MIC):

Five millilitres of broth cultures of isolates that were resistant by the disc diffusion method were incubated for 5 h at 37°C after which they were thoroughly mixed. 2.3.5. triphenyl tetrazolium chloride (Sigma Co., St. Louis, U.S.A.) was dissolved in sterile distilled water to give a concentration of 0.1 per cent. 0.05 ml of nutrient broth was delivered into each of 8 wells in a row in a sterile WHO microtiter plate. 0.05 ml of the antibiotic under test was then added to the first well and serial dilutions were made using a sterile graduated Pasteur pipette (Morse guage No. 55) and 0.05 ml of the antibiotic solution was transferred at each dilution. This was followed by the addition of 0.025 ml of 2.3.5. triphenyl tetrazolium chloride. Later, 0.025 ml of each suspension was added. This gave an initial antibiotic concentration of 5.0 mcg/ml, 2.5 mcg/ml in the first, and second wells respectively and 1.25 mcg/ml in the third well and so

forth. The procedure was repeated for the control S. aureus (NCTC 6571). The microtitre plates were then covered and incubated at 37°C for 18-24 h and the results were recorded. The minimum inhibitory concentration (MIC) of each antibiotic was taken as the minimum amount of the antibiotic needed to inhibit the growth of the Staphylococcus strain under test and it was indicated by no change in the colour of the indicator.

RESULTS

Antibiotic susceptibility patterns: Of the 9 S. aureus and S. intermedius strains isolated from humans, 2 were resistant to penicillin (≤ 12 mm) and the minimum inhibitory concentration (MIC) of penicillin to the 2 strains was 2.5 mcg/ml. Resistance to penicillin was also found in one S. aureus strain of caprine origin and 3 S. aureus strains from other animals (Table 5.3). Their MICs ranged from 0.15 to 0.009 mcg/ml. Of the 10 strains resistant to tetracycline, 2 were isolated from humans, 3 from goats and 5 from other animals. The MICs of tetracycline to these strains ranged from 5.0 to

0.009 mcg/ml. Resistance to lincomycin was found in only one strain of S. aureus isolated from goats. Resistance to vancomycin was found amongst 2 isolates. The MICs of [redacted] lincomycin and vancomycin was not done due to the non-availability of the powdered drugs. All other strains showed a relatively high rate of sensitivity to oxacillin, kanamycin, minocycline, fosfomycin, cefamandol and other cephalosporins (Tables 5.2a, 2b, 2c, 3, Appendix 3.B).

DISCUSSION

Recently, resistance to a variety of antibiotics by staphylococci has generated considerable interest. Infact some strains of S. aureus tend to be epidemic in character and they can even spread rapidly within human or animal populations (Moeller, Smith, Shoemaker and Tjalma, 1963; Pulverer, Gho and Spieckerman, 1971). Locksley, Cohen, Quinn, Tompkins, Coyle, Kirihara and Counts (1982) recently reported on the evolution and transmission of a multiple antibiotic-resistant S. aureus within the United States of America which led to a nocosomial epidemic in which 17

of 35 patients died. Other nocosomial infections resulting from methicillin-resistant strains of S.aureus have been reported (Klimek, Marsik, Bartleth, Weir, Shea and Quintiliani, 1976; Peacock, Marsik and Wenzel, 1980; Grieble, Krause, Pappas and Discostanza, 1981). The relationship existing between skin disease and antibiotic pressure in the colonization and shedding of resistant S. aureus (Bernstein and McDermott, 1960) might have influenced the spread of nocosomial strains of S. aureus. In addition, Crossley, Lendesman and Zaske (1979) have suggested that skin disease provides a means for establishing transient colonization of S. aureus on the hands of hospital personnel. Thus extensive efforts are often necessary to identify patient and personnel carriers of antibiotic-resistant S. aureus as these bacteria are capable of prolonged carriage (Boyce, Landry, Deetz and Dupont, 1981). Although none of the S. aureus strains examined herein was resistant to methicillin, it should be noted that methicillin-resistant S. aureus strains have been found to account for 3 to 28 per cent of nocosomial staphylococcal infections in some European

hospitals (Bulow, 1971; Kayser,1975; Bojan and Er, 1977). In the United States of America, the incidence of methicillin-resistant S. aureus has been reported in at least 22 hospitals in 17 states (Barnett, McGhee and Finland, 1968; Saroglou, Cromer and Bisno, 1980). Whilst these cases may be of nocosomial origin, naturally occurring strains of methicillin-resistant S. aureus have been reported (Smith and Counts, 1960; Fedorko and Katz, 1960). Thus, methicillin-resistant staphylococci probably existed before even methicillin itself became available (Cetin and Ang, 1962). Methicillin-resistance may even occur where the drug is not used (Ojo, 1972). Furthermore, methicillin resistance may be accompanied by a simultaneous change or shift in phage types (Jessen, Rosendal, Bulow, Faber and Eriksen, 1969; Parker and Hewitt, 1970). Whilst staphylococcal resistance to penicillin is generally coded for on a plasmid and only rarely on the chromosome (Dyke and Richmond, 1967; Peyrù, Wexler and Novick, 1969), Asheshov (1966), Poston (1966), Sawai, Mitsuhashi and Yamagishi (1968) have reported the occurrence of chromosomally located penicillinase gene

in some strains of S. aureus. Although screening for plasmids in most of the strains was not undertaken in this present study, the development of resistance to penicillin by S. aureus of human and animal origins is of clinical importance as some rare strains of staphylococci that possess a heritable resistance to penicillin G characterized as "intrinsic" and mediated through a mechanism other than inactivation of the ring do exist (Dyke, Jevons and Parker, 1966; Smith, Hamilton-Miller and Knox, 1969).

In a survey of staphylococcal infections in some of the hospitals in Lyon, France, Brun, Fleurette, Forey and Gonthier (1981) found that hospital isolated staphylococci had a greater resistance to antibiotics. The authors also in another survey found that all the strains of staphylococci they examined were sensitive to cephalothin and pristinamycin. This is also true of all the strains of S. aureus and S. intermedius of human and animal origins examined herein. Generally, cephalothin is remarkably free of close-related side effects and is therefore the most sensitive cephalosporin for parental therapy of serious

staphylococcal infection (Eickhoff, 1972). Cephalothin is not inactivated by staphylococcal penicillinase and is active against both staphylococci susceptible to penicillin G and resistant to it (Klein, Eickhoff, Tilles and Finland, 1964). In a study of purulent conjunctivitis cases in Nigeria, Onile et al. (1976) found that most of the strains of S. aureus examined were commonly sensitive to gentamycin, chloramphenicol and methicillin in decreasing order. Orcfici, Scopetti, Martino, Tubaldi and Serra (1981) also found that cephalothin, vancomycin, gentamycin and erythromycin showed activities higher than 95 per cent against S. aureus strains isolated from clinical cases in Italy. This is relatively similar to the antibiograms of the S. aureus strains examined in this present study (Table 5.3) as a 100 per cent sensitivity to chloramphenicol, cephalexin and other cephalosporins was found amongst the strains. In veterinary medicine, however, macrolide antibiotics notably erythromycin, oleandomycin, tylosin and spiramycin are primarily used as therapeutic agents against Gram-positive bacteria and Mycoplasma species.

Furthermore, lincomycin and clindamycin and the streptogramins - pristinamycin and viginiamycin to which most of the strains examined here were sensitive are used either as therapeutic agents or growth promoters. Although marked resistance to these antibiotics are not common in the veterinary literature, Devriese (1976) has however noted that bovine, poultry and porcine S. aureus strains he examined, showed significant resistance to macrolide antibiotics. Resistance of S. aureus to macrolide antibiotics, lincomycin and streptogramin-B type antibiotics such as viginiamycin is known to be due to methylation of 23S ribosomal ribonucleic acid (RNA), a component of the 50S ribosome subunit the target site of the antibiotics (Lai and Weisblum, 1971) which results in a lower binding affinity of these structures for erythromycin and related antibiotics. In clinical practice, isolates of erythromycin-resistant S. aureus strains can be divided into constitutive and inducible methylators. The constitutive methylators usually appear to be highly resistant to the macrolides, the lincomycins and the streptogramin-B type antibiotics whereas the

inducible strains can be induced by subinhibitory concentrations of erythromycin and eventually other macrolides to a high level of resistance to all these antibiotics including the inducer(s). However, resistance to erythromycin followed almost immediately after the introduction of the antibiotic. In this investigation, only a relatively few S. aureus strains were however resistant to erythromycin (Table 5.3). Resistance to erythromycin has also been found to occur in other animals. Thus resistance to erythromycin in S. aureus of bovine and poultry origins (Huebner and Scheetz, 1956; Kunter, 1975; Devriese, 1980), slaughtered pigs (Kusch, Siems and Sinell, 1972) have been reported. Interestingly, however, the antibacterial spectrum of licomycin and erythromycin (Table 5.3) appeared similar. This is in agreement with the findings of McGhee, Smith, Wilcox and Finland (1968) who noted that despite the differences in the biochemical properties of lincomycin and erythromycin, the antibiotics possessed very similar antibacterial activities. Although lincomycin may not be the drug of choice in the treatment of staphylococcal infections (Eickhoff, 1972), nonetheless,

therapeutic results when treating infection caused by susceptible staphylococci appeared to be comparable to those obtained with penicillin (Price, O'Grady, Shooter and Weaver 1965). Resistance to tetracycline, in this study, was common amongst S. aureus strains isolated from humans and animals (Table 5.3). The strains of S. aureus of poultry origin which showed resistance to tetracycline (Appendix 3.B) may be due to the incorporation of tetracycline in their feeds as Smith and Crobb (1960) noted that S. aureus skin strains were prevalent on poultry farms that used feeds containing this antibiotic. In addition, Harry (1967) postulated that staphylococcosis in poultry may be the consequence of the colonization of the skin on the upper respiratory tract by S. aureus followed by mechanical damage or other unknown factors that favour tissue invasion. In addition to the incorporation of tetracycline in some feeds, tetracycline resistance in some of the strains examined herein may however be plasmid-borne because two strains of S. aureus (UI 118, UI 145) screened for tetracycline, cadmium, streptomycin and chloramphenicol plasmids had tetracycline

plasmid sizes of 21.5 and 4.5 Kbp respectively. Furthermore, these tetracycline plasmids showed restriction patterns for the endonuclease Sau3a and Hind 3 identical with PMK 148, originally isolated from S. simulans. Generally, the genetic markers involving resistance to tetracycline, sulfanilamide and streptomycin although independent appear to be closely linked. Kasaga, Hashimoto and Mitsuhashi (1968) in their transductional studies found that tetracycline and sulfanilamide resistance showed 100 per cent cotransduction with some other markers. However, whether the genetic determinants are on the chromosome or on a plasmid appears to be a property of the host strain as indicated by transduction of the genes between the two strains involved (Kasuga and Mitsuhushi, 1968).

Table 5.1 Symbols and concentrations of antibiotics used for sensitivity testing of S. aureus strains

Symbol	Antibiotic	Concentration
Pen_{10}	Penicillin	10 units
Ox_{1}	Oxacillin	1 microgramme (mcg)
CF_{10}	Cefalothine	10 mcg
K_{30}	Kanamycin	30 "
GM_{10}	Gentamycin	10 "
NN_{10}	Tobramycin	10 "
C_{30}	Chloramphenicol	30 "
TE_{30}	Tetracycline	30 "
MI_{30}	Minocycline	30 "
D_{30}	Deoxycline	30 "
E_{15}	Erythromycin	15 "
L_{2}	Lincomycin	2 "
PR_{15}	Pristinamycin	15 "
FF_{50}	Fosfomycine	50 "
RA_{30}	Rifampicin	30 "
VA_{30}	Vancomycin	30 "
MA_{30}	Cefamandol	30 "
SXT	Trimethoprim/ Sulfamethazole	1.25 + 23.5 "
FOX_{30}	Cefoxin	30 "
CXM_{30}	Cefuroxim	30 "
CTX_{30}	Cefotaxim	30 "

Table 5.2a. Zone inhibition (mm) of chemotherapeutic agents used against coagulase-positive staphylococci isolated from humans

Strain No.	Antibiotics	Pen_{10}	OX_{1}	CF_{10}	K_{30}	GM_{10}	NN_{10}	C_{30}	TE_{30}	MI_{30}	D_{30}	E_{15}	L_{2}	PR_{15}	FF_{50}	RA_{30}	VA_{30}	MA_{30}	SXT	FOX_{30}	CXM_{30}	CTX_{30}
M221		16	20	30	26	26	27	24	27	28	28	29	21	28	31	36	18	25	26	27	29	27
M222		38	25	40	22	27	26	23	23	28	28	29	16	29	33	38	18	38	32	30	29	32
M223		20*[1]	23	27	30	26	31	30	33	30	32	27	18	25	48	38	24	29	29	28	36	33
M224		36	22	35	26	26	25	25	29	28	30	31	21	30	28	35	24	39	33	29	30	29
M225		32	23	30	21	21	23	23	10	23	14	27	17	27	36	34	NT	33	R	26	26	26
M226		6*	18	26	21	20	22	23	23	25	26	25	16	25	27	30	16	21	NT	24	25	25
M232		12*[1]	18	21	23	24	24	19	26	26	27	27	19	25	26	36	13	33	37	25	27	26
M337		6*	16	22	21	21	22	21	9	14	25	NT	18	24	26	33	16	20	26	23	26	24
M253		39	26	39	33	NT	25	24	25	26	29	26	18	27	35	32	16	39	23	30	24	23
S. aureus (Lyon, OHS)		29	20	30	21	22	23	20	22	24	24	25	17	24	26	30	15	29	24	26	28	26

NT: Not tested; R: Resistant; * Penicillinase production; [1]: Late

Table 5.2b. Zone inhibition (mm) of chemotherapeutic agents used against coagulase-positive staphylococci isolated from goats

Strain No.	Antibiotics	Pen$_{10}$	OX$_{1}$	CF$_{10}$	K$_{30}$	GM$_{10}$	NN$_{10}$	C$_{30}$	TE$_{30}$	MI$_{30}$	D$_{30}$	E$_{15}$	L$_{2}$	PR$_{15}$	FF$_{50}$	RA$_{30}$	VA$_{30}$	MA$_{30}$	SXT	FOX$_{30}$	CXM$_{30}$	CTX$_{30}$
UI 27		36	24	37	23	24	24	23	24	26	28	26	19	27	25	33	17	34	28	26	28	27
UI 96		25*[1]	13	32	29	30	30	25	R	26	13	28	R	25	20	28	18	30	22	23	25	21
UI 118		6	17	26	20	21	21	20	10	24	14	26	17	23	32	28	16	21	27	26	29	24
UI 145		31	27	39	29	29	31	29	10	30	21	30	20	30	37	41	21	30	24	30	33	29
UI 150		40	24	44	29	29	30	30	32	35	34	35	25	35	33	42	20	38	R	37	33	30
UI 153		31	25	38	24	24	24	23	28	28	31	29	19	25	24	35	17	35	29	26	29	26
UI 154		39	26	39	25	25	27	26	27	28	29	31	22	29	31	35	18	36	26	26	33	29
UI 160		38	27	39	23	23	24	23	26	26	27	28	19	27	37	34	17	36	29	27	28	27
UI 161		37	23	37	22	NT	23	22	24	26	27	29	20	27	38	34	16	33	29	25	29	26
UI 166		15*[1]	20	31	26	21	25	23	26	23	27	28	22	33	33	34	29	28	27	25	32	26
UI 327		26	15	31	25	22	26	26	R	26	13	23	12	26	36	37	17	28	31	26	31	26
UI 331		38	20	37	22	23	23	22	24	25	23	28	18	28	32	38	17	36	29	26	30	26
UI 334		29	22	35	20	20	20	21	23	24	25	27	17	28	33	26	9	32	28	24	27	9
S. aureus (Lyon, CHS)		29	20	30	21	22	23	20	22	24	24	25	17	24	26	30	15	29	24	26	28	26

NT: Not tested; R: Resistant; * Penicillinase production; [1]: Late.

Table 5.2c. Zone inhibition (mm) of chemotherapeutic agents used against coagulase-positive staphylococci isolated from dogs

Strain No.	Antibiotics	Pen_{10}	OX_{1}	CF_{10}	K_{30}	GM_{10}	NN_{10}	C_{30}	TE_{30}	MI_{30}	D_{30}	E_{15}	L_{2}	PR_{15}	FF_{50}	RA_{30}	VA_{30}	MA_{30}	SXT	FOX_{30}	CXM_{30}	CTX_{30}
D238		32	23	31	19	19	19	19	R	11	9	23	16	23	37	29	14	31	24	23	25	24
D240		36	25	37	22	23	23	23	24	27	27	26	18	25	42	31	15	36	21	33	33	30
D241*		13*	23	32	23	24	25	24	26	28	27	29	17	27	34	29	16	27	6	32	33	30
D242		13*	24	33	24	NT	27	23	6	16	17	28	19	28	22	33	16	27	6	32	34	31
D243		10*	22	33	27	28	29	10	28	29	30	30	21	32	36	36	17	27	28	29	36	34
S. aureus (Lyon, OHS)		29	20	30	21	22	23	20	22	24	24	25	17	24	26	30	15	29	24	26	28	26

R: Resistant; NT: Not tested; *Penicillinase production.

Table 5.3. Percentage sensitivity of coagulase-positive staphylococci isolated from man and animals to various antibiotics

HOSTS \ Antibiotics	Pen_{10}	OX_{1}	CF_{10}	K_{30}	GM_{10}	NN_{10}	C_{30}	TE_{30}	MI_{30}	D_{30}	E_{15}	I_{2}	PR_{15}	FF_{50}	RA_{30}	VA_{30}	MA_{30}	SXT	FOX_{30}	CXM_{30}	CTX_{30}
Human	77.8	100	100	100	88.9	100	100	77.8	100	100	88.9	100	100	100	100	88.9	100	88.9	100	100	100
Goats	92.3	100	100	100	100	100	100	76.9	100	100	100	92.3	100	100	100	92.3	100	92.3	100	100	92.3
Other animals	81.3	100	100	100	100	100	100	87.5	100	87.5	93.8	93.8	100	100	100	87.5	100	93.8	100	100	100
S. aureus (OHS, Lyon)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : Susceptible

CHAPTER 6

ISOLATION OF CAPRINE PHAGES AND PHAGE TYPING OF S. AUREUS STRAINS

INTRODUCTION

Following the discovery of staphylococcal bacteriophages (Callow, 1922), the basic studies of Burnet and Lush (1935), Williams and Rippon (1952), Blair and Carr (1953) led to the widespread use of phage typing as an epidemiological aid in the control and prevention of staphylococcal infections (Fisk; 1942; Seto and Wilson, 1958). Although Wilson and Atkinson (1945) foresaw the need for the collective use of a variety of staphylococcal phages in a basic typing set, several additional phages to the basic set (Parker and Rountree, 1971) are now in use in several countries.

In the veterinary literature, Slanetz and Jawetz (1941), Smith (1948) were probably the early authors who investigated the use of phage typing of S. aureus from

bovine mastitis. Following the low typability of the staphylococcal strains isolated from milk by St. George, Russell and Wilson (1962), Markham and Markham (1966) suggested that it was necessary to develop a separate phage set for most strains of S. aureus in view of the apparent host specificity of staphylococci. However, some years before the suggestions of these authors, Seto, Kaesburg and Wilson (1956) had developed 6 phages, 2 of which were derived from phage 42D by propagation in different strains of staphylococci of bovine origin. Ojo and Lawson also isolated phages from S. aureus associated with tick pyaemia of lambs (unpublished). Thus several attempts have been made to isolate specific phages from S. aureus strains obtained from different hosts. In addition to the basic set of phages for typing human strains of S. aureus, bovine- and poultry- adapted phages are now available for typing S. aureus of animal origin. However, not all animal strains of S. aureus are susceptible to human or bovine phages hence the need to isolate, where practicable, specific phages for each host such as was done in this study.

MATERIALS AND METHODS

(A) Isolation of a caprine phage: During the course of phage typing the S. aureus strains examined herein, strain UI 150 was found to be lysogenic. Isolated plaques were removed and added to 1 ml of glucose-nutrient broth to which has been added 0.04 ml of Calcium chloride ($CaCl_2$ M/10) in seven tubes. The eighth tube was used as a control. The tubes were later incubated in a 30°C water bath for at least 18 h with occasional observations for the appearance of lysis. After obtaining lysis in one of the tubes, the isolated phages were harvested and passaged after which the stock solution was centrifuged at 5,000 revolutions per minute for about ten minutes. The supernatant fluid was filtered through a sintered glass ("5/3") filter giving the filtrate as a phage suspension. The phage was serially propagated until a satisfactory titre of 1,000 times (10^3 x RTD) routine test dilution of the stock phage was achieved. The phages were then stored at 4°C until required.

(B) Phage typing of S. aureus strains with caprine and human sets of phages.

(1) Propagation of phage 55: The propagating strain 55 was grown overnight in glucose-nutrient broth incubated at 30°C. Two millilitres of the lysotyping broth were added to each of six tubes to which were also added 0.08 ml of Calcium chloride ($CaCl_2$ M/10). The seventh tube (control) had 5.0 ml of broth and 0.2 ml $CaCl_2$ M/10. 0.2ml of the stock of phage 55 at 100 times routine test dilution (RTD x 100) was added to the first tube. After careful mixing, 0.5ml was pipetted off and added to the second tube. This procedure was repeated up to the sixth tube after which 0.5 ml of the suspension was discarded. One drop of the overnight broth culture of propagating strain 55 was added to all the tubes starting with the control tube down to the first tube. All the tubes were later incubated in a 37°C water bath after which they were observed for lysis on an hourly basis. After obtaining reasonable amounts of lysis, the phage was titrated serially undiluted and also as a diluted (10^2) form with 5 ml of broth and 0.05 ml of $CaCl_2$ M/10 being used for

the diluted phage. Toluene was later added to the new phage stock which was later kept at 4°C. Other propagating phages such as phages 52, 53 and 81 were treated using different dilutions as described above before actual phage typing of the strains examined herein was carried out.

2. Phage typing

Strains. All the S. aureus strains isolated from humans and animals were tested (Table 3.1a).

Lytic activity of caprine phage (G1). Drops of the phage at routine test dilution (10^4 x dilution of stock) were deposited onto a lawn of an overnight plate culture of each of the S. aureus strain grown at 37°C. The plates were then incubated overnight at 30°C.

Phage typing with International sets of human phages: Lysotyping agar plates were flooded with 4-6 h broth cultures of the strains under test. Excess fluid was removed and the plates were allowed to air dry. One drop of each of the typing phages diluted to 100 times the routine test dilution (RTD x 100) was placed on the seeded

plate with a graduated Pasteur pipette (1/50 ml). A template was held underneath the plate. Each plate was allowed to air dry again and then incubated at 30°C overnight. Lytic reactions were read semiquantitatively and microscopically. A ± reaction was used to indicate fewer than twenty isolated plaques, a + reaction indicated 20 to 50 plaques and ++ reaction indicated more than 50 plaques. LC and LSC (complete/confluent and semi-confluent lysis) were used to indicate complete lysis and to differentiate them from more than 50 distinct plaques. Staphylococcal cultures which did not show lysis with any of the phages used were reported as "non-typable" (NT).

RESULTS

Lytic activities of caprine phage (G1). None of the S. aureus strains isolated from humans, giant rats (Cricetomys gambianus) rabbits, dogs and sheep was susceptible to the newly isolated caprine phage G1. However, 3 caprine strains of S. aureus were susceptible to the caprine phage G1 and they produced confluent lysis. These caprine strains of S. aureus were untypable with

the human set of phages (Table 6.1).

Phage types of human strains of S. aureus: Six strains were typable with the human set of phages. Four strains were susceptible to phages of Group I whilst none was susceptible to the Group II phages (Table 6.2). Susceptibility to phage Group III and the miscellaneous group was found amongst 3 S. aureus strains. Thus the 6 S. aureus strains isolated from humans that were phage typable belonged to phage groups I, III and the mixed group. Four of the human S. aureus strains were however susceptible to each of phages 81, 52 and 77 whilst three strains were also susceptible to phages 80, 84 and 85 (Table 6.3).

Phage types of S. aureus strains isolated from animals: Nine of the 14 caprine strains of S. aureus examined herein were susceptible to the human set of phages at 100 x RTD. The strains were generally lysed by phages belonging to Groups II, III and the miscellaneous group. Seven of the caprine strains of S. aureus were susceptible to phage 29 whilst 8 strains were lysed by phage 54. Six strains were susceptible to each of phages 3A and 3C.

One strain was susceptible to phage 187 whilst 5 strains were lysed by phage 81. None of the caprine strains of S. aureus was lysed by phage 42D. Susceptibility to experimental phages D11 and HK2 was found amongst 4 strains (Table 6.4). The S. aureus strain isolated from rabbits was lysed by phages 3A, 3C, 55 and 71 only. Thus the rabbit strain of S. aureus belonged to phage group II. The S. aureus strain isolated from giant rats (Cricetomys gambianus) susceptible to human phages belonged to phage group III (Appendix 4.B). Thus of the 22 S. aureus strains isolated from different animals, 11 (50 per cent) were susceptible to human set of phages.

DISCUSSION

In essence, phage typing is a test of susceptibility of a culture of S. aureus to a variety of phages having relatively narrow host ranges at predetermined dilutions. This susceptibility can be measured semiquantitatively and relationships between different cultures from a common outbreak are established on the similarity of their patterns of susceptibility to these phages. Since most of animal strains of S. aureus were not lysed by the

human phages at routine test dilution (RTD) or those that were typable gave wide patterns that were unstable and difficult to interpret (Parker, 1972), the basic set of phages for typing human staphylococci have generally been found not applicable to animal S. aureus (Oeding, 1978). Non-typability has particularly been observed with staphylococci from dogs, pigeons (Oeding, Marandon, Hajek and Marsalek, 1970), horses (Oeding et al., 1974), sheep (Oeding et al., 1976). In man, Williams and Rippon (1952), Blair and Carr (1953), Nahmias, Sakurai, Blumberg, Doege and Sulzer (1961) noted that a relatively high percentage of S. aureus of human biotype was resistant to phages and thus unidentifiable by the phage method. This may be true of some S. aureus strains from humans which were found untyable with the International set of human phages. However, other human strains of non-host specific biotype were susceptible to a variety of human phages (Table 6.3). The caprine strain of S. aureus of human biotype examined herein was also susceptible to the human set of phages. Thus susceptibility of S. aureus strains to human set of phages may

vary from strain to strain and from host to host.

With the discovery of new species of coagulase-positive staphylococci (Hajek, 1976; Devriese, Hajek, Oeding, Meyer and Schleifer, 1978) that are known to be resistant to human set of phages, attempts have been made to isolate phages for typing these staphylococcal strains. Kawano, Shimizu and Kimura (1982) isolated 5 experimental phages from pigeons with which 10 (8.2 per cent) of 122 strains of S. intermedius isolated from dogs, horses, mink and foxes were typed. In this present study, of the 14 caprine strains of S. aureus examined, 3 (21 per cent) were typable with the newly isolated caprine phage G1. Perhaps the lytic spectrum of this new phage may be restricted to caprine strains of S. aureus as none of the other S. aureus of human or animal origin was susceptible to the phage. With a new set of phages for typing avian staphylococci, Shimizu (1977a) was able to type more than 80 per cent of chicken staphylococci isolated in Japan. Hajek and Horak (1978) using the phages of Shimizu, obtained a typability rate of 94 per cent of S. aureus strains of poultry origin. Witte,

Hummel, Meyer, Exner and Wundrak (1977) also isolated a poultry phage (A1591) with which they obtained a typability rate of 71 per cent with S. aureus strains isolated from Bulgaria, West Germany, Belgium and the Netherlands. However, using the International set of phages for typing human strains of S. aureus for typing poultry strains of S. aureus, divergent results were obtained by several authors. Whilst Shimizu (1976) obtained a typability rate of 3.0 per cent, Genigeorgis and Sadler (1966); Devriese, Devos, Beumer and Maes (1972) obtained 14.3 and 15.8 per cent respectively with the use of human phages for typing poultry strains of S. aureus. In this present investigation, the poultry strains examined were not susceptible to human set of phages (Appendix 4.B). In addition to the development of poultry phages, new typing phages for bovine strains of S. aureus have been developed (Slanetz and Bartley, 1962; Lorbacher and Blobel, 1968). Lobacher and Blobel (1968) were able to characterize 25 per cent of the 257 bovine cultures which were untypable with the modified phage sets of Davidson (1961) by using some newly-isolated

phages from bovine S. aureus strains. An international set of bovine phages is currently being used for typing animal strains of S. aureus (Davidson, 1972). The phage types of staphylococci that cause bovine mastitis are often found amongst those which are pathogenic to man and which have been found on the skin and nasal mucosa of human beings. It is thus possible that milkers may play a part in initiating and spreading infection amongst milking cows. Thus in an attempt to trace the sources of sporadic cases of bovine mastitis at the Teaching Farm of the University of Nigeria, Nsukka, Mohan (1980) found that the strains of S. aureus recovered from the nasopharynx of the attendants showed phage patterns which were similar to some of the animal strains of S. aureus which also belonged to lytic group I. Also, in the study of the S. aureus strains isolated from milk in Finland, Korianem (1969) found that lytic group I strains were more closely associated with acute mastitis than other strains although the author obtained only 42.7 per cent typability with human set of phages. However, Hajek and Marsalek (1976) obtained 100 and 98 per cent typability of bovine

strains with human and bovine phages respectively. The authors also obtained 58.7 per cent typability of ovine strains of S. aureus with human set of phages. In this present study, the ovine strain of S. aureus examined was not susceptible to either caprine phage G1 or to the set of human phages used (Appendix 4.B). This is in agreement with the findings of Markham and Markham (1966) who reported non-typability of ovine strains of S. aureus. A 4 per cent typability rate of ovine S.aureus was however obtained by Hajek and Horak (1978) with human set of phages at routine test dilution as compared with the relatively high typability of 58.7 per cent earlier obtained by Hajek and Marsalek (1975).

In the study of the characteristics of canine strains of S. aureus isolated in Nigeria, Ojo (1972) found that the commonest phage types associated with the strains were 75 and 81. Rajulu, Foltz and Lord (1960) in another study, found that 19 of the 50 strains of coagulase-positive staphylococci isolated from dogs showed phage patterns that were characteristic of human S. aureus. However, Live and Nichols (1961) in a study of outpatient

clinic dogs, found that only 4.9 per cent of the 445 animals that they examined carried strains in their nares that were typable with the set of phages for S. aureus of human origin. This is in agreement with the findings of Garner and Coles (1965) who noted that only 4.9 per cent of the 382 dogs examined carried in their nasal passages, S. aureus typable with phages of the international series. The strain of S. aureus of canine origin examined herein was not typable with the set of human phages used (Table 6.1a). Thus the apparently low typability of S. aureus strains of canine origin prompted Coles (1963) to investigate the development of specific phages for canine strains of S. aureus. The author isolated three phages from lysogenic strains of staphylococci. Blouse and Meekins (1968); Jackel, Lorbacher and Blobel (1969) also isolated some specific canine phages for S. aureus of canine origin. In this present study, although none of the S. aureus strains isolated from man, poultry, sheep giant rats (Cricetomys gambianus) and rabbits was susceptible to the new caprine phage used, it is interesting to note that the phage types

of the strain of S. aureus isolated from a rabbit are identical with the phage types that have been found associated with some rabbit diseases in Belgium (Devriese, personal communication).

Generally, cultures sensitive to phage 187 are rarely sensitive to other phages at routine test dilution. Thus phage 187 rarely occurs in lytic patterns with other phages (Smith, 1972). This is in agreement with the findings in this present study whereby the caprine strain of S. aureus that was susceptible to phage 187 was not lysed by other phages (Table 6.4). Furthermore, Oeding (1974), noted that phage 187 susceptible strains of S. aureus contained N-acetylgalactosamine instead of N-acetyl glucosamine in their teichoic acids. However, the caprine strain susceptible to phage 187 in this study was found to possess ribitol glucosamine teichoic acid when tested both biochemically and serologically (Tables 7.3; 8.2b).

Although no particular phage types were prevalent amongst the S. aureus strains isolated from humans, susceptibility to phage 80/81 complex was found in some

strains. None of the strains examined herein was obtained from an epidemic outbreak. Phage 80/81 is a potential epidemic strain and since no routine phage typing is done in Nigeria, it is difficult to say it does not cause any epidemic (Scott-Emuakpor cited by Ojo, 1972).

However, Poole and Baker (1966) reported an outbreak of infection with phage 80/81 in a veterinary field station where human personnel were predominantly infected though lesions were found on goats and sheep used for experimental purposes. The authors also found that all the human strains of S. aureus typable with human phages belonged to lytic group III and the mixed group. Orcifici, Scopetti, Martino, Tubaldi and Serra (1981) in a survey of some patients in some Italian hospitals found that 18 (27 per cent) of the strains examined belonged to the mixed group whilst several other strains were refactory to group I phages. Also, Krynski and Becla (1981) in a four-year survey of S. aureus strains isolated in Poland found that staphylococci belonging to phage types 94/96, 96 and 71/96 were the chief cause of septicaemia, purulent dermatitis and furunculosis. Furthermore, these authors noted that

a strain of S. aureus that was susceptible to phage HK2 at routine test dilution (RTD) was responsible for epidemics in some hospitals for babies. In this present study, some stains of S. aureus isolated from humans and goats refractory to phages 71, 94 and 96; D11 and HK2 were obtained from apparently non-epidemic cases. Epidemic strains of S. aureus are often refractory to phage 80/81 complex (Poole and Baker, 1966). Thus in the epidemiological studies of the phage 80/81 complex in animals, Skaggs and Nicol (1961) isolated strains of S. aureus phage type 80/81 from the skin lesions and milk of cattle in a herd in which the dairy attendants were found to have infections caused by as well as to be the carriers of the same strain of staphylococci. Drury and San Clemente (1962) highlighted the public health importance of the 80/81 phage complex in their experimental introduction of S. aureus of phage type 80/81 into the udders of cows. These authors also noted that S. aureus strain susceptible to phage 80/81 may persist in the bovine udder indefinitely and may even escape detection unless suitable laboratory procedures are carried out on suspicious milk samples.

However, S. aureus of phage 80/81 may also be infectious for dairy cattle under natural conditions (Zinn, Anderson and Skaggs, 1961) and human attendants can harbour S. aureus phage 80/81 with possible transfer to animals (Wallace, Quisenberry and Tanimoto, 1962).

Table 6.1 Lytic activities of human and caprine phages with *S. aureus* strains isolated from man and animals

Origin of strain	No. of strains	No. typable with human set of phages*	Number sensitive to caprine phage G1
Man	8	6	0
Goat	14	9	3
Sheep	1	0	0
Giant rat	3	1	0
Rabbit	1	1	0
Poultry	2	0	0
Dog	1	0	0

*See Table 6.2

Table 6.2. Susceptibility patterns of S. aureus strains isolated from different hosts with human set of phages

PHAGES AT ROUTINE TEST DILUTION (RTD) X 100

Host	Phage type:	29	52	52A	79	80	3A	3C	53	71	6	42E	47	53	54	75	77	83A	84	85	42D	81	94	95	96	187	88	89	90	92	D11	HK2	Total Number Typable
	Phage group:	GROUP I					GROUP II						GROUP III								IV	MISCELLANEOUS (NOT CLASSIFIED)											
Human		3*	4	4	2	3	0	0	0	0	0	4	3	4	3	1	4	3	5	3	0	5	1	3	1	0	1	2	1	3	2	3	6
Goats		7	5	1	2	4	5	5	0	1	6	0	3	6	8	0	4	2	1	2	0	5	5	1	0	1	1	5	0	1	1	5	9
Giant Rat (Cricetomys gambianus)		1	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
Rabbit		0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

*Total number susceptible

Table 6.3. Susceptibility patterns of human S. aureus strains with human set of phages

Strain No.	PHAGES AT RTD X 100																														
	29	52	52A	79	80	3A	3C	55	71	6	42E	47	53	54	75	77	83A	84	85	81	94	95	96	42D	187	88	89	90	92	D11	HK2	ECOVAR
		GROUP I				GROUP II					GROUP III							MISCELLANEOUS						GR IV	MISCELLANEOUS					GROUP		
M221	LC	LC	++	-	-	-	-	-	-	-	±	±	-	-	-	+	-	-	-	-	-	±	-	-	-	-	-	-	-	±	Human
M222	LSC*	LC	LC	LSC	LC	-	-	-	-	-	LSC	LSC	++	LSC	LSC	LSC	+	LSC	LSC	LC	-	LSC	-	-	-	-	-	-	++	LSC	LSC	ND
M223	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Human
M224	-	LC	LSC	-	LC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++	-	LC	-	-	-	-	++	-	-	-	-	Human
M225	-	-	-	-	-	-	-	-	-	-	+	-	-	LSC	-	-	+	-	-	+	-	-	-	-	-	-	-	-	++	-	-	NHS3
M226	LC	LC	LSC	+±	LC	-	-	-	-	-	-	+	+	-	-	LSC	+	LSC	LSC	LSC	++	LSC	++	-	-	++	++	+	++	LSC	LSC	Human
M253	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
M337	±	-	-	-	-	-	-	-	-	-	+	-	+	±	-	++	-	+	++	-	-	-	-	-		-	-	-	-	-	-	NHS3

*LSC: Semi complete lysis; LC: complete lysis; ++: 50 plaqu

+: 20 - 50 plaques; ±: 20 plaques;

-: No plaque formation; ND: Not determined; NHS: Non host ific.

Table 6.4 Susceptibility patterns of caprine strains of S. aureus with human set of phages

Strain No.	PHAGES AT RTD X 100																														Ecovar
	29	52	52A	79	80	3A	3C	55	71	6	42E	47	53	54	75	77	83A	84	85	81	94	95	96	42D	187	88	89	90	92	D11	HH2	
		GROUP I					GROUP II					GROUP III								MISCELLANEOUS				GROUP IV		MISCELLANEOUS GROUP						
UI 27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Ovine
UI 96	±	++	+	++	LSC*	-	-	-	-	-	-	-	LC	LC	-	LC	-	LC	LC	++	-	LSC	-	-	-	++	LC	LC	LC	-	-	Bovine
UI 118	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	NHS4
UI 120	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±	-	LC	-	-	-	-	-	-	-	-	-	-	-	-	ND
UI 145	++	-	-	+	-	-	-	-	-	±	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	±	-	NHS1
UI 150	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NT
UI 153	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Ovine
UI 160	+	LSC	-	-	-	++	LC	-	-	++	-	+	++	LC	-	++	-	-	-	LC	LC	-	-	-	-	-	LSC	-	-	-	LSC	Ovine
UI 166	-	-	-	-	-	LC	LC	LSC	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Human
UI 327	++	LSC	-	-	-	++	LSC	-	-	++	-	±	++	LC	-	++	-	-	-	-	LC	-	-	-	-	-	++	-	-	-	++	Ovine
UI 331	±	LSC	-	-	++	+	LC	-	-	+	-	++	++	LC	-	-	-	-	-	LSC	LC	-	-	-	-	-	++	-	-	-	LSC	Ovine
UI 334	+	LSC	-	-	-	++	LSC	-	-	+	-	-	++	LC	-	LSC	-	-	-	LSC	LC	-	-	-	-	-	LSC	-	-	-	LSC	Ovine
UI 154	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Ovine
UI 161	++	LC	-	-	-	LC	LC	-	-	++	-	LC	LC	LC	-	LSC	-	-	-	-	LC	-	-	-	-	-	-	-	-	-	-	Ovine

*LSC: Semi complete lysis

CHAPTER 7

SEROLOGICAL TYPING OF S. AUREUS STRAINS

INTRODUCTION

Staphylococci are known to contain several agglutinogens located in the cell walls and cross-absorption experiments coupled with slide agglutination technique have shown that the majority are species-specific whilst some are shared (Grov and Rude, 1967). In serotyping animal strains of S. aureus, factor sera produced against human staphylococci are currently being used. Generally only S. aureus strains are routinely serotyped where facilities are available. Although serotyping of S. epidermidis is not done on a wider basis, the organism are known to contain species-specific agglutinogens (Aasen and Oeding, 1971). The 2 main serotyping systems useful for S. aureus had been the Cowar-Mercier-Pillet system and the Oeding-Haukenes system (Haukenes, 1967).

In characterizing animal strains of S. aureus, serotyping has also been employed. Grun (1958b) found that an antigen in bovine staphylococci was also present

in human strains of S. aureus. White, Rattray and Davison (1962) reported the presence of some human type agglutinogens in S. aureus strains isolated from cases of bovine mastitis. Marandon and Oeding (1967) further demonstrated another antigen in S. aureus from milk of bovine mastitis. Often when outbreaks of staphylococcal infection occur in animals, the causative agents are usually of an adapted biotype. Furthermore, epidemiological surveys often reveal a certain number of strains which are different; some being of another biotype, for example, of the human one, other having intermediary characteristics. In situation of this type, serological determination of the type of cell wall teichoic acid and the presence of protein A found to be a valuable supplement to the biochemical subdivision of the strains (Oeding, 1978) were carried out for further characterization of the coagulase-positive staphylococci described herein.

MATERIALS AND METHODS

Strains: S. aureus strains listed in Table 3.1 were serotyped.

Affinity to fibrinogen using passive hemagglutination method:

One drop of sensitized red cells (bio Merieux,

Charbonnieres les Bains, France) and another drop of control red cells were added onto two different spots on a clean, grease-free slide. To each of the drops, one or two colonies of the strains grown on trypticase-soya agar was added. The mixture was then carefully spread with the tape-red end of a Pasteur pipette after which the slide was gently rocked. Large clumping appearing within 15 seconds in the sensitized red cell suspension was indicative of the presence of S. aureus. Absence of clumping with the control red cells made reading interpretable.

Protein A (I) Slide hemagglutination method: Onto a perfectly clean glass slide were placed, on two different spots, one drop of test red cell suspension (bioMerieux, France) and one drop of control red cell suspension. Several colonies of each S. aureus strain on trypticase-soya agar were emulsified in each suspension. An agglutination which appeared in the rest cell suspension in less than 2 min was indicative of protein A production by the S. aureus strain. No agglutination appeared in the control cell suspension. Negative results were confirmed as described in step II.

(II) Microtitre plate method: An overnight broth culture of the test organism was suitably diluted in brain heart infusion broth to give 1 in 2, 1 in 4 and 1 in 8 dilutions. To the first row of a clean, microtitre plate (Greiner) were added: 2 volumes of overnight suspension, and to the next column were added 1 volume of brain heart infusion broth (Oxoid) and 1 volume of broth suspension of the strain. The third column contained 3 volumes of brain heart infusion broth to which was added 1 volume of broth suspension of the strain to give a 1 in 4 dilution. A 1 in 8 dilution was obtained by diluting the third column containing 1 in 4 dilution, 1 in 2. The dilutions were made such that equal volumes of diluted and undiluted broth suspensions were in the columns of the microtitre plate. The same procedure was repeated for the second row of wells. After shaking the dropper bottles properly, one volume of each of Aure A - test red cells and Aure - A control red cells (bio Merieux, France) were added to the first and second rows respectively. A broth control was also set up. The suspensions were mixed for two minutes using a shaker after which the plates were left

on the bench for 2 h. Hemagglutination was recorded when sedimentation pellet was observed in the test cells and none in the control red cells.

Immunological detection of ribitol teichoic acid: (poly AB). Sterile 1.2 per cent Agar Noble (Difco) containing 0.85 per cent sodium chloride was layered onto clean immunological slides. After being allowed to solidify, circular wells were cut with a cork borer. The system used consisted of 6 peripheral and one central wells which were 3 mm in diameter and 4 mm apart. The central well was filled with undiluted antiserum to S. aureus Wood 46. Four peripheral wells were filled with bacteria to be tested (harvested growth from overnight culture on Oxoid nutrient agar) and the other peripheral wells were filled with purified control poly AB preparation (0.5mg/ml). The antiserum and poly AB were added with a finely-drawn Pasteur pipette by allowing the fluid to run down the side of the well. Bacteria were removed from the nutrient agar plate and added to the wells with the aid of a small loop. The plates were then placed in a humid chamber and kept at $4^{\circ}C$ for 18 h after which they were

left on the bench for 6-8 h before being examined for typical precipitin lines.

Serotyping (A) Preparation of factor sera: The method described by Oeding (1957) was employed for the preparation of factor sera (Appendix 5.B).

(B) Agglutination reactions: S. aureus strains were first seeded onto nutrient agar incubated at 37°C for 18 - 24 h after which some colonies were harvested and inoculated into 8 ml of serotyping broth (Appendix 5.A). The cultures were later incubated in a 37°C water bath-shaker for three to four hours and later overnight at 37°C. The cultures were then centrifuged at a low speed of 3,000 revolutions per minute for ten minutes. After discarding the supernatant, the deposit was resuspended in 1 ml of 0.5 per cent formalinized broth and mixed. Using calibrated Pasteur pipettes, one drop of each of the factor sera used (Table 7.2) and one drop of the bacterial suspension were placed carefully on a clean, grease-free glass plate on a Kline agitator. Ellipsoidal mechanical agitation was performed at room temperature for 20 min after which

the glass was rocked gently to mix the reactants. The stability of the bacterial suspension was checked in saline. Agglutination was observed with the naked eye. Results were coded as follows:

++++: numerous large agglutinates in a slightly opalescent clear liquid,

+++: small, clearly visible agglutinates;

+ : rare agglutinates;

(+): doubtful reaction and o: negative reaction.

The above procedure was repeated for confirmatory purposes and results compared. Only consistent results obtainable twice were taken as positive or negative reactions.

Detection of thermostable antigens: Suspensions used for agglutination reactions were autoclaved at 1.1kg/cm^2 for 30 min. After cooling and mixing, each bacterial suspension was serotyped as described before using only a_5, c_1, h_2, k_1, k_1k_2 and p factor sera.

RESULTS

Affinity for fibrinogen: All the S. aureus strains isolated from humans had affinity for fibrinogen by the passive

hemagglutination method. Thirteen of the 14 caprine strains of coagulase-positive staphylococci examined also had affinity for fibrinogen. The S. aureus strains isolated from giant rats (Cricetomys gambianus), sheep, rabbits and monkey also had affinity for fibrinogen (Table 7.4).

Protein A: Using the slide method, eight of the 9 strains of coagulase-positive staphylococci isolated from humans produced protein A. The strain that gave negative results with the slide technique was also negative with the microtitre plate method (Table 7.2). Of the 14 caprine strains of coagulase-positive staphylococci examined herein, 8 (57.1 per cent) produced protein A whilst the remaining 6 strains gave negative results with both the slide and microtitre plate methods. Two S. intermedius strains of canine origin produced protein A (Table 7.1). The three strains of S. aureus isolated from giant rats (Cricetomys gambianus) produced protein A. The S. aureus strain isolated from a rabbit failed to produce protein A when examined by the slide and the microtitre plate methods (Table 7.4).

Poly AB (β-N-acetyl glucosaminyl ribitol teichoic acid): Whilst all the S. aureus strains isolated from rabbits, monkey, sheep and giant rats (Cricetomys gambianums) produced poly AB, only five of the 9 S. aureus strains isolated from humans produced poly AB. Eleven of the 15 strains of S. aureus of caprine origin examined produced poly AB (Table 7.1).

Serological characteristics: None of the S. aureus strains isolated from humans agglutinated, a_4, e, h_1, i_1i_2 and 18 factor sera but agglutination was found with other antisera (Table 7.2). Three strains agglutinated each of a_5, b_1, h_2, n, 263-1 and O antisera respectively. Agglutination with k_1k_2 and 1 antisera was found with two strains. Of the first group of factor sera, only one strain agglutinated c_1 antiserum whilst agglutination with m factor serum was found in another strain (M226). Of the third group of factor sera, agglutination with 263-2 and p-factor sera was found with only one human strain of S. aureus. Generally, a_5 agglutinogen was found in only 2 strains, b_1 in 4 strains, h_2 in 3 strains, K_1K_2

and 263-1 agglutinogens were found in 3 strains. Of the 4 strains that did not produce poly AB, their serotypes were found to be h_2; a_5, b_1, K_1K_2, n, 263-1, 1; b_1, 263-1, O and K_1, K_1K_2 respectively. The only strain that did not produce protein A had serotype b_1, n, p (Table 7.2). The S. intermedius strain isolated from humans had serotype K_1, K_1K_2. K_1K_2 agglutinogen was also found in the S. intermedius strain of canine origin (Table 7.4).

Using the human factor sera, all the coagulase-positive staphylococci isolated from humans were serotypable.

Within the group I factor sera, agglutination with a_4 antiserum was found in only one of the 14 caprine strains of coagulase-positive staphylococci examined. a_5 factor serum was agglutinated by four strains whilst b_1 and c_1 antisera were agglutinated by 3 and 4 caprine strains of coagulase-positive staphylococci respectively (Table 7.3). Using the group II factor sera, agglutination was found with only h_2 and m factor sera. Five strains agglutinated h_2 antiserum and only one strain agglutinated

m antiserum. Using the group III factor sera, agglutination was found with 263-2, 1, o and p factor sera. Four strains agglutinated o antiserum and three other strains agglutinated 1 antiserum. Of the fourteen caprine strains of coagulase-positive staphylococci examined, 4 were found untypable serologically, whilst h_2, o and a_5 agglutinogens were common amongst the caprine strains serotypable. The S. hyicus subsp. hyicus strain (UI 120) examined had serotype a_5, c_1. Two strains that did not produce poly AB had serotypes a_5, c_1 and a_5, h_2, m, 263-2 whilst the other caprine strain of coagulase-positive staphylococci (UI 150) was untypable (Table 7.3).

The coagulase- positive staphylococcal strains isolated from sheep and other animals possessed both thermolabile and thermostable agglutinogens. They had different serotypes but a_5, c_1 and o agglutinogens were prevalent amongst the strains (Table 7.4).

DISCUSSION

Like phage typing, serotyping is a useful epidemiological marker system. However, some differences exist

with respect to the antigenic components of staphylococci of different sources (Marandon and Oeding, 1967). Also, whilst nearly 100 per cent of human S. aureus strains have been found typable by means of the slide agglutination technique (Cohen, 1972), different typability rates have been obtained by several authors for animal strains of S. aureus by using human factor sera. In bovine strains from infection and carriers, Malik and Singh (1960), Nag and Prasad (1965), Marandon and Oeding (1967) reported a typability rate of between 66 to 99 per cent. With canine staphylococci, Shimizu (1968) found none of the S. aureus strains examined serotypable. However, up to 85 per cent typability rates were found by Live and Nichols (1965), Hahn and Blobel (1968), Oeding, Marandon, Hajek and Marsalek (1970). Generally, comparatively few antigenic studies have been carried out on animal strains of S. aureus. Animal staphylococci in addition to sharing some agglutinogens with human S. aureus, may possess some agglutinogens not yet represented in human strains. Pillet, Isbir and Mercier (1950), Grun (1958a), Malik and Singh (1960), Live and Nichols (1965) noted that

animal strains of S. aureus agglutinated by human typing sera generally yielded weaker agglutination reactions and less characteristic serological patterns. However, Adegoke, Devriese, Godard, FIeurette, Brun and Ojo (1983a) using the simplified serotyping method of Fleurette and Modjadedy (1976) found that caprine strains of S. aureus not only reacted strongly with human factor sera, but also possessed some human-type agglutinogens with definite serotypes. In addition to serotyping S. aureus strains isolated from human and animals, serological assay for some staphylococcal products are often useful for the characterization of the strains encountered in practice. Thus characterization of protein A of S. aureus is not only useful for routine diagnostic purposes (Flandrois, Fleurette and Eyraud, 1975), it is also of epidemiological (Hoff and Hoiby, 1975) and taxonomic importance (Lachica, Genigeorgis and Hoeprich, 1979). In assaying for protein A, passive hemagglutination is the most widely used method. However, immunofluorescence (Lachica et al., 1979) and gel immunoprecipitation (Grov, Mylestad and Oeding, 1964) are also useful

for the assay of protein A. In the study of the characteristics of several strains of S. aureus, Hajek and Marsalek (1975) found that of the 77 strains of S. aureus isolated from humans, 92.2 per cent produced protein A. The authors also found the production of protein A in cow, sheep and poultry to be 40.2, 6.5 and 2.5 per cent respectively. In the biotyping and determination of protein A in S. aureus strains isolated from skin lesions of man, Pospisil, Skalka and Kabatova (1981) found that all the 150 strains examined produced protein A. In this present study, eight of the nine S. aureus strains isolated from humans produced protein A. Pospisil et al. (1981) also noted that whilst only 2 of the 15 bovine strains of S. aureus produced protein A, none of the coagulase-negative staphylococci they examined produced protein A. The absence of protein A from the coagulase-negative staphylococci examined by these authors is in agreement with the findings herein and those of Jensen, Neter, Gorzynski and Anzai (1961) and Aasen and Oeding (1971). Thus with the paucity of data on the occurrence of protein A in S. aureus of animal origin

(Lachica et al., 1979) and the irregular occurrence of protein A in bovine strains (Marandon and Oeding, 1967), it is interesting to note that several of the caprine strains of S. aureus examined herein possessed protein A (Table 7.3).

Although staphylococci may be associated with a variety of diseases in man and animals, the relative ease with which S. aureus protein A can be coupled to immune sera has been exploited in the immunological diagnosis of some infections. Hampton and Wasilauskas (1980) found staphylococcal coagglutination technique (CoA) to be rapid and sensitive for the detection of bacterial antigens in cerebrospinal fluid. Shuka and Gough (1979) also described the use of S. aureus protein A in immune electron microscopical detection of plant virus particles.

The ability of staphylococcal protein A to combine with the Fc part of immunoglobulins predominantly IgG of most mammalian species has also been used to detect influenza virus antibodies by slide coagglutination (Borel, Muhlestein and Gasser, 1980).

Affinity of S. aureus to fibrinogen by the passive hemagglutination technique has been found useful for the

detection of S. aureus. Flandrois and Carret (1981) noted that passive hemagglutination test was more suitable than plasma agglutination tests for the determination of the affinity of S. aureus to fibrinogen. Although most of the S. aureus strains examined herein had affinity for fibrinogen by the passive hemagglutination method (Table 7.1), the use of this test to detect clumping factor on the surface of S. aureus is yet to be used on a large scale despite the obvious advantages pointed out by some authors (Duthie, 1954; Bruckler, Schaeg and Blobel, 1974; Switalski, 1976). However, serotyping of S. aureus continues to be done where facilities for the preparation of factor sera and other necessary materials are available. Furthermore, whilst antibiotic sensitivities of S. aureus strains may change with antibiotic usuage and the emergence of resistant strains and phage susceptibilities may also change by interaction with wild phages by lysogenisation, transduction and by loss of a prophage, staphylococcal serotypes are relatively more stable (Pillet, Isbir and Mercier, 1950, Kretzschmar and Kretzschmar, 1962). Infact,

several comparative studies between phage types and serotypes have been carried out. Oeding and Williams (1958), Cohen, Smith and West (1963), Kretzschmar (1969), noted that S. aureus strains susceptible to phage 187 had agglutinogen K either alone or in combination with other agglutinogens. In this present study, the caprine strain of S. aureus susceptible to phage 187 (Table 6.4) possessed b_1 and o agglutinogens (Table 7.3). Thus the association of K agglutinogen with S. aureus susceptible to phage 187 may not be true of all animal strains of S. aureus. Comparative results between phage types and serotypes have also been obtained by other authors. Hofstad and Oeding (1962), Hofstad (1964) noted a correlation between agglutinogen 263-1 and 80/81 complex. This finding has been corroborated by several authors (Cohen et al., 1966; Kretzschmar, 1969; Galinski and Krynski, 1970). A correlation between agglutinogen 263-1 and the 80/81 phage complex was found amongst two S.aureus strains isolated from humans (Tables 6.3; 7.2). However, the only caprine strain of S. aureus susceptible to the 80/81 phage complex did not agglutinate 263-1 antiserum

but it agglutinated a_4, a_5, b_1 and o antisera. This caprine strain of S. aureus was the only one that possessed a_4 agglutinogen amongst all the strains of S. aureus examined in this study. Thus correlation between 263-1 agglutinogen and the 80/81 phage complex may be common amongst human strains of S. aureus.

Hitherto, antigenic structure, sensitivity to bacteriophages and other characteristics have been used to differentiate S. aureus of human and less frequently of animal origin (Oeding, Maran on, Hajek and Marsalek, 1970). Human-type agglutinogens a_5, o, h_2 and 1 found herein amongst some animal S. aureus strains are in agreement with the findings of Slanetz and Bartley (1962) of Marandon and Oeding (1967) who examined strains from other animals. Furthermore, in the examination of several biotypes of S. aureus for the presence of polysaccharide A, Oeding (1974) found that only 48 per cent of the porcine strains studied contained poly AB (β-N-acetyl-glucosamine ribitol teichoic acid). However, 11 of the 14 caprine strains of S. aureus examined possessed poly AB (Table 7.3). Production of poly AB by other animal strains of S. aureus was relatively

irregular (Table 7.4).

Although relatively good serotypability rates have been obtained with the S. aureus strains of human and animal origins examined herein, nevertheless, phage typing of S. aureus is presently of wider use. However, as more strains from different hosts become serotypable and the simplified techniques of Fleurette and Modjadedy become more standardized and acceptable world-wide, both phage typing and serotyping may continue to be used for epidemiological and epizootiological investigations of S. aureus infections.

Table 7.1 Production of protein A and poly AB by coagulase-positive staphylococci isolated from humans and animals

Host	Affinity for fibrinogen	Poly AB	PRODUCTION OF: Protein A		Total number tested
			Slide	Tube	
Man	9*	5	8	0	9
Goats	13	11	5	2	14
Sheep	1	1	1	0	1
Giant rats (Cricetomys gambianus)	3	3	3	0	3
Dogs	3	1	2	1	5
Poultry	1	1	1	0	2
Monkey	1	1		±	1
Rabbits	1	1	0	0	1

*: Number showing positive reactions

0: Negative reaction; ±: Weak reactions

Table 7.2 Serological characteristics of coagulase-positive staphylococci isolated from humans

Strain No.	Protein A	Affinity for fibrinogen	Poly AB	FACTOR SERA																	
				a_4	a_5	b_1	c_1	e	h_1	h_2	k_1	k_1k_2	i_1i_2	m	n	263-1	263-2	l	o	p	18
				GROUP I								GROUP II				GROUP III					
M221	+	+	-	-	-	-	-	-	-	+++*	-	-	-	-	-	-	-	-	-	-	-
M222	+	+	+	-	-	-	-	-	-	++*	-	-	-	-	-	-	-	-	-	-	-
M223	+	+	-	-	++*	+++	-	-	-	-	-	+++	-	-	++	++	-	+++	-	-	-
M224	+	+	+	-	++*	-	-	-	-	-	-	-	-	-	-	+++	-	-	-	-	-
M225	-	+	+	-	-	++	-	-	-	-	-	-	-	-	++	-	-	-	+++	-	-
M226	+	+	+	-	++*	-	-	-	-	++*	-	-	-	+++	-	-	+++	++	-	-	-
M232	+	+	-	-	-	+++	-	-	-	-	-	-	-	-	-	++	-	-	++	-	-
M253	+	+	-	-	-	-	-	-	-	-	+++*	+++*	-	-	-	-	-	-	-	-	-
M337	+	+	+	-	-	-	++	-	-	-	-	-	-	-	++	-	-	-	++	++	-

*Thermostable agglutinogen (after autoclaving: 115/ lb/sq. in for 30 mins.)

+++: Numerous agglutinates;

-: Negative reaction.

Table 7.3 Serological characteristics of coagulase-positive staphylococci isolated from goats

Strain No.	Protein A	Affinity for fibrinogen	Poly AB	FACTOR SERA																				Serotype
				a_4	a_5	b_1	c_1	e	h_1	h_2	K_1	K_1K_2	i_1i_2	m	n	263-1	263-2	l	o	p	18	Water	
				GROUP I							GROUP II							GROUP III						
UI 27	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		NT
UI 96	+	+	+	+++	+++*	+++	-	-	-	-	-	-	-	-	-	-	-	-	+++	-	-	-		a_4, a_5, b_1, o
UI 118	+	+	+	-	-	+++	-	-	-	-	-	-	-	-	-	-	-	-	+++	-	-	-		b_1, o.
UI 120	-	-	-	-	++*	-	++*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		a_5, c_1*
UI 145	+	+	+	-	-	++	-	-	-	-	-	-	-	-	-	-	-	-	+++	++	-	-		b_1, o, p
UI 150	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		NT
UI 153	+	+	+	-	-	-	-	-	-	+++*	-	-	-	-	-	-	-	++	++	-	-	-		h_2, l, o.
UI 160	+	+	+	-	-	-	++	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-		c_1, o
UI 166	+	+	-	-	++*	-	-	-	-	+++*	-	-	-	++	-	-	+++	-	-	-	-	-		a_5, h_2, m, 263-2
UI 327	-	+	+	-	++	-	-	-	-	++*	-	-	-	-	-	-	-	++	-	-	-	-		a_5, h_2, l.
UI 331	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		NT
UI 154	+	+	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		NT
UI 161	-	+	+	-	-	-	-	-	-	+++*	-	-	-	-	-	-	-	-	-	-	-	-		h_2
UI 334	-	+	+	-	-	-	-	-	-	+++*	-	-	-	-	-	-	-	++	-	-	-	-		h_2, l.

*Thermostable agglutinogen; +++: Numerous agglutinates; - : Negative reaction;
NT; Not typable; d: doubtful reaction.

Table 7.4 Serological characteristics of coagulase-positive staphylococci isolated from sheep, rabbits, giant rats (Cricetomys gambianus) and other animals

Strain No.	Host	Protein A	Affinity for fibrinogen	Poly AB	FACTOR SERA: GROUP I a_4	a_5	b_1	c_1	e	h_1	h_2	k_1	K_1K_2	i_1i_2	m	n	263-1	263-2	GROUP II 1	o	p	GROUP III 18	Water	Serotype
SUI 116	Sheep	+	+	+	-	-	+++	-	-	-	-	-	++	-	-	-	-	-	++	+++	++	-	-	b_1, k_1k_2, l, o, p
Rb 314	Rabbit	-	+	+	-	-	-	-	-	-	+++*	-	-	-	-	-	-	-	-	-	-	-	-	h_2
R167	Giant rat	+	+	+	-	++*	-	++*	-	-	-	-	-	-	-	-	-	-	-	-	+++	-	-	a_5, c_1, p
R168	" "	+	+	+	-	++*	-	++*	-	-	-	-	-	-	-	-	-	-	-	-	+++	-	-	a_5, c_1, p
R169	" "	+	+	d	-	++	-	-	-	-	-	-	-	-	-	-	-	-	-	++	++	+++	-	a_5, o, p, 18
D238	Dog	+	+	+	-	-	-	++*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	c_1
D240	"	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NT
D241	"	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NT
D242	"	+[1]	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NT
D243	Dog	+	+	-	-	++*	-	-	-	-	-	-	++*	-	-	-	-	-	-	-	-	-	-	a_5, k_1k_2
Mo335	Monkey	+	+	+	-	-	++	+++*	-	-	-	-	-	-	-	-	-	-	-	+++	-	-	-	b_1, c_1, o
A295	Poultry	+	+	+	-	-	-	++	-	-	-	-	-	-	-	-	-	-	-	+++	-	-	-	c_1, o

* : Thermostable agglutinogen; +++ : Numerous agglutinates

\- : Negative reactions; NT : Not typable;

d : doubtful reaction; 1 : Microtitre plate tes.

CHAPTER 8

CHARACTERISTICS OF UNCLASSIFIABLE STRAINS OF STAPHYLOCOCCI

INTRODUCTION

Generally the identification of Staphylococcus species found in practice is important as some strains are frequently encountered in infections which often demonstrate a high level of resistance to a variety of antibiotics (Devriese, and Derycke, 1979: Archer and Tenenbaum, 1980). Often a few other strains are unclassifiable by using some phenotypic characteristics. However, the application of molecular systematics has facilitated the identification of several strains of staphylococci (Kloos and Wolfsholl, 1980).

In the eighth edition of Bergey's Manual of Determinative Bacteriology, S. aureus S. saprophyticus and S. epidermidis were the only staphylococcal species recognized (Baird-Parker, 1974). However, S.saprophyticus and S. epidermidis have been found to be genetically

heterogenous and so Kloos and Schleifer (1975a), Schleifer and Kloos (1975a) amployed phenotypic and molecular characterization procedures in the amended descriptions of these staphylococcal species. Thus, acid production from a variety of carbohydrates, assays for cell wall peptidoglycan and teichoic acids often employed in the descriptions of novel species of staphylococci.were also done in this present study.

MATERIALS AND METHODS

Strains. Fourteen strains of coagulase-negative and three coagulase-positive staphylococci isolated from goats and sheep which were unclassifiable by using simple phenotypic markers were screened for chemotaxonomic and molecular characteristics.

Preparation of cell walls. Thirty millilitres of yeast extract-glucose peptone broth (Appendix 6.A) was inoculated with each strain under test after which the medium was incubated at 35°C overnight using a shaker. The broth was checked for purity prior to its addition to 1,500 ml of fresh yeast extract-glucose peptone broth. The medium was

later reincubated as above and centrifuged at 4,5000 r.p.m. after incubation for 20 min. Glass pearls were generously added to the deposit with occasional addition of distilled water. The cell suspension was distintegrated in a homogenzer (Vibrogen-Zellmuhle, Buhler, Tubingen) for 20-30 min. The preparation was then examined microscopically for the presence of broken cell walls. The glass beads used were extracted by mild sunction pressure. The suspension was centrifuged again at 1,800 rpm for 20 min after which 5 ml of 0.005 M phosphate buffer (pH 7.8)were added. The preparation was mixed with an "ultra-turrax" mixer and heated to boiling (100^{o}C) for 20 min. It was later cooled to 35^{o}C, 1.0 ml of toluence and a few granules of trypsin (Merch, Darmstadt, W. Germany) were added. The suspension was incubated at 35^{o}C overnight after which it was centrifuged at 1,800 rpm for 20 min. The deposit was washed 4 times mixing with the "ultra-turrax" mixer with every washing. One millilitre of distilled water was later added to the suspension and mixed as above. Extraction of water from the suspension was done by 1 h period of

freezing (-20°C) followed by intense freeze-drying (Wkf, Lu5) overnight. The Petri dish containing the suspension was covered with perforated aluminium foil. After freeze-drying for 24 h, the preparation constituted the cell wall used for the determination of peptidoglycan types and teichoic acid composition.

Determination of cell wall peptidoglycan types: 0.2 millilitre of 4N hydrochloric acid (HCl) was added to 2.0 mcg of cell wall in a small bottle. The preparation was mixed after which it was sealed and heated for 1 h at 100°C in a hydrolyzer. After cooling, the suspension was dried in an aerated water bath (40°C). A little amount of double-distilled water was then added and mixed after which the partial acid hydrolysates were spotted onto a Schleicher-Schull 2043 b Mgl paper in a two-dimensional descending paper chromatography. Isopropanol-acetic acid-water solvent system was applied in the direction of the machine and α-picoline-ammonium hydroxide-water solvent was applied in the other direction with a running time of 2 x 24 (48 h) for each direction. The temperature of the chromatography chamber

was maintained at 27 to 28°C. Chromatogram sheets were later dried at 80 to 100°C briefly after which they were sprayed in a fume chamber with Nihydrin.

Determination of cell wall teichoic acid composition: The method described by Wolin, Archibald and Baddiley (1966) was used. Fifteeen microgrammes of cell walls were extracted in 1.5 ml of 2N hydrochloric acid (HCl) for about 24 h at room temperature. The extracted cell walls were later centrifuged and supernatants obtained were hydrolyzed at 100°C for three hours. The hydrolysates were spotted on Whatman No. 1 paper and separated in propanol- ammonium hydroxide-water solvent by ascending chromatography. Glycerol, ribitol and anhydroribitol and their derivatives were detected by using periodate-Schiff reagents for glycols. Based on the products of hydrolysis, the teichoic acid type was recognized as described by Work (1971). The occurrence of sugar or polyol phosphates and the lack of unsubstituted polyols in the acid hydrolysates of the cell extracts indicated that an atypical teichoic acid was present. In some cases where both glycerol and ribitol were found in the acid

hydrolysates, the teichoic acids were checked more carefully.

Determination of pH after anaerobic growth:

Fifteen millilitres of yeast extract-glucose broth in 160 x 16 mm diameter tubes were used for growing the strains under test. The tubes were incubated in a static position for five days at 30°C after which the pH of each solution was determined colorimetrically with a pH meter (Reibold, Wein).

Determination of lactic acid configuration: After determining the pH of each suspension, the cells were centrifuged and the clear supernatant was used for lactic acid determination. L-lactate dehydrogenase from rabbit muscle (Boehringer, Manheim) was applied to estimate the content of L-lactate in each culture medium. Oxidation of lactate to pyruvate was determined by the spectrophotometric measurement of the product of reaction at 340 and 360 nm. The D-lactate content of each broth was estimated by using the D-lactate dehydrogenase obtained from Leuconostoc mesenteroides cells. L. mesenteroides (ATCC 12291) was grown in a stationary culture in 10 litre

batches of MRS broth (Man, Rogosa and Sharpe, 1960) at 30°C for 12 to 16 h. A thick suspension of washed cells in 0.002 M phosphate buffer (pH 7.5) was disintegrated with glass beads in a cell mill. The soluble supernatant obtained after the removal of the glass beads and after centrifugation at 20,000 rpm for 20 min was fractionated with ammonium sulphate. The fraction precipitating between 50 to 75 per cent saturation was collected (Dennis, 1962). The precipitate was later dissolved in water to give a suspension containing 3 to 6 mg/ml protein. The quantitative content of the L-and D-lactate of the broth cultures was determined in samples diluted appropriately to give an increase of extinction at 360 nm of 0.15 to 0.30. The L- and D- lactate content of an uninoculated broth was substracted from each reading.

Isolation of deoxyribonucleic acid (DNA). Thirty millilitres of yeast extract-glucose broth without glycine was used for the growth of the unclassifiable strains for 18 to 24 h at 35°C. The broth suspensions were later checked for sterility microscopically and

later seeded into 300 ml of fresh medium after which it was reincubated at 35°C for 6 h using a shaker. A few granules of penicillin was added and the suspension was left at 35°C for 1 h after which it was centrifuged at 4,500 rpm for 20 min. To the deposit were added a few ml of saline-EDTA (Ethylenediamine tetra acetic acid, pH 8.0) lysostaphin (1 mcg/ml) and a few granules of lysozyme (Serva, Heidelberg). Protoplast-like slime formation became obvious after the addition of a few drops of 25 per cent sodium lauryl sulphate (SDS). Twenty millilitres of 5M sodium chloride (NaCl) was added to the suspension to give a final concentration of 1 M NaCl. After mixing properly, the suspension was heated for 20 min at 60°C after which it was cooled down under running tap water. An equal amount of chloroform (1:1) was added to the suspension after which it was shaken for 15 to 20 min followed by centrifugation at 4,500 rpm for 20 min. To one volume of the supernatant was added about half a volume of iso-propanol. The preparation was later transferred to 4°C to precipitate more deoxyribonucleic acid (DNA). To the deposit was added 30 to 60 ml of twice

distilled water after which it was transferred to a 60°C water bath shaker until the preparation appeared homogenous. The DNA meshwork was washed in 10 to 50 ml of 0.1xSSC (Standard-Sodium citrate) buffer (pH 7.0) and mixed properly. The preparation was further dissolved at 37°C for about 1 to 3 h with regular observation for the appearance of dissolved meshwork of the DNA preparation.

10 x SSC buffer was added to the preparation in a ratio of 1 to 11 (50 ml of DNA suspension + 5 ml of 10 SSC buffer) and the above processes of dissolving and washing the DNA were repeated until the preparation appeared clear. Chloroform was then added. Preparations with high protein content required longer periods for clearing. To concentrate the DNA, 9 ml of 10 x SSC buffer was added to about 50 ml of homogenous suspension of the DNA meshwork. A final concentration of 1 x SSC buffer was achieved with the addition of 9.5 ml of 5 M NaCl (Sodium chloride) in twice distilled water.

CONCENTRATION OF DNA. 20 millilitres of 4 per cent N-cetyl-N, N, N-trimethyl ammonium bromide (Merck, CTAB

Darmstadt, West Germany) was added to the preparation so that its final concentration was 1 per cent. The concentration of the CTAB was further adjusted to 0.6 M with twice distilled water. The preparation was later centrifuged and the deposit was suspended in 1 M NaCl for 18 hours at 35°C. The same processes of dissolving and centrifuging until the preparation was clear were repeated. More 5 M NaCl had to be added to the completely-dissolved DNA fragments to bring the concentration to 1 M NaCl after which a few amount of bovine ribonuclease (Serva, Heildeberg) was added. The suspension was transferred to a 35°C water bath shaker for 10 to 15 min. Few amount of proteinase K (Merck, Darmdstadt) was added and the preparation was allowed to stay for 45 min in a 35°C water bath shaker. The suspension was later dissolved in 0.1 x SSC buffer as described before after which it was dialysed in 1 x SSC buffer (pH 7.0) over-night at 4°C using a mixer. Using 1 x SSC buffer as a blank control, the ratio of the DNA to the protein content of the DNA sample was determined spectrophotometrically (Beckman, Model 24).

RADIO ACTIVE LABELLING OF DNA

The Ependorff reaction vials to be used for radioactive labelling of the DNA were first siliconized by adding 1 ml of silicon (Serva) into each vial (15 vials for 1 ml) with washing and rinsing of the inner sides after which they were transferred to a hydrolyzer at 100°C for 1 h. Each DNA preparation was then labelled in siliconized Eppendorff reaction vials with 6-^{3}H thymidine (0.5 m c_i, New England Nuclear) and 5 µg of 5-fluorode oxyuridine (Sigma). Dilutions with distilled water and DNase polymerase were made for each DNA preparation and the radioactive reagents used after which the vials were centrifuged prior to the addition of DNase polymerase. The vials were later heated to 15°C for 60 min (using a water chamber). The labelled DNA preparation was passaged through a Sephadex G column previously washed with 10 m mol Tris HCl in 0.1 M mol Na_2EDTA (pH 8.0) after which special radioactivity counts of each DNA preparation were obtained with the addition of 5 ml of scintillation water. The labelled DNA preparations in Eppendorff vials were later centrifuged

and the deposits were dried in a dessicator. Each preparation was dissolved in 500 ul of 0.1 x SSC buffer with occasional shaking (about 30 mins). 100 ul of 10 x SSC buffer was then added to bring the final concentration from 0.1 to 1 x SSC. To 5 ul of this labelled DNA preparation, were added 1 ml of twice-distilled water and 10 ul of scintillation water in a scintillation bottle. The DNA peak was then measured as aforementioned using a checking time of 4 min (10^4 counts/min).

DNA-DNA HYBRIDIZATION. Nitrocellulose filters (Sartorius, 50 mm Ø and 0.2 um pore size) were soaked in 3 x SSC buffer and heated to 60°C for 10 mins to remove any ultra violet absorbing substance which might interfere with the DNA binding assay. DNA solution was diluted with 0.1 x SSC buffer to give 20 µg/ml after which it was denatured at room temperature for 30 min with 1.0 ml of 6N NaOH. Later, the preparation was transferred to an ice-bath for at least 1 h. After adjustments with twice-distilled water, 3 x SSC and 18 x SSC buffers, the DNA preparation was neutralised with

1.00 ml of 6 N HCl (final pH 7.0 - 7.5). The denatured DNA preparation was immediately loaded onto the filters by mild sunction. After washing the nitro-cellulose filters with 3 x SSC buffer, they were air dried with vacuum pressure overnight at room temperature. They were then heated at 80°C for 4 h and stored at 4°C until required.

Measurement of filter-bound DNA. The amount of DNA bound to the filters was determined by the optical density at 260 nm of each DNA solution before and after passage through the filter. The concentration of the DNA was also measured with diphenylamine (Burton, 1968) as follows: release DNA with 0.5 ml of 5N perchloric acid ($HClO_4$) from pre-punched mini-filters (Ø5 mm) obtained from above. After covering the tubes with aluminium foil, they were incubated at 70°C for twenty minutes. The preparation was transferred to an ice-bath after which 1.0 ml of diphenylamine reagent was added. Control tubes of known DNA concentrations (10, 20, 30, 40 µg DNA) and a DNA-free tube were set up. After shaking, the tubes were incubated at 35°C for 16 to 20 h. The colour-

intensity of each preparation was measured photometrically at wavelength of Hg 578. Two tubes were used for each DNA filter.

Preincubation of filters. The small discs (minifilters Ø5 mm) punched out of the larger ones as well as filter-bound DNA controls were separately placed in Eppendorff reaction vial . Each vial was preincubated with 500 µl (0.5 ml) of preincubation medium (Denhardt, 1966) in 3 x SSC buffer at 60°C for 4 h. 2 ml of 10 per cent formamide (Merck) was added to the 3 x SSC reassociate buffer prior to incubation in order to give restrictive hydridization condition by lowering the melting point of the DNA. Adequate amount of each labelled DNA was diluted with hybridization fluid (3 x SSC in 10% formamide). 4 µg of each DNA was diluted with 4 ml (enough for 20 vials) of the hybridization fluid after which each labelled DNA solution was sheared by three passages through a French pressure cell (American Inst. Co., Maryland, USA) at 1,000 cells pressure. Shortly before use, the labelled DNA was further diluted to 2 µg/ml and heat-denatured at 100°C for 10 mins in a

water bath followed by quick cooling down at 4°C in an ice bath. Excess preincubation medium was removed from the labelled mini-filters by rinsing them on both sides with 1.0 ml of 3 x SSC (pH 1.0) after which they were blotted dry thoroughly at 80°C for 1 h.

RADIOACTIVITY COUNT. The amount of labelled input DNA bound to a certain DNA_f was measured by counting the radioactivity on the membrane filter using 3.0 ml of scintillating fluid with a liquid scintillator counter (Beckman Model LS 230). The percentage ratio between ^3H-labelled DNA which hybridized with unlabelled, immobilized DNA_f of each of the strains examined herein and the relative amount of DNA_s which hybridized with the DNA_f of the same strain as the labelled strain was taken as the homology value of the strain (Tables 8:3. 4; Appendix 6.B).

Determination of the DNA base content. The guanine plus cytosine (G + C) content of the DNA of some of the strains examined was determined spectrophotometrically (Gilford, Instruments, Ohio, USA) from the purified DNA preparation

according to the method of Ulitzur (1972) (Fig. 8.A). A melting point range of 60° to 75°C for 70 minutes was used. The melting point (Tm) of the DNA in the hybridization buffer was determined spectrophotometrically and was also manually calculated (Fig. 8.B). The G + C ratio was then determined by using the formula of Deley (1970).

Determination of the FDP (Frucose-1, 6-diphosphate) class. Two loopfuls of each bacterial strain grown overnight on a sugar-free medium were suspended in 1 millilitre of Tris-HCl-buffer (0.5 M; pH 8.6) in an Eppendorff reaction vial. The suspension was mixed thoroughly and centrifuged in an Eppendorff centrifuge for 30 seconds after which the supernatant was discarded. To the deposit were added: 0.1 ml of 0.5 M $HClO_4$ (Perchloric acid) with mixing followed by 0.2 ml of 0.5 M Tris-HCl-buffer (pH 8.6); 50 µl of 0.4 M EDTA (Ethylene diamine tetra acetic acid pH 8.6 - 8.8); 50 ul of 0.56 M hydrazin sulphate ($N_2H_5SO_4$, pH 8.6) and 50 µl of 25 ug/ml of fructose-1, 6-diphosphate. The vials were incubated in a 37°C water bath for 30-60 min with occasional shaking every 15 min after which 0.1 ml of 20 per cent trichlor-acetic acid

(TCA) was added. The vials were centrifuged for half a minute. Into small glass tubes were added 0.1 ml of each supernatant followed by 0.1 ml of 0.75 N NaOH (Sodium hydroxide) after which the mixture was incubated at room temperature for ten minutes. 0.1 ml of 0.1 per cent 2,4-diphenyl-hydrazine (Sigma) was then added. The suspension was then incubated for 20 mins in a 37°C water bath after which 0.7ml of 0.75 N NaOH was added. Purple colour (positive) was indicative of Aldolase class I and brown (negative) Aldolase II.

Acid production from carbohydrates. Carbohydrate fermentation tests were carried out as described in Chapter 4.

Novobiocin susceptibility test. This was carried out as described in Chapter 3.

RESULTS

Cell wall peptidoglycan types. Three cell wall peptidoglycan types were found amongst the strains examined. Peptidoglycan type L-Lys-Ala-Gly_4 was found in 6 strains of coagulase-negative staphylococci isolated from goats and sheep (Table 8.1). Seven coagulase-negative

staphylococcal strains of caprine origin had peptidoglycan type L-Lys-Gly_{5-6} which was also found amongst three caprine strains of coagulase-positive staphylococci (Tables 8.2a; 2b). One caprine strain of coagulase-negative staphylococci possessed peptidoglycan type L-Lys-Gly_4 (L-Ser) (Table 8.2a).

Cell wall teichoic acids. Ribitol and glycerol teichoic acids were found in five caprine strains of coagulase-negative staphylococci. These strains also had similar peptidoglycan type L-Lys-Gly_{5-6} (Table 8.2a). Two strains had glycerol with glucose teichoic acids whilst another strain of coagulase-negative staphylococci of caprine origin possessed glycerol, glucose, galactosamine and N-acetylglucosamine teichoic acids. The two strains that had glycerol and glucose teichoic acids had different types of cell wall peptidoglycan (Table 8.2a).

One caprine strain of coagulase-positive staphylococci possessed glycerol and glucosamine teichoic acids whilst the two other strains examined had ribitol and glucosamine teichoic acids (Table 8.2b).

pH in glucose broth. After anaerobic growth in glucose

broth, the pH of the broth cultures tested varied from strain to strain. Of the caprine strains of coagulase-negative staphylococci that possessed identical type of cell wall peptidoglycan, the pH ranged between 4.79 and 4.94 whilst the two coagulase-negative staphylococcal strains of ovine origin had pH of 6.66 and 4.98 respectively (Table 8.1). Four caprine strains of staphylococci with identical cell wall peptidoglycan and teichoic acids had a pH of 6.07 to 6.18. Varied results were obtained with other strains (Table 8.2a). The pH of the coagulase-positive staphylococci tested was almost identical (Table 8.2b).

Lactic acid configuration. Lactic acid configuration type L was common amongst the caprine and ovine strains of coagulase-negative staphylococci that possessed peptidoglycan type L-Lys-Ala-Gly_4 (Table 8.1). Four other caprine strains of coagulase-negative staphylococci that had peptidoglycan type L-Lys-Gly_{5-6} and identical cell wall teichoic acids produced little amounts of L-(+)-lactate. Four strains produced varying amounts of L-(+)- and D-(-)- lactate (Table 8.2a). L-(+)-lactate was common

amongst the coagulase-positive staphylococci examined (Table 8.2b).

Aldolase class. Aldolase class I characteristic of staphylococci was found in all the strains examined.

Acid production from carbohydrates. Production of acid from a variety of carbohydrates were found amongst the strains examined. Some strains with similar cell wall peptidoglycan and teichoic acids produced acid from almost the same type of carbohydrates (Tables 8.1, 2a, 2b).

Deoxyribonucleic (DNA) base content. The guanine plus cytosine (G:C) ratio determined for three caprine strains of coagulase-negative staphylococci ranged from 32.9 to 34.6% (Tables 8.1; 2a; Appendix 6.B).

Novobiocin susceptibility: Most of the coagulase-negative staphylococci examined were resistant to novobiocin.

DNA-DNA hybridization. Strain UI 109 had a homology value of 60 per cent making it closely related to S. gallinarum whilst strain UI 143 was not related to S. xylosus, S. gallinarum or S. aureus. Strain SUI 58 with a homology value of 50 per cent appeared closely related

to S. lentus whilst strains UI 1 and UI 136 are homologous to S. sciuri (Tables 8.3,4).

DISCUSSION

In medical and veterinary microbiology, some strains of staphylococci are often encountered which may not be identifiable to species-level by using simple phenotypic markers. Thus Szynkiewicz, Krynski, Binek and Becla (1981) reported the incidence of some strains of staphylococci isolated from dogs in Poland which were difficult to classify. Of the eight coagulase-negative, enterotoxigenic strains of cocci isolated by Lotter and Genigeorgis (1975) from a number of sources, five strains could not be identified with certainty. The strains were found to have produced phosphatase, acetoin and heat-stable nuclease. In addition, the five strains produced acid from mannitol and the guanine plus cytosine (G:C) content of their deoxyribonucleic acid (DNA) ranged between 32.7 and 37.6. Thus the authors found the strains to have got some typical characteristics of S. aureus, S. epidermidis or S. saprophticus. Furthermore, the

strains produced heat-stable nuclease, a feature characteristic of S. aureus (Baird-Parker, 1974). Although the characteristics of the coagulase-negative staphylococci examined herein appeared similar to those described by Lotter and Genigeorgis (1975) and also possessed the characteristic features of staphylococci, most of them could not be identified with certainty (Tables 8.1, 2a).

At present, the nomenclature of staphylococci has been unified at two valid names S. aureus Rosenbach 1884 (Cowan, 1956) and S. epidermidis Winslow and Winslow, Evans, 1916 (Hugh and Ellis, 1968). Recently several new species of staphylococci have been isolated from man and animals (Schleifer and Kloos, 1975a; Hajek, 1976; Devriese, Hajek, Oeding, Meyer and Schleifer, 1978; Kloos and Schleifer, 1983). Prior to the detailed characterizarion of these new species of staphylococci, they were probably "unclassifiable" by using simple phenotypic markers. Schleifer and Kocur (1973) isolated some strains of staphylococci (Group III) which were found to possess some peculiar characteristics. The strains were not

named as the authors suggested further extensive comparative studies of the strains. The strains were characterized by the possession of unique peptidogylcan type L-Lys-Ala-Gly_4 and different cell wall teichoic acids. In this present study, peptidoglycan type L-Lys-Ala-Gly_4 associated with S. sciuri and S. lentus (Kloos, Schleifer and Smith, 1976) was also found amongst some caprine and ovine strains of coagulase-negative staphylococci which appeared to have got some characteristics similar to S. sciuri and S. lentus yet possessed some other peculiar characteristics which make them distinct and thus remain unnamed (Table 8.1). Kloos, Zimmerman and Smith (1976) isolated five strains of staphylococci from sheep skin which appeared to be related to S. xylosus and S. sciuri. The strairs were designated as unnamed Staphylococcus sp2 and they were characterized by their orange pigment and small colony diameter, unique umbonate colony profile, lack of acid production from D-(+)-galactose and a combination of other characteristics. Furthermore, the authors isolated forty-eight strains of staphylococci from a variety of animal species that

appeared to be somewhat intermediate in relationship between S. xylosus and S. cohnii. The strains were also designated as unnamed Staphylococcus sp 3. The strains were found to be different from S. xylosus in having only glistening colonies with an entire edge, no or occasionally very weak anaerobic growth in a thioglycollate medium, no reduction of nitrates, no or only weak production of acid aerobically from D-(+)-galactose and no acid from D-(+)-mannose, D-(+)-xylose, [illegible]-lactose, sucrose or D-(+)-turanose with cell wall peptidoglycan type L-Lys-Gly_{5-6} or L-Lys-Ala-Gly_4. Kloos et al. (1976) also isolated some unnamed strains of Staphylococcus sp 5 which were characterized by a unique colony morphology and pigment patterns. The strains also possessed the unique character combination of nitrate reduction, slight resistance to novobiocin, moderate caseinolytic, gelatinase, lecithinase and phosphatase activities and lipolytic activities on triolein and tributyrin and also had peptidoglycan type L-Lys-Gly_{3-5}, L-[illegible]$er_{0.6-1.5}$. Nonetheless, the strains of coagulase-negative staphylococci examined here differed from the aforementioned ones both

in morphological and biochemical characteristics (personal observations). The strains examined here also differed from those described by Gramoli and Wilkinson (1978). These authors could not identify with certainty four of the 13 coagulase-negative, weakly heat-stable deoxyribonuclease producing staphylococci isolated from various sources. The authors noted that one of the unidentified strains possessed some characteristics which were similar to S. epidermidis by having a glucose-containing glycerol teichoic acid and a high serine content in its peptidoglycan but unlike S. epidermidis, it failed to produce large colonies and acetoin. Much as there are uncertainties in the identification to species level of some coagulase-negative staphylococci, a few coagulase-positive staphylococcal strains possess some unique characteristics different from S. aureus (Hajek, 1976; Devriese et al., 1978). Kloos, Zimmerman and Smith (1976) even isolated some unnamed strains of coagulase-positive staphylococci (Staphylococcus sp 1) from animal skin. These strains were found to be different from the typical S. aureus by having glycerol and galactosamine as components of teichoic acids, weaker

anaerobic growth in a thioglycollate medium, no or only weak haemolysin activity on bovine blood agar, usually no or only weak aerobic acid production from maltose and D-mannitol and lack of pigmentation.

Generally several phenotypic characterization procedures are employed in the identification of the Staphylococcus species (Baird-Parker, 1974). Lactic acid configuration used in this present study facilitated the grouping of the "unclassifiable" coagulase-negative staphylococci isolated from goats and sheep (Tables 8.1; 2a). Some strains produced only L(+)-lactate while other strains produced both D-(-)- and L(+)-lactate although in varying amounts.

The lowering of the pH values of glucose broth when staphylococci are grown therein anaerobically to below pH 5.0 is known to be typical characteristic of staphylococci (Evans, Bradford and Niven, 1955). However, although some strains examined here satisfied this characteristic, several other ones which are "sensu stricto" staphylococci produced higher values (Table 8.2a). Also, the knowledge of the guanine plus cytosine (G + C)

content of the deoxyribonucleic acid (DNA) forms a relatively important basis for taxonomic considerations as it has contributed immensely to the understanding of the relationship of staphylococci to other Micrococcaceae. Staphylococci have a low GC content of about 30 to 41 per cent whilst micrococci have a high GC content which ranges from 54 to 75 per cent (Kocur, 1973). The GC content of the deoxyribonucleic acid (DNA) of the strains examined here are thus in agreement with those of typical staphylococci. In differentiating S. aureus from the newly-described coagulase-positive staphylococci, cell wall composition has been found useful (Hajek, 1976; Devriese et al., 1978). Strains of S. aureus and S.hyicus do not contain or contain only traces of L-serine in their peptidoglycan while most strains of S. intermedius have serine. Furthermore, ribitol teichoic acid which is a typical constituent of the cell walls of S. aureus is replaced by glycerol teichoic acid in S. intermedius and S. hyicus. Other differences as to the allosteric specificity of L-lactate dehydrogenase, esterase pattern and the catabolism of lactose or galactose are useful for

differentiating these strains of coagulase-positive staphylococci. Also S. aureus strains usually ferment glucose to DL-lactic acid whereas S. intermedius and S. hyicus strains produce only L-lactate (Kloos and Schleifer, 1981). In this present study, the unidentifiable caprine strain of coagulase-positive staphylococci (UI 150) had peculiar glycerol containing glucosamine teichoic acids and also produced L-lactate. The cell wall teichoic acids are quite different from those of other coagulase-positive staphylococci. Furthermore, other phenotypic characteristics of this strain are quite distinct from S. aureus, S. intermedius and S. hyicus (Devriese, personal communication).

In the DNA-DNA hybridization studies of some strains of staphylococci, Schleifer, Meyer and Rupprecht (1979) found that the relative binding of DNA strains of various species of staphylococci to S. sciuri (Sc 116) at an optical criterion of 60°C was very low and in the range of 5 to 21 per cent. Thus, the relatively fairly good homology exhibited by some coagulase-negative staphylococci examined herein (Table 8.3) may be characteristic of some "new species" of staphylococci that may be related to S. sciuri or S. lentus.

Table 8.1. Cell wall peptidoglycan type and lactic acid configuration of some "unclassifiable" strains of coagulase-negative staphylococci isolated from goats and sheep

Strain	Host	Novobiocin sensitivity 30 mcg	Lactic acid configuration	pH of glucose medium (anaerobic growth)	Type of peptidoglycan	G:C Ratio	Oxidase test	FDP-aldolase class	Maltose	Trehalose	Melibiose	Cellibiose	Xylose	Mannose	Sucrose	Turanose	Raffinose	Arabinose	Mannitol	Fructose	Ribose
UI 1	Goat	R[1]	L	4.79	L-Lys-Ala-Gly_4	ND	+	I	*+	+	+	+	+	+	+	+	-	-	+	+	+
UI 4	"	R	L	4.94	L-Lys-Ala-Gly_4	ND	+	I	+	+	+	+	+	+	+	+	-	-	+	+	+
UI 17	"	R	L	4.86	L-Lys-Ala-Gly_4	ND	+	I	+	+	+	+	+	+	+	+	-	-	+	+	+
UI 136	"	R	(L)	4.81	L-Lys-Ala-Gly_4	ND	-	I	+	+	-	+	+	+	+	±	-	+	+	+	+
SUI 58	Sheep	R	L	4.98	L-Lys-Ala-Gly_4	ND	-	I	+	+	-	+	+	+	+	+	-	+	+	+	+
SUI 66	"	R	L	6.66	L-Lys-Ala-Gly_4	33.4	+	I	-	-	-	-	+	-	-	-	-	+	-	-	-

[1]: Resistant; ND: Not determined; *Acid production in phenol red broth;

±: Weak reaction; +: Strong reaction.

Table 8.2a. Teichoic acids and other variable characteristics of some "unclassifiable" coagulase-negative staphylococci isolated from goats

Strain No.	Novobiocin sensitivity 30 mcg	Oxidase Test	Lactic acid configuration	pH of glucose	Type of peptidoglycan	Cell wall teichoic acids	G:C Ratio	FDP-Aldolase class	Maltose	Trehalose	Melibiose	Cellibiose	Xylose	Mannose	Sucrose	Turanose	Raffinose	Arabinose	Mannitol	Fructose	Ribose
UI 64	R	-	(L)	6.12	L-Lys-Gly_{5-6}	Ribitol + Glycerol		I	-	+*	-	-	-	+	+	+	-	-	+	+	+
UI 19	R	-	(L)	6.18	L-Lys-Gly_{5-6}	Ribitol + Glycerol		I	+	+	±	-	±	±	±	±	±	±	±	±	±
UI 20	R	-	(L)	6.17	L-Lys-Gly_{5-6}	Ribitol + Glycerol		I	+	+	+	-	+	+	+	+	+	+	+	+	+
UI 63	R	-	(L)	6.07	L-Lys-Gly_{5-6}	Ribitol + Glycerol	34.6	I	+	+	-	+	+	+	+	+	-	+	+	+	+
UI 85	S	-	D,L	4.55	L-Lys-Gly_{5-6}	Glycerol + Glucose		I	+	+	-	-	-	-	±	+	-	-	+	+	-
UI 75	R	-	(D,L)	6.36	L-Lys-Gly_{5-6}	Ribitol + Glycerol		I	+	+	-	-	-	-	+	+	-	-	+	+	+
UI 143	R	-	D,L	4.80	L-Lys-Gly_{5-6}	Glycerol + Glucose + galactosamine + N-acetylglucosamine	32.9	I	+	+	-	+	+	+	+	+	-	+	+	+	±
UI 119	R	-	L(D)	4.80	L-Lys-Gly_4 (L-Ser)	Glycerol + Glucose		I	+	+	-	+	+	+	+	+	-	+	+	+	-

*Acid production in phenol red broth; ± : weak reactions;
+: strong reactions; - : No reaction.

Table 8.2b. Variable characteristics of some coagulase-positive staphylococci isolated from goats

Strain No.	Novobiocin sensitivity 30 mcg	Oxidase Test	Lactic acid configuration	pH of glucose	Type of peptidoglycan	Cell wall teichoic acids	FDP-Aldolase class	Maltose	Trehalose	Melibiose	Cellibiose	Xylose	Mannose	Sucrose	Turanose	Raffinose	Arabinose	Mannitol	Fructose	Ribose
UI 118	S	-	L	4.69	L-Lys-Gly$_{5-6}$	Ribitol + glucosamine	I	+*	+	-	-	-	+	+	+	-	-	+	+	+
UI 150	S	-	L	4.86	L-Lys-Gly$_{5-6}$	Glycerol + glucosamine	I	-	+	-	-	-	+	+	-	-	-	-	+	+
UI 166	S	-	ND	ND	L-Lys-Gly$_{5-6}$	Ribitol + glucosamine	I	+	+	-	-	-	+	+	+	-	-	+	+	-

*Acid production in phenol red broth;

ND: Not determined.

Table 8.3 DNA-DNA hybridization values amongst some caprine strains of staphylococci

Source of filter bound DNA	Percentage relative binding of labelled DNA from:			
	S. lentus (K 21)	*S. gallinarum* VIII	UI 143	*S. aureus* (ATCC 12600)
S. lentus (K 21)	100			
S. gallinarum VIII	-	100	26	23
UI 143	-	25	100	29
S. xylosus (KL 162)	-	12	17	13
S. aureus (ATCC 12600)	-	10	15	100
S. sciuri (Sc 116)	-	8	10	13
UI 63	-	20	24	26
UI 119	20	60	25	25
UI 1	36			
UI 136	32			
SUI 58	50			
S. sciuri (SC 116)	32			

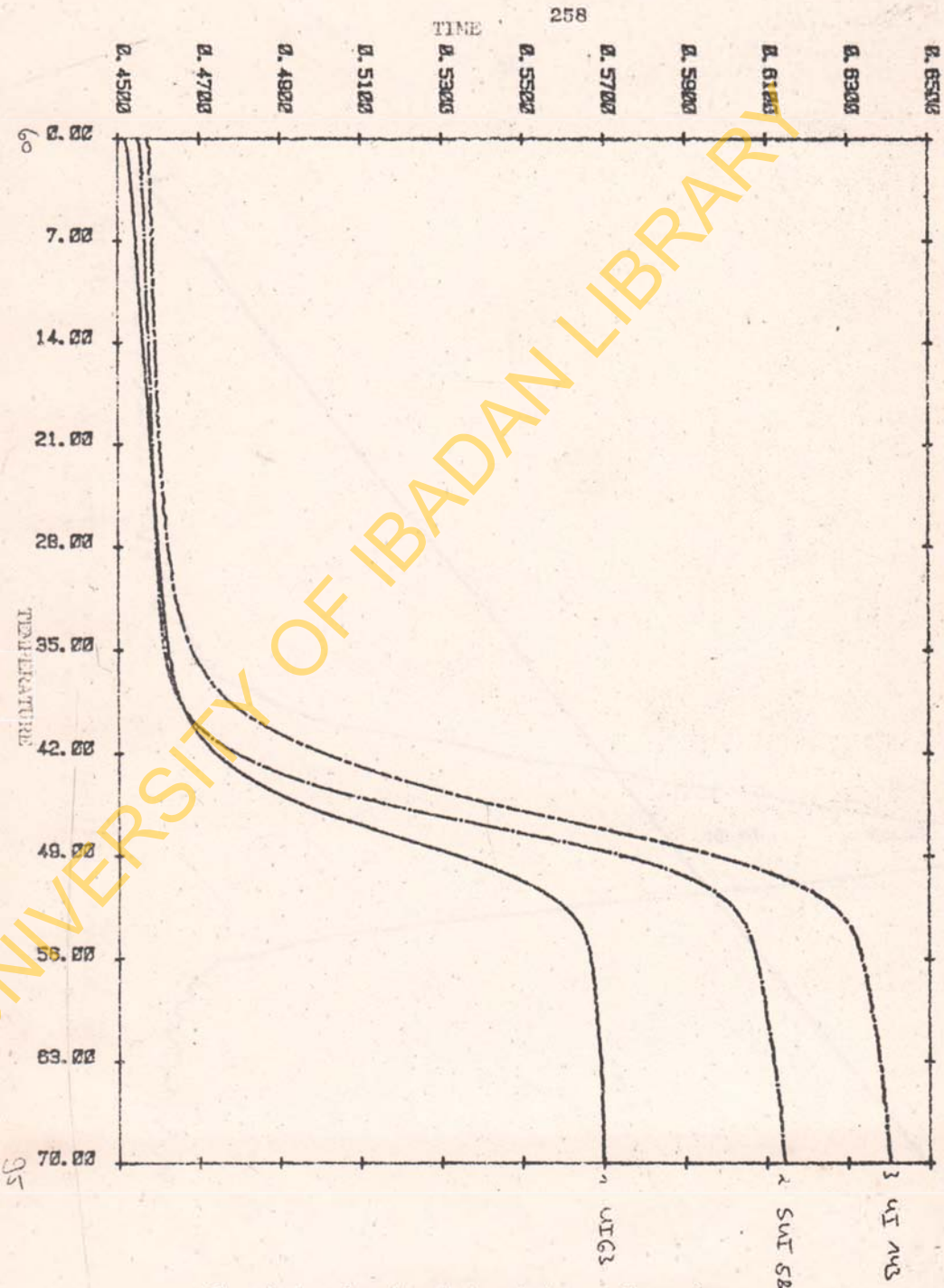
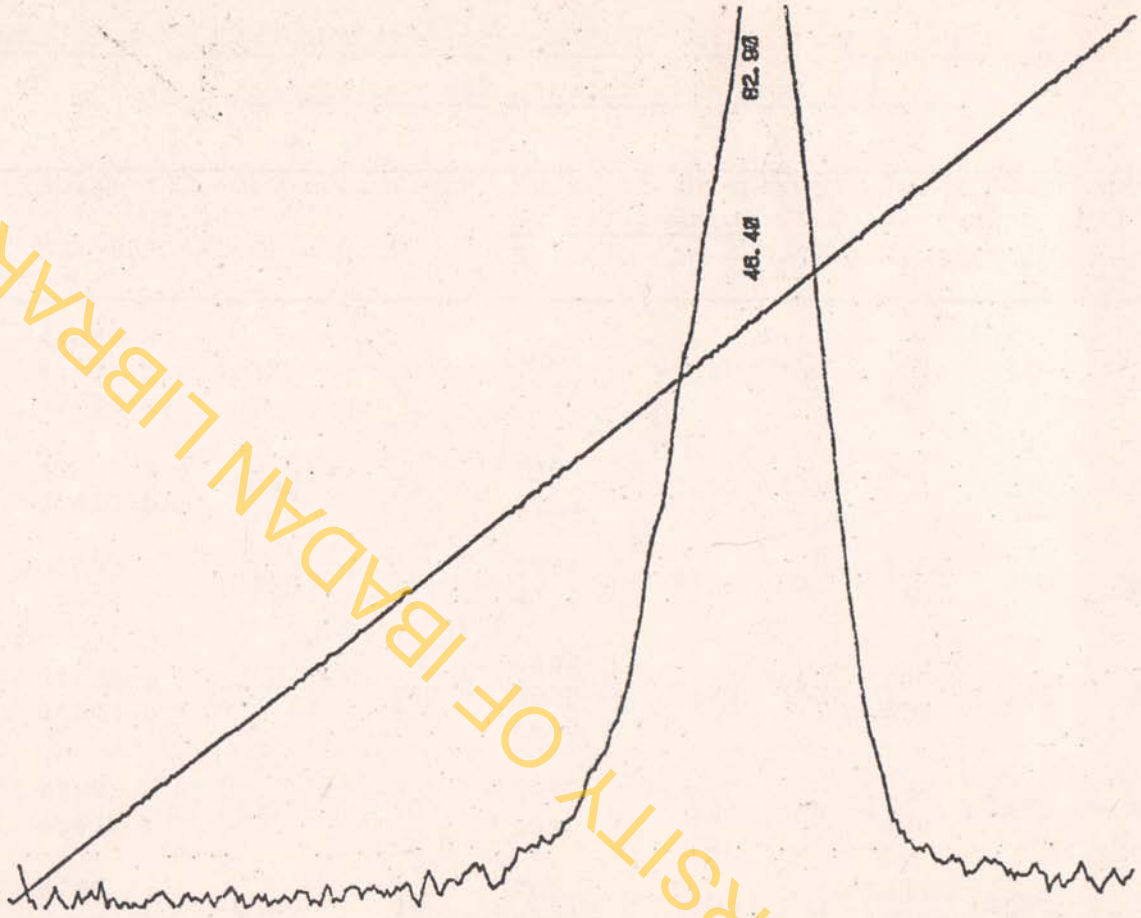


Fig. 8.A: Spectrophotometric readings for the determination of GC ratio of some staphylococcal strains



UI 143 Fig. 8.B: Spectrophotometric determination of melting point (Tm) of a DNA solution of sample UI 143. The Tm = 82.9°C.

Table 8.4 Radioactivity counts and homology values of some staphylococcal strains of animal origin

Filter \ DNA	Sp.A: 442, 060 c.p.m./ug UI 143 - E. 83705 cpm = 0.19 ug			Sp.A: 68540 cpm/ug S. gallinarium VIII E.11420 cpm = 0.17ug			Sp.A: 10936 cpm/ug S. aureus (ATCC 12600) E: 3747 cpm = 0.34 ug		
UI 143	44343 a 45701 b 37834 c	42329	100%*	1622 1543	1547	25%	340 394	343	29%
UI 63	10250 a 10420 b	10038	24	1388 1193	1255	20	342 327	310	26
UI 119	12053 a 9367 b	10413	25	3534 4012	3738	60	315 330	298	25
S. gallinarum VIII$_1$	11404 a 11541 b	11175	26	6144 5828 6653	6173	100	306 279	268	23
S. sciuri Sc 116	4789 a 4563 b	4379	10	595 519	522	8	179 174	152	13
S. aureus ATCC 12600	6285 a 6669 b	6180	15	706 551	593	10	1011 1212 1392	1181	100

* : See Appendix 6.B for calculations.
a, b : Scincillator counts for radioactive filters
c : Homologue count.

CHAPTER 9

SUMMARY AND CONCLUSION

Generally, the close contact of domestic animals with human beings is often conducive to the interchange of staphylococci between them. Live (1972) isolated some strains of S. aureus of human biotype in dogs and also S. aureus of canine origin in man. In this study, S. intermedius usually associated with animals was isolated from the nares of a human being keeping pet dogs. This is in agreement with the suggestion of Moeller, Smith, Shoemaker and Tjalma (1963) who noted that a high proportion of dogs carried S. aureus in their nasal passages with possible transfer of the bacteria between man and animals.

There is a paucity of data on the characteristics of caprine strains of staphylococci. With this present study, it is now known that caprine strains of S. aureus belong to biotype C or the ovine ecovar.

Korukov, 1978). In the examination of some faecal strains of S. aureus isolated from goats, Dimitracopoulos, Sakellarion and Papavassilou (1976) found that most of the strains they examined were fibrinolytic. In this present study, only 2 of the 14 caprine strains of S. aureus tested were fibrinolytic (Table 4.3a). Thus the relatively high rate of kinase activity found by these authors might be due to the action of proteases or staphylokinase. In addition to the biotyping characteristics of caprine strains of S. aureus, nasal carriage of staphylococci by goats consist essentially of S. sciuri, S. lentus and S. xylosus (Table 3.1b).

For the first time, caprine phages have been isolated and found to be relatively specific for S. aureus of caprine origin which were untypable with the human set of phages. Whilst Meyer (1967) suggested that staphylococci susceptible to phages of groups I, II or III of the International sets of human phages were of human biotype, in this study, the good typability of caprine strains of S. aureus with phages developed for human strains does not imply that the strains are epidemiologically related

to human strains. Only one strain (UI 166) showed the typical biotype characteristics of human strains of S. aureus.

In the novel descriptions of several species of coagulase-negative staphylococci isolated from persons living in England, United States of America and Switzerland, Schleifer and Kloos (1975a) found that the characteristics of these bacteria were remarkably similar. The characteristics of the coagulase-negative staphylococci isolated from humans and animals in this present study are similar to those described by these authors. However, but for the anaerobic growth in thioglycollate medium and aerobic acid production from turanose, xylose and maltose by S. sciuri and S. lentus characterised herein, biochemical and other physiological properties of these organisms are similar to those described by Kloos, Schleifer and Smith (1976). Morphologically, differentiation between S. sciuri and S. lentus on agar media after two days' incubation was inconclusive as both species almost had identical sizes

and colony profiles (Figs. 3.a, c; Table 3.4). This is however, in contradiction with the description of these organisms. In this study, the apparent low activity of most coagulase-negative staphylococci isolated from humans and animals on Tween 80 after two days coupled with the non-production of hyaluronidase were found to be useful in differentiating these staphylococci from S. aureus strains although nine of the 21 S. xylosus strains isolated from goats and sheep hydrolyzed Tween 80 but none of them produced hyaluronidase. Pazdiora and Serbus (1970) also noted that coagulase-negative staphylococci had lower activity on Tween 20, 40, 60 and 80 when compared with coagulase-positive strains. However, these authors did not identify their strains of staphylococci to species level

In the study of the staphylococcal strains isolated from different hosts, a variety of phenotypic markers were found useful for the identification of the species encountered. S. sciuri and S. lentus were found to have got some common characteristics. However, the production

of acid from melibiose by S. lentus distinguished these strains from S. sciuri. The differentiation of S. gallinarum and S. lentus was facilitated by the application of urease activities and oxidase test. Although the strains of S. intermedius of human and canine origins examined herein coagulated rabbit plasma, the inability of these strains to produce hyaluronidase and acetoin distinguished them from S. aureus. Thus not all staphylococci that coagulate one type of plasma or the other can be referred to as S. aureus. Although the strains of S. aureus examined here were obtained from non-epidemic cases, the carriage of phage type 29/52/81 and 263-2 agglutinogen by a strain of S. aureus isolated from a man is of epidemiological importance as it may be capable of causing similar infections reported by Cohen (1972). Presently, in serotyping S. aureus strains of animal origin, factor sera produced against human staphylococci are used. Before the improvement

in the understanding of the agglutinogens of staphylococci, animal and human strains of S. aureus were believed to have got different agglutinogens (Mercier, Pillet and Chabanier, 1950). However, it is now known that some specific agglutinogens particularly C_1, h_2 and 1 are common to human and some animal strains of S. aureus. In addition to these, a_5 and o agglutinogens were found herein to be common amongst human and animal strains of S. aureus. Some strains of S. intermedius and S. hyicus subsp. hyicus isolated from humans and animals respectively which were found untypable by using the International set of phages for typing human strains of S. aureus were serotypable with the simplified serotyping technique of Fleurette and Modjadedy (1976). Furthermore, the S. intermedius strains of human and canine origins had a common k agglutinogen (particularly k_1k_2) which

probably may be peculiar to S. intermedius strains.

Serological investigations have shown that human S. aureus strains regularly contain glucosaminyl ribitol teichoic acid (Haukenes, 1962; Hofstad, 1965) and S. aureus susceptible to phage 187 generally should not contain this teichoic acid (Oeding, 1974). However, the caprine strain of S. aureus found here to be susceptible to phage 187 produced glucosaminyl ribitol teichoic acid when examined biochemically and serologically. Furthermore, β-N-acetyl-glucosaminyl ribitol teichoic acid (poly Aβ) was found amongst 10 of the 14 caprine strains of S. aureus tested. Thus caprine strains of S. aureus may in addition to the possession of human-type agglutinogens possess teichoic acids which are similar to those of S. aureus of human origin. In addition to the possession of typical teichoic acid of S. aureus, the caprine strain of S. aureus susceptible to phage 187 had no k agglutinogen but had c_1 and o agglutinogens. This is in contradiction to the findings of Oeding and Williams (1958) who associated k agglutinogen either alone or in combination with other agglutinogens in S. aureus strains

susceptible to phage 187. Perhaps since the studies of these authors did not include caprine strains of S. aureus, the receptor for phage 187 may not be entirely the k agglutinogen (Cohen, 1972). Invariably, more strains susceptible to phage 187 need to be examined biochemically and serologically for a logical conclusion of the characteristics of these strains of S. aureus which may be met in medical and veterinary microbiology.

With the proven advantages of serotyping (Cohen, 1972) coupled with the simplified technique employed in this study which facilitated the serotyping of several strains of coagulase-positive staphylococci (S. aureus, S. intermedius and S. hyicus) isolated from humans and animals, it is hoped that serotyping of staphylococci will be of wider application.

SUGGESTIONS FOR FURTHER STUDIES

Coagulase-positive staphylococci. One of the caprine strains of coagulase-positive staphylococci found here to have some peculiar characteristics should be studied further. In addition, more of this strain should be isolated and characterized using further phenotypic and molecular procedures. The determination of its esterases and the immunological distance of its catalase in relation to other coagulase-positive staphylococci should be done.

Coagulase-negative staphylococci. Whilst the DNA-DNA homology studies of some of the coagulase-negative staphylococci examined herein revealed some relationship to either S. xylosus, S. gallinarum and S. sciuri, further hybridization studies of these strains may reveal their identity in terms of their relationship or otherwise to known staphylococci species. In addition, coagulase-negative staphylococcal strains isolated from lesions should be characterized.

Bacteriocin-like substance production. The production of a bacteriocin-like substance by some strains characterized herein should be investigated further. The bacteriocin-like substance should be extracted, purified and characterized. Furthermore, the lytic spectrum of the substance should be further investigated as it may turn out to be "antibiotic-like".

Enterotoxin: Production of enterotoxins by the strains of S. aureus isolated from humans and animals should be investigated.

Pathogenicity tests. Apart from S. hyicus (Devriese, Vlaminck, Nuyten and Kerrsmaecher, 1983), most of the recently described coagulase-negative staphylococci are yet to be tested in susceptible hosts. Thus attempts should be made to satisfy Koch's postulates by using the neonatal mouse gain assay (Kinsman and Arbuthnott, 1980) since several of the strains examined herein were isolated from infectious processes.

Phage typing. The newly isolated caprine phage should be characterized electron-microscopically and the lytic spectrum determined with wider range of hosts.

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APPENDIX 1.a

PHYSIOLOGICAL CHARACTERISTICS OF STAPHYLOCOCCI

Media:

Blood Agar (Oxoid, CM 55)

"Lab-lemco" beef extract	10 g
Peptone	10 g
Sodium chloride	5 g
Agar	15 g
Distilled water	1000 ml
pH 7.4	

The constituents were dissolved in 1,000ml of distilled water and later autoclaved at 1.2kg/cm^2 for 15 min. The medium was allowed to cool down and kept in a water bath at 45^{o}C after which 50 ml of sterile sheep blood was added to the blood agar base properly mixed and poured. The plates were later examined for contamination after overnight incubation at 37^{o}C.

Nutrient Agar (Oxoid, CM 3)

"Lab-lemco" powder	1.0 g
Yeast extract	2.0 g
Peptone	5.0 g
Sodium chloride	5.0 g
Agar No. 3	15.0 g
Distilled water	1000 ml
pH 7.4.	

The constituents were dissolved in 1,000 ml of distilled water by boiling after which the mixture was autoclaved at 1.2kg/cm^2 for 15 min. The medium was allowed to cool down and kept in a water bath at 45°C. About 15 ml of the medium was poured into each Petri dish (817 cm in diameter) and allowed to set. After overnight incubation at 37°C, contaminated plates were removed.

P agar (Kloos et al., 1974)

Peptone	10.0 g
Yeast extract	5.0 g
Sodium chloride	5.0 g
Glucose	1.0 g
Agar	15.0 g
Distilled water	1,000 ml

The constituents were dissolved in 1000 ml of distilled water after which the mixture was autoclaved as described for the other media.

Brain Heart Infusion Broth (Oxoid, CCM 225)

Calf brain infusion solids	12.5 g
Beef heart infusion solids	5.0 g
Proteose peptone (Oxoid, L46)	10.0 g
Sodium chloride	5.0 g
Dextrose	2.0 g
Disodium phosphate (anhydrous)	2.5 g
Distilled water	1,000 ml

pH 7.4 (approx.)

37 g of the dehydrated medium was dissolved in 1 litre of distilled water. After mixing properly, the medium was distributed into 5.0 ml amounts and sterilized by autoclaving at $1.2 kg/cm^2$ for 15 mins. 15.0 g of agar was added to the medium prior to dissolving the Brain Heart Infusion broth in distilled water (Brain Heart Infusion Agar).

Lysostaphin medium

Peptone	2.0 g
Yeast extract	1.0 g
Sodium chloride	1.0 g
Glucose	0.4 g
Agar	2.4 g
Distilled water	200 ml
pH 7.2 - 7.4	

The constituents were dissolved in 200 ml of distilled water and the pH adjusted to between 7.2 and 7.4. The mixture was autoclaved at 1.2kg/cm^2 for 15 min. and cooled to 45 - 50^{o}C after which 2 ml of sterile lysostaphin (1 mg/ml) was added. After proper mixing , plates were poured.

Medium for the production of bacteriocin-like substance

Peptone	10.0 g
Yeast extract	5.0 g
NaCl	5.0 g
Agar	6.0 g
Distilled water	1,000 ml
pH 7.2 to 7.4.	

The constituents were dissolved in 1 litre of cold distilled water after which the medium was distributed in 30 ml amounts and steriled by autoclaving at 1.2 kg/cm^2 for 15 minutes.

Scheme for the identification of novobiocin-resistant Staphylococcus species from farm animals and man.

Species	Oxidase	Urease	Protease (strong reaction)	Acid from					
				Cellobiose	Xylose	Arabinose	Raffinose	Melibiose	Sucrose
Species associated with animals and man									
S. saprophyticus	-	+	-	-	-	-	-	-	+
S. cohnii	-	D	-	-	-	-	-	-	-
S. xylosus	-	+	-	-	+	D	-	-	+
Species associated with animals									
S. sciuri	+	-	+	+	D+	D	-	-	+
S. lentus	+ or w	D-	D	+	+	D+	+	D+	+
S. gallinarum	-	+	-	D+	+	+	+	+	+

Symbols: + : 90% or more strains positive ; - : 90% or more strains negative ; D : between 25 and 75% positives ; D- or D+ : more than 75% but less than 90% negative or positive ; w : weak reaction.

APPENDIX 1.e

Scheme for the identification of novobiocin-sensitive Staphylococcus species associated with farm animals and man.

Species	Clumping factor	DNase (strong reaction)	Colony size	Urease	Haemolysis	Protease (strong reaction)	Anaerobic growth	Acid from Trehalose	Mannitol	Maltose	Sucrose
Species associated with animals											
S. hyicus subsp. *hyicus*	-*	+	L	+	-	+	+	D+	-	-	+
S. hyicus subsp. *chromog.*	-	-	L	+	-	+	+	+	D	D	+
S. caprae†	-	-	SM	+	w	-	+	+	-	D+	-
Species associated with animals and man											
S. aureus and *S. intermedius*	+*	+			+						
S. simulans	-	-	L	+	- or w	-	+	+	D	- or w	+
S. epidermidis	-	-	SM	D+	- or w	-	+	-	-	+	+
S. haemolyticus	-	-	ML	D-	+ or w	-	D-	+	D+	+	+
S. warneri	-	-	SM	+	- or w	-	+	+	D+	D+	+
Species associated with humans§											
S. hominis	-	-	SM	+	- or w		- or w	D	D	+	+
S. capitis	-	-	S	-	-		+ or w	-	+	-	D
S. auricularis	-	-	S	-	-		- or w	+ or w	-	D	D

*Symbols : + : 90% or more strains positive ; - : 90% or more strains negative ; D : between 25 and 75% positives ; D- or D+ : more than 75% but less than 90% negative or positive ; w : weak reaction.
L : large (⩾ 7 mm) ; M : medium (5-6 mm) ; S : small (⩽ 4 mm).

†Based on data from Devriese et al. (in press)

*Positive in *S. aureus*, variable in *S. intermedius*

§Data taken from Kloos & Schleifer 1983

Appendix 1.d

Growth characteristics of coagulase-negative staphylococci isolated from sheep

	COLONY								
	Size (mm) 2 days	3 days	Colour Yellow	Creamy White	Consistency Moist	Dry	Edge Irregular	Round	Number of strains tested
S. sciuri	6-7	7-9	7	30	37	0	21	16	37
S. lentus	7	7.5-8.5	0	16	16	0	11	5	16
S. xylosus	5-6	6.5-8.0	0	6	5	1	6	0	6
S. saprophyticus	4-5	6-6.5	3	0	3	0	3	0	3

Appendix 1.e

Growth characteristics of coagulase-positive staphylococci isolated from humans

COLONY	Size (mm) 2 days	5 days	Colour Yellow	Cream/White	Consistency Moist	Dry	Edge Irregular	Round	
M221	4.0	5.5	+*		+		+		S. aureus
M222	4.5	6.0		+	+		+		S. aureus
M223	5.0	7.0		+	+		+		S. aureus
M224	4.0	5.5	+		+		+		S. aureus
M225	4.5	5.0	+			1	+		S. aureus
M226	3.5	5.0		+	+		+		S. aureus
M232	4.5	5.0		+	+		+		S. aureus
M253	4.0	5.5		+	+		+		S. intermedius
M337	4.5	7.0		+	+		+		S. aureus

*Positive results; 1 : Fairly dry.

APPENDIX 2.A

EXTRACELLULAR PRODUCTS AND BIOCHEMICAL CHARACTERISTICS

Media

Urea agar base (Oxoid, CM 53)

Peptone	1.0 g
Dextrose	1.0 g
Sodium chloride	5.0 g
Disodium phosphate	1.2 g
Potassium dihydrogen phosphate	0.8 g
Phenol red	0.012 g
Agar	15.0 g
Distilled water	95 ml

pH 7.1

About 2.4 g of the constituents was added to 95 ml of distilled water and soaked for 15 min. The mixture was autoclaved at 1.2kg/cm^2 in for 20 min and later cooled to 55^{o}C after which 5.0 ml of sterile 40 per cent (w/v) urea solution was added. After mixing, the medium was distributed in 5 ml amounts into sterile test tubes and allowed to set in the slope portion. Checks for contamination were also carried out.

Brewer Thioglycollate Medium (Difco)	
Beef Infusion form	500.0g
Sodium chloride	5.0g
Dipotassium phosphate	2.0g
Proteose peptone (Difco)	10.0g
Bacto-Peptone	5.0g
Sodium thioglycollate (Difco)	0.5g
Bacto-agar	0.5g
Bacto-Methylene Blue	0.002 g
pH at 25°C	7.2

To rehydrate the medium, 40.5 grams of the powder was suspended in 1000 ml of cold distilled water after which the suspension was heated to boiling to dissolve the medium completely. The medium was distributed in 10 ml amounts and later autoclaved at 1.2kg/cm^2 for 15 mins. and cooled to 25°C. The medium was stored in the dark at room temperature until required. Adequate precautions were taken to ensure that most of the uppermost portion of the medium did not change to a green colour prior to usage.

Calcium Caseinate Agar (Merck)

Meat extract	3.0 g
Peptone from meat	5.0 g
Sodium chloride	5.0 g
Casein according to Hammersten	2.5 g
Calcium hydroxide	0.15g
Agar-agar	13.5 g
Distilled water	1000 ml

pH at 30°C 7.0 ± 0.2

The constituents were dissolved in 1,000 ml of freshly-distilled water. The mixture was boiled gently for 10 min and later filtered after which it was autoclaved at 1.2kg/cm² for 15 mins and plates poured.

Tween 80 hydrolysis medium

Nutrient agar	28.0 g
Calcium chloride	100.0mg
Tween 80 (Merck)	10.0 ml
Distilled water	1000 ml

pH 7.4

The constituents were dissolved in 1000 ml of distilled water. After mixing properly, plates were poured.

Bile Aesculin Agar (Difco)

Bacto-Beef extract	3.0 g
Bacto-Peptone	5.0 g
Aesculin	1.0 g
Bacto-Ox gall	40.0 g
Ferric citrate	0.5 g
Bacto-agar	15.0 g
Distilled water	1000 ml

pH at 25°C 6.6 ± 0.2

64 grams of the dehydrated medium was suspended in 1,000 ml of distilled water. The mixture was heated to boiling to dissolve completely and later dispensed in 6.0 ml amounts. After autoclaving at 1.2kg/cm^2 for 15 mins, the medium was allowed to set in the slope position.

Nitrate Reduction Medium (Kloos and Schleifer, 1975a)

Beef extract of lab lemco	3.0 g
Peptone	5.0 g
Potassium nitrate	1.0 g
Distilled water	1000 ml.

The constituents were dissolved in 1 litre of distilled water and distributed in 5.0 ml amounts. The medium was autoclaved at 1.2kg/cm^2 for 15 mins.

Baird-Parker Medium (Oxoid, CM 275)

Typtone (Oxoid, L42)	10.0 g
"Lab lemco" powder (Oxoid L29)	5.0 g
Yeast extract (Oxoid L21)	1.0 g
Sodium pyruvate	10.0 g
Glycine	12.0 g
Lithium chloride	5.0 g
Agar No. 3 (Oxoid L13)	20.0 g
Distilled water	1000 ml

pH 6.8 (approx.)

63.0g of the medium was suspended in 1 litre of distilled water and later heated to boiling to dissolve the medium completely. The medium was dispensed into flasks and sterilized by autoclaving at 1.2kg/cm^2 for 15 mins and cooled at 50^{o}C. The yolk of one egg was added to 100 ml of distilled water. After mixing the yolk suspension, 5 ml of it was added to 100 ml of molten Baird-Parker medium. The preparation was mixed properly before plates were poured. Prepared plates were later stored at 4^{o}C.

Phenol Red Broth (Difco)

Bacto-beef extract	1.0g
Proteose peptone No. 3	10.0g
Sodium chloride	5.0g
Bacto-phenol red	0.018g
Distilled water	1000 ml

16 grams of the dehydrated medium was dissolved in 1 litre of cold distilled water. After sterilizing 30 ml amounts of the medium by autoclaving at 1.2kg/cm^2 for 15 mins, 1.5 ml of filter-sterilized 20% carbohydrate solution was added under asceptic precautions.

Gel for thermostable nuclease production

DNA (Difco)	300.0mg
Agar (Difco)	10.0g
Calcium chloride $2H_2O$ (14.70 mg/10M or 0.01M)	1ml
Tris buffer (0.05 M, pH 9.0)	1000 ml
Sodium chloride	10.0g
O-toluidine blue 0.1 M	3 ml

The constituents were dissolved in the buffer by boiling after which 3 ml of 0.1m O-toluidine blue was

added. The gel preparation was used for the thermostable nuclease production using the poured-plate technique.

DNase agar (Oxoid CM 321)

Tryptose	20g
Deoxyribonucleic acid	2g
Sodium chloride	5g
Agar	12g
Distilled water	1000 ml

pH 7.3 (approx.)

39 grams of the dehydrated medium was suspended in 1 litre of distilled water and brought to boil until it dissolved completely. The medium was then sterilized by autoclaving at 1.2kg/cm^2 for 15 minutes.

Phosphate buffered saline (PBS)

Sodium chloride	8.0g
Potassium chloride	0.2g
Disodium hydrogen phosphate	1.15g
Potassium dihydrogen phosphate	0.2g
Distilled water	1000 ml

pH 7.3

After dissolving the chemicals in 1 litre of distilled water, the suspension was sterilized at 1.1kg/cm^2 for 10 minutes.

APPENDIX 2.B

Characteristics of *Staphylococcus aureus* isolated from giant rats (*Cricetomys gambianus*), rabbits and poultry

Strain No:	UI167	168	169	UI 314	A295	A297	*S. aureus* ATCC 25923
Host:	Giant Rats			Rabbit	Poultry		
Coagulase	+	+	+	+	+	+	+
Clumping factor	+	+	+	+	−	−	+
Staphylokinase	−	−	−	−		−	−
Fibrin protease	−	−	−	−	−	+	−
Proteinase 48h	+	+	+	+	+	+	±
Desoxyribonuclease	+	+	+	+	+	+	±
Thermostable nuclease	±	+	+	+	+	+	+
Phosphatase 48h	+	+	+	+	+	+	+
Hyaluronidase	+	+	+	+	+	+	+
Oxidase	−	−	−	−	−	−	−
Nitrate reduction	+	+	+	+	+	+	+
Urease (7 days)	+	+	+	+	+	±	+
Aesculin (48 h)	±	±	±	+	±	−	−
Tween 80 hydrolysis (48 h)	+	+	+	−	+	+	+
Egg yolk reaction	+	+	+	+	+	+	+
Acetylmethylcarbinol	+	+	±	+	+	−	+
Penicillinase production	−	−	−	−	−	−	NT
Crystal violet reaction	C	C	C	C	A	C	

NT : Not tested

APPENDIX 2.C

Carbohydrate reactions of Staphylococcus aureus and Staphylococcus intermedius of canine origin

Strain No.[a]	D238	D240	D241	D242	D243	S. aureus ATCC 25923
Maltose*	+	+	+	+	+	+
D-(+)-Trehalose	+	+	+	+	+	+
Melibiose	-	-	-	-	-	-
Raffinose	-	-	-	-	-	-
D-(+)-Cellibiose	-	-	-	-	-	-
D-(+)-Xylose	-	-	-	-	-	-
L-(+)-Arabinose	-	-	-	-	-	-
D-(+)-Mannose	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+
D-(+)-Turanose	+	-	+	+	+	+
Fructose	+	+	+	+	+	+
D-(-)-Ribose	+	+	+	+	+	+
D-(+)-Mannitol	+	+	-	+	+	+
Acetylmethycarbinol	-	-	-	-	-	+

*: Aerobic acid production in phenol red broth (1-3 days)

a: See Table 4.4 for Strain identification.

APPENDIX 2.D

Characteristics of coagulase-negative staphylococci isolated from poultry

Strain No:	A186	188	189	192	193	279	339	A200	278	282	286	298	A302	306	A280	A304	A309	A315	
*Species:			S. lentus					S. xylosus					S. gallinarum		S. cohnii	S. sciuri	S. warneri	S. hyicus subsp. hyicus	S. lentus ATCC 29070
CHARACTERS																			
Deoxyribonuclease	-	-	±	-	-	±	-	-	-	-	-	-	-	-	-	±	±	+	±
Thermostable nuclease	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Phosphatase 48 h	+	+	+	+	+	+	+	±	+	+	+	+	+	+	±	+	-	+	-
Proteinase 48 h	±	-	±	-	-	-	±	-	-	-	-	-	-	-	-	+	-	+	-
Staphylokinase	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Fibrin protease	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+
Urease production (7 days)	±	±	±	-	-	±	±	+	-	-	+	±	+	+	+	±	±	+	-
Aesculin hydrolysis (48 h)	+	+	+	+	+	+	+	-	+	±	±	+	-	±	+	+	-	-	+
Egg yolk reaction	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	±	±	-
Oxidase test	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	-	-	+
Nitrate reduction	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+
Tween 80 hydrolysis (48 h)	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-
Acetylmethylcarbinol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hyaluronidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-

±: weak reactions - : Negative reactions

+: Strong reactions * Appendix 1b

APPENDIX 2.E

Characteristics of coagulase-negative staphylococci isolated from antelope and other animals

Strain No:	UI 342	UI 204	207	312	UI 313
Animal:	Antelope	Cow			Pig
Desoxyribonuclease	±	+	+	-	+
Thermostable nuclease	-	-	-	-	+
Phosphatase 48 h	+	+	+	+	+
Proteinase 48 h	-	+	+	+	+
Staphylokinase	+	-	-	-	-
Fibrin protease	-	+	+	-	±
Urease production (7 days)	+	+	+	+	+
Aesculin hydrolysis (48 h)	+	-	-	-	-
Egg yolk reaction	-	-	-	-	+
Oxidase test	+	-	-	-	-
Nitrate reduction	+	+	+	-	+
Tween 80 hydrolysis	-	-	-	-	+
Acetylmethylcarbinol	-	-	-	-	-
Hyaluronidase	-	-	-	-	+
Species	*S. sciuri*	*S. hyicus* subsp. *chromogenes*		*S. spidermidis*	*S. hyicus* subsp. *hyicus*

APPENDIX 3.A

ANTIBIOTIC SUSCEPTIBILITY TESTING

Media

Mueller Hinton Agar (Oxoid, CCM 337)	
Beef infusion	300.0g
Casein hydrolysate	17.5g
Starch	1.5g
Agar No. 1	10.0g
Distilled water	1000 ml

pH 7.4 (approx.)

35 grams of the dehydrated medium was suspended in 1 litre of distilled water after which it was brought to boil to dissolve the medium completely. The medium was later sterilized by autoclaving at 1.2kg/cm^2 for 15 minutes.

APPENDIX 3.B

Zone inhibition (mm) of chemotherapeutic agents used against coagulase-positive staphylococci isolated from giant rats (*Cricetomys gambianus*), rabbits, monkey and poultry

Antibiotic	Strain No: UI167	168	169	UI 314	Mo 335	A295	A297*	*S. aureus* (Lyon OHS)
	Host: Giant Rats			Rabbit	Monkey	Poultry		
Pen_{10}	40	29	42	40	34	37	39	29
OX_{1}	22	20	29	26	23	23	20	20
CF_{10}	32	34	40	41	31	37	42	30
K_{30}	24	20	23	27	23	23	27	21
GM_{10}	28	21	23	27	23	NT	26	22
NN_{10}	28	22	24	26	23	23	28	23
C_{30}	25	22	23	26	23	21	25	20
TE_{30}	25	24	24	25	24	10	10	22
MT_{30}	25	23	27	28	25	25	28	24
D_{30}	32	25	26	27	26	14	11	24
E_{15}	25	23	28	33	27	6	31	25
L_{2}	16	10	18	22	16	6	21	17
PR_{15}	27	26	27	32	20	26	32	24
FF_{50}	36	33	43	25	38	37	38	36
RA_{30}	34	32	35	43	30	33	37	30
VA_{30}	10	9	17	20	17	17	18	15
MA_{30}	34	33	39	36	35	35	40	29
SXT	27	26	27	27	30	28	28	24
FOX_{30}	25	25	29	28	25	26	31	26
CXM_{30}	26	26	30	31	30	28	30	28
CTX_{30}	22	24	27	27	28	25	28	26

*S. hyicus subsp. hyicus (API System, France).

APPENDIX 4.A

PHAGE TYPING

Media

Lysotyping broth

"Lab-lemco" beef extract (Oxoid L29)	8.0g
Bacto-peptone (Difco)	5.0g
Proteose-peptone (Difco)	5.0g
Sodium chloride analytical (Merck)	5.0g
Distilled water	1000 ml

pH 7.4

The constituents were dissolved in cold distilled water after which the medium was sterilised by autoclaving at 1.2kg/cm^2 for 30 mins.

Glucose Medium

Nutrient broth (Difco)	20.0g
Sodium chloride (Merck)	5.0g
Glucose-Bacto-Agar (Difco)	8.0g
Distilled water	1000 ml

The glucose Bacto-agar was dissolved by autoclaving at 120°C for 30 mins. 10% NaOH was used to adjust the pH to 7.4 after which the medium was distributed in 500 ml amounts in Roux bottles to which were added 5 ml of 1 M Magnesium sulphate ($MgSo_4$) and 20 ml of M/10 Calcium chloride ($CaCl_2$). After mixing properly, the medium was sterilized by autoclaving at $1.2kg/cm^2$ for 30 minutes and plates poured for use.

Appendix 4.B. Susceptibility patterns of S. aureus strains isolated from rabbits, giant rats (Cricetomys gambianus), sheep and poultry with human phages

PHAGES AT RTD X 100

Strain No.	Host	29	52	52^{A}	79	80	3^{A}	3^{C}	55	71	6	42^{E}	47	53	54	75	77	83	84	85	81	94	95	96	42^{D}	187	88	89	90	92	D11	HK2
		GROUP I					GROUP II				GROUP III										MISCELLANEOUS				GRP IV	MISCELLANEOUS GROUP						
RB 314	Rabbit	-	-	-	-	-	LC*	LC	LC	LC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R167	Giant Rat	++	-	-	-	-	-	-	-	-	++	+	+	±	-	++	-	-	-	-	LSC	-	-	-	-	-	-	-	-	-	-
R168	"	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R169	"	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A295	Poultry	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A297	Poultry[8]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SUI 116	Sheep	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*LC: Complete lysis; LSC: Semi-complete lysis; H: 50 plaques;

+: 20 - 50 plaques; ± : 20 plaques.

APPENDIX 5.A

SEROLOGY

Media

Serotyping broth

Nutrient broth (Oxoid, No. 2)	25.0g
Glucose	2.0g
Sodium chloride	20.0g
Distilled water	1000 ml

pH 7.0 approx.

The constituents were dissolved in 1 litre of distilled water after which the medium was distributed in 8.0 ml amounts and sterilized by autoclaving at 1.1kg/cm^2 for 20 mins.

Glucose-nutrient broth

Nutrient broth (Oxoid No.2)	25.0g
Agar noble	12.0g
Glucose	2.0g
Distilled water	1000 ml.

The constituents were dissolved in 1 litre of cold distilled water and later autoclaved at 1.1kg/cm^2 for 20 mins.

Agar Noble-nutrient broth

Nutrient broth (Oxoid No. 2)	25.0g
Agar Noble	12.0g
Distilled water	1000 ml.

The constituents were dissolved in 1000 ml of distilled water and later autoclaved at 1.2kg/cm^2 for 20 mins.

IDG for poly AB

Tris HCl buffer	250ml
NaCl	2.1g
$CaCl_2$	0.025g
Agar noble	3.0g
Distilled water	1000 ml

The constituents were dissolved by gentle heating and later poured as gel.

APPENDIX 5.B

Preparation of factor sera

(A) Rabbit inoculation. Lyophilized bacteria of the reference strain known to possess specific agglutinogens were tested for purity on blood agar plates from where nutrient agar plates were inoculated. After 18 h incubation at 37°C, the growth was suspended in a 1 per cent solution of 40 per cent formalin and kept at 37°C for 24 h. After centrifugation and sterility control on blood agar plates, a suspension in saline of approximately 5 x 10^9 bacteria per millilitre (ml) was made. Four New Zealand white rabbits were injected intravenously on three successive days with an interval of five days to the next series (about four weeks needed). Doses of 0.1, 0.2 and 0.4 ml were given in the first series, 0.4, 0.6, 0.8ml in the second, and 0.8, 1.0 and 1.0 ml in the third. The rabbits were bled after the third series and sera obtained were inactivated at 56°C for 30 min after which 5 ml portions of the serum to which two drops of 1 per cent solution of merthiolate have been added were distributed in small glass tubes with rubber stoppers and frozen until required. Generally,

a serum showing strong agglutination at a dilution of 1 in 1,000 to 1 in 2,000 with live 5 h bacteria of the homologous strain is satisfactory.

(B) Absorption of factor sera. Nutrient agar (Oxoid) plates 14 centimetres in diameter, were seeded separately with overnight broth cultures of S. aureus Cowan I and 2095 (absorbing strains for C_1). The plates were incubated at 37°C for 18 - 24 h. Harvested growths from the plates were washed in 5.0 ml of physiological saline twice after which the suspensions were centrifuged at 4,500 rev/min for 15 mins. Five to ten plates giving confluent growths are generally used for the absorption of 10 ml of diluted serum. After carefully pipetting off the supernatants from the suspension of the absorbing strains, 10 ml of 1 in 10 dilution of antiserum (immunization serum) in saline (7.2 ml of sodium azide-physiological saline added to 0.8 ml of serum 3647) was suspended in the bacterial deposit by means of a Pasteur pipette. The tubes were later placed in an incubator at 37°C for 2 h with intermitent shaking and then left at 4°C overnight. The preparation was later centrifuged

at 4,000 rev/min for 15-30 mins after which the supernatant was carefully pipetted off and used as factor serum C_1. After the absorptions have been completed, the factor serum was checked with the vaccine strain and the strains used for absorption. After the factor serum has been freed of antibodies against the absorbing strains, it was then checked by agglutination with specific type strains and heated for 20 mins at 60°C after which two drops of 1 per cent methiolate were added to 5 ml portions of the serum.

APPENDIX 6.A

CHEMOTAXONOMIC AND GENETICAL STUDIES

Media

Glucose-peptone-yeast extract broth

Glucose	5.0g
Peptone	10.0g
Yeast extract	5.0g
NaCl	8.0g
Glycine	2.5g
Distilled water	1000 ml.

pH 7.2

The constituents were dissolved in 1 litre of cold distilled water after which the medium was sterilized at 1.2kg/cm^2 for 15 minutes.

Glycine was not added to the medium used for the isolation of deoxyribonucleic acid (DNA).

1 x SSC buffer (Dialysing buffer)

NaCl (0.15 M)	8.75g
Trisodium citrate (0.015 m)	4.4115g
Double-distilled water	1000 ml

pH 7.0

The constituents were dissolved in 1 litre of distilled water and the pH was adjusted to 7.0.

Isopropanol-acetic acid solution

Isopropanol	75 parts
Acetic acid	10 parts
Distilled water	15 parts

The constituents were mixed together thoroughly and stored in a brown bottle.

α - picoline solution

α- picoline	70 parts
25% NH_4OH	2 parts
Distilled water	28 parts

The constituents were carefully mixed together and later stored in a dark-brown bottle.

Burton's reagent

Diphenylamine (Merck)	1.5g
Sulphuric acid (H_2SO_4)	1.5ml
Acetyaldehyde (16 mg/ml)	0.5 ml
Acetic acid	100 ml.

The constituents were dissolved in 100 ml of acetic acid. The reagent was then stored in a dark brown bottle

and shielded from light with the use of aluminium foil.

Denhardt's preincubation medium

0.02% Ficoll (M.W 400,000 Sigma)

0.02% polyvinylpyrrolidon (Sigma, M.W. 360,000)

0.02% bovine serum albumin (Fraction 5).

The constituents in adequate proportions were dissolved in 3 x SSC buffer and stored at 4°C until required.

APPENDIX 6.B

G + C Ratio determination from Spectrophotometric readings

Tm of sample UI 143 = 82.9°C (Fig. 8.B).

Using DeLey's formula,

GC = 2.44 (Tm - 69.4)

= 2.44 (82.9 - 69.4)

= 2.44 x .35

= 32.9

Thus the G + C ratio of sample UI 143

= 32.9 Mol. %

Calculation of homology values for some staphylococcal strains

Scincillator counts for UI 143 were 44343 for the 45701 radioactive filters and 37834 for the homologue (Table 8.4)

Mean count for these readings = 127878/3

= 42626

But the count for the free (background)

filter = 297

Actual count for sample UI 143

= 42626 - 297

= 42329

Using count 42329 as 100% homology,

The binding rate for count 10038 (UI 63)

$$= \frac{10038 \times 100}{42329} \%$$

= 24%

Zbl. Bakt. Hyg., I. Abt. Orig. A 255, 234–238 (1983)

Biotypes, Serotypes and Phage Types of Caprine Strains of Staphylococcus aureus

Biotypen, Serotypen und Lysotypen capriner Stämme von Staphylococcus aureus

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Received January 31, 1983

Summary

Nine of 11 caprine strains of *Staphylococcus aureus* examined belong to biotype C. They were typable serologically using antisera developed for typing human strains of *Staphylococcus aureus*. H_2 was found to be the dominant thermostable antigen whilst I and 0 were the most common thermolabile antigens. Some strains possessed protein A and poly Aβ (β-glucosaminyl ribitol teichoic acid).

Phage typing of the strains was achieved with an international set of phages for typing human strains of *Staphylococcus aureus* and they belong to phage groups II, and the mixed group.

Zusammenfassung

9 von 11 untersuchten, von Ziegen stammenden *Staphylococcus aureus*-Stämmen gehörten zum Biotyp C. Sie ließen sich unter Verwendung von zur Typisierung humaner *S. aureus*-Stämme entwickelten Antiseren serologisch typisieren. Als dominantes thermostabiles Antigen wurde H_2 festgestellt, während I und 0 die häufigsten thermolabilen Antigene waren. Einige Stämme wiesen Protein A und Poly Aβ (β-Glucosaminyl-Ribitol-Teichonsäure) auf.

Die Lysotypie der Stämme erfolgte mittels eines internationalen Phagensatzes zur Typisierung von *S. aureus*-Humanstämmen; sie gehörten zu den Phagengruppen II und der gemischten Gruppe.

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Introduction

Staphylococci from various hosts have been systematically examined by several workers (*Baird-Parker*, 1965; *Schleifer* and *Kloos*, 1975; *Devriese* and *Oeding*, 1976). Thus in an attempt to trace the origin of the strains of *Staphylococcus aureus* isolated from goats (*Adegoke* and *Ojo*, 1981), strains which gave positive "clumping factor reaction" with the method described by *Devriese* and *Hajek* (1980) were biotyped, serotyped and phage typed.

Materials and Methods

Isolates. The characteristics and antibiotic susceptibility patterns of the strains used in this investigation have been reported (*Adegoke*, 1981).

Biotyping Tests. The method for determining the crystal violet type as described by *Meyer* (1967) was employed. The strains were designated into biotypes (*Hajek* and *Marsalek*, 1971).

Staphylokinase (Kinase). Staphylokinase was tested in fibrin plates with and without dog plasma (*Devriese* and *Van de Kerckhove*, 1980).

Protein A. Detection of protein A was carried out after the methods described by *Flandrois* et al. (1975).

Poly Aβ. Immunological assay for poly Aβ (β-glucosaminyl ribitol teichoic acid) was carried out as described by *Oeding* (1959).

Serotyping. Immune sera prepared as described by *Flandrois* et al. (1978) were used for serotyping the strains of *Staphylococcus aureus* with the technique proposed by *Fleurette* and *Modjadedy* (1976).

Phage typing. Phage typing was carried out using the method of *Blair* and *Williams* (1961) with the phages of the International Set for typing *Staphylococcus aureus* of human origin.

Results

Table 1 shows the serological and phage types as well as the biotypes of caprine strains of *Staphylococcus aureus*. The biotyping characteristics are similar to biotype C described by *Hajek* and *Marsalek* (1971).

Of the 9 strains which had poly Aβ (β-glucosaminyl ribitol teichoic acid), 7 also possessed protein A.

Although only six of the strains could be grouped into the serogroups proposed by *Modjadedy* and *Fleurette* (1974), caprine strains of *Staphylococcus aureus* posses human-type agglutinogens (Table 1).

Strains typable with human phages belong to phage groups II, and the mixed group.

Discussion

Hitherto, the acceptability of serotyping *Staphylococcus aureus* has been in doubt. However, with the proven advantages of serotyping (*Cohen*, 1972) and with the simplified serotyping techniques proposed by *Fleurette* and *Modjadedy* (1976), it is hoped that as strains of *Staphylococcus aureus* from more hosts are typable, serotyping will be of wider use.

Table 1. Biotypes, Serotypes and Phage Types of Caprine Strains of *S. aureus*

Isolate No.	Clumping Factor Reaction	β-Haemolysin	Crystal Violet Test	Kinase	*Hajek and Marsalek* Biotype	Coagulation (6 h) Human Plasma	Coagulation (6 h) Bovine Plasma	Protein A	Poly Aβ	Serotyping Thermostable Agglutinogen	Serotyping Thermolabile Agglutinogen	Fleurette's Sero Group	Phage Type
UI 96	+	+	A	−	C	ND	+	+	+	a_3	a_4, b_1, o	C+D	29±/52/79/80/53/54/77/84/85/81/95/+
UI 27	+	+	C	−	C	+	+	+	+	NT	NT	−	NT
UI 145	+	+	C	−	NT	+	−	+	+	−	b_1, o, p	C+D	29/85/+
UI 153	+	+	C	−	C	+	+	+	+	h_2	1, o	H	NT
UI 154	+	+	C	−	C	+	+	+	d	NT	NT	−	NT
UI 160	+	+	C	−	C	+	+	+	−	−	C_1, o	−	29+/52/80/3A/3C/6/53/54/77/81/94/+
UI 161	+	+	C	−	C	+	+	−	+	h_2	−		29/52/3A/3C/6/47/53/54/72/94/+
UI 327	+	+	C	−	C	+	+	−	+	a_2, h_2	1	H	29/52/80/3A/3C/6/53/54/94/+
UI 331	+	+	C	−	−	+	+	−	+	NT	NT	−	29/52/80/3A/3C/6/47/53/54/81/94/+
UI 332	+	+	C	−	C	+	+	−	+	h_2	1	H	29/52/3A/3C/6/47/53/54/77/81/94/+
UI 166	+	+	C			+	−	+	−*	a_1, h_2	m, 263-2,1	A	3A/3C/55/71+

NT: Not typable
ND: Not done
d: doubtful

No specific biotype, serotype or phage type have been associated with caprine strains of *Staphylococcus aureus*. *Dimitracopoulos* et al. (1976) found that most of the goat strains of *Staphylococcus aureus* examined were fibrinolysin positive. This can be due to the action of proteases or to staphylokinase. Thus it is not known whether or not caprine strains of *Staphylococcus aureus* differ from the major pathogenic animal staphylococci which belong to different species (*Devriese* and *Hajek*, 1980) and biotypes (*Hajek* and *Marsalek*, 1971). However, the biotyping characteristics of most caprine strains of *Staphylococcus aureus* examined here have now been found to be similar to biotype C described by *Hajek* and *Marsalek* (1971).

Protein A, a species-specific antigen (*Forsgren*, 1970) is of epidemiological importance in human strains of *Staphylococcus aureus* (*Hajek* and *Marsalek*, 1976). With the paucity of data on the occurrence of protein A in *Staphylococcus aureus* of animal origin (*Lachica* et al., 1979) and the irregular occurrence of protein A in bovine strains (*Marandon* and *Oeding*, 1967) it is interesting to note that many of the caprine strains of *Staphylococcus aureus* examined possessed protein A.

Furthermore, in the examination of several biotypes of *Staphylococcus aureus* for the presence of polysaccharide A, *Oeding* (1974) found that only 48% of the porcine strains examined contained poly Aβ. However, 9 of 11 of our strains have been found to contain poly Aβ. Thus caprine strains of *Staphylococcus aureus* may have teichoic acid and other group precipitinogens which are similar to those of human beings.

Hitherto, antigenic structure, sensitivity to bacteriophages and other characteristics have been used to differentiate *Staphylococcus aureus* of human and less frequently animal origin (*Oeding* et al., 1970). Human-type agglutinogens h_2, l were found here to be common amongst caprine strains of *Staphylococcus aureus*. This is in agreement with the findings of *Grun* (1958), *Marandon* and *Oeding* (1967) who examined strains from other animals.

The good typability of caprine strains of *Staphylococcus aureus* with phages developed for human strains does not imply that the strains are epidemiologically related to human strains. Only 1 strain (UI 166) showed the typical biotype characteristics of human strains of *Staphylococcus aureus*.

We are grateful to the Belgian Agency for Co-operation and Development (A.B.O.S.) Brussels, Belgium, Miss *A. van de Kerckhove* and Mr. *Lucein Serras*, Faculty of Veterinary Medicine, University of Ghent, Ghent, Belgium; Mrs. *S. Reat*, Laboratoire de Bactériologie, Université Claude Bernard, Lyon, France and Prof. Dr. *K. H. Schleifer* for reading the manuscript.

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PROVINCE DE BRABANT

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February 8, 1983

Rue du Remorqueur 28
1040 BRUXELLES

Mr. Gabriel ADEGOKE
c/o Professor SCHLEIFER
Lehrstuhl Mikrobiologie
Arcisstrasse 16
D. München.

Dear Mr. Adegoke,

Being now in possession of a good stock of the phage we isolated from your goat Staphylococcus aureus strain, I am sending you the results of assays on the series of strains I received from you and Dr. Devriese for phage-typing. As you can see, this phage (called G1) lysed three goat strains, two of which were untypable with the human set of phages whereas the other was slightly sensitive to phage 187. I think I shall join it to the set of typing phages when typing animal strains to see if it is further useful. Of course, I shall give you this phage whenever you wish.

Hoping that you are having a good and interesting time in Munich,

Sincerely yours,

C. Godard

Dr. C. GODARD.