THE STAPHYLOCOCCI

Edited by

JAY O. COHEN

Center for Disease Control
Atlanta, Georgia

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FOREWORD

A little over a decade ago I was rash enough to prophesy that the output of literature on staphylococci was likely to decrease. Those of us who witnessed the tremendous impact of antibiotics felt that interest in pathogenic bacteria would wane. To some extent this has proved true; but the essence of research is in the word "search," and bacteriology has simply taken another direction to push forward its vigourous shoots. From being simply a handmaiden of medicine, bacteriology has returned to its origins as a science in its own right. Pasteur would undoubtedly have been pleased to know that this cyle had been completed within a century, and that bacteriology is once more a fundamental biological science using the tools of contemporary chemistry.

In that spirit, this book summarises the advances made in the understanding of staphylococci during the past dozen years or so. In terms of exact knowledge in the field of pure (i.e., not immediately applicable) science progress has been rapid and considerable. Yet throughout this book there is an underlying thread that still connects the main theme of staphylococci with disease and disease processes. This, too, is pure science. In truth, comparatively little is known of how most other bacteria produce disease in man and animals. Perhaps it is not surprising that staphylococci refuse to yield all their secrets.

This book was produced by a group of experts, all internationally known in their respective fields. Such an approach means that each contributor describes his own creed in the form of an interim statement. Of course, in science all statements are interim; only the future can tell whether one or another view will prevail. In my opinion, this is one of the features that lend extra strength to the book. Reading through it carefully, chapter by chapter, one becomes aware of the differences in outlook among the contributors and yet there are no contradictions or seemingly incompatible ideas. Inevitably, some of the ideas will shift, or fall by the wayside, while others will acquire new interpretations, but in the meanwhile they are a spur to fresh efforts. Furthermore, the role of experts is to indicate a surefooted way through the maze of literature, which only the ultraspecialist can find. The techniques described are neeessarily concise but give the essential details required to enable the reader to formulate his own opinions.

It is a little sad to reflect that the name given to the main pathogenic species by Ogston, its discoverer, is not used here. Traditionally, in medical microbiology generic names often undergo revision, but the second half of the binomial, which is descriptive of the typical disease caused by a pathogen, remains more stable. We understand of course that trinomials are illegitimate (harsh words!), but *Staphylococcus pyogenes* would be so much better than *Staphylococcus aureus*, not only because it has correct priority, but also because pigmentation is an extremely variable characteristic in some strains of this species. Taxonomy is designed to serve a utilitarian purpose; it is a kind of shorthand. Sorting out the various species is a biological aim,

but here again we have the invisible thread in trying to define *S. aureus*, which commonly causes pyogenic lesions, and *S. epidermidis* which, although it may be involved in disease, plays an entirely different role. In this respect, the findings that DNA from *S. aureus* will not hybridise with DNA from *S. epidermidis* carries more conviction than the earlier observations of allegedly shared haemolysins or other factors. Both these species have now been effectively and accurately split off from the vast numbers of other micrococci.

The recent great advances in genetics have shattered certain firmly held convictions in taxonomy. Many of the "biochemical tests" of old can now be manipulated genetically and their taxonomic value has to be seen in the statistical sense rather than as absolute criteria. This applies to the many demonstrable enzymes and I suspect will also hold for body constituents that are still held to be stable. The reorientation of ideas has been expressed brilliantly by Ernst Mayr who defined a species as a genetic unit consisting of a large intercommunicating gene pool. In this view the individual is merely a vessel holding a small portion of the gene pool for a relatively short time. Staphylococci are not the handiest organisms for genetic studies, but in the past few years a great deal has been learnt about both the chromosomal and extrachromosomal locations of genes. The stimulus was drug resistance and the very action of the antibiotics provided the methodology. That lysogenization alters the phenotypic behaviour of an organism was known for some time. Since staphylococci do not appear to conjugate, plasmid transfer presumably occurs solely by transduction. Nevertheless, this mechanism may well be a very important factor in the emergence and spread of antibiotic-resistant staphylococci. This, however, is only our worm's-eye view. Taking a broader perspective, the exchange of genes that control enzymes useful to the organism for establishing itself in a particular environment will mould the organism within the pool, now known as a species, in a seemingly Lamarckian fashion. However, this plasticity is available on at least two levels: the single, large, and persistent linkage group, the chromosome, carries the less flexible requirements, while the plasmids carry facilities more immediately at hand.

Typing extends bacterial identification within the species. In fact, as a term it is a misnomer, since the "typing" methods so far described, namely phage typing, serotyping, and the newer resistogram typing, all seek to identify individual strains as they pass from one host to another. The main aim is to fingerprint the organism for epidemiological purposes. However, it is now realized that phage per se is not a reliable indicator of identity without some independent epidemiological evidence to buttress it. Thus, for organisms isolated from sources distant in time and space, similar phage types do not necessarily indicate identity. Nor can phage typing provide direct evidence of virulence. This old misconception is exploded by the book in no uncertain terms. Similarities in pattern, however, do indicate relationships, and it is noteworthy that the animal biotypes, particularly those of bovine origin, can be defined in this way. It is generally believed that serotyping is based on more stable markers. The patient and elegant work described herein reveals the flaws of phage typing, which for so long was the last court of appeal. Thus lysogenization, plasmid transfer, and loss of prophage can all lead to changes in phage type without a corresponding change in serotype. Important phage complexes like 80/81 can be subdivided into distinct serotypes. It is too early to say how often and under what circumstances serotype can change, but this, undoubtedly, is also capable of happening. Resistogram typing, when fully worked out, may complement those two older methods and define isolates with still greater precision.

It is stated by one of the authors that the "major reason for the existence of this book . . . is the universal interest in controlling and eradicating the organism," and

it is implied that the best way of achieving this would be through a fuller understanding of the factors controlling virulence. There are, however, two intrinsic difficulties in the identification of bacterial determinants of virulence. First, with rare exceptions, virulence is determined not by one factor, but by several, and the loss of any single factor can result in partial or complete loss of virulence. The second difficulty lies in the fact that virulence for a given animal species can only be determined *in vivo*. Selection and phenotypic changes *in vitro* may lead either to loss of virulence determinants or, what is equally important, to the acquisition of factors that do not occur *in vivo*. The time-honoured method of "association" with certain characteristics, for example, haemolysins and other enzymes, or surface antigens, may indicate no more than a description of the species in statistical terms. As in a court of law, "association" may render an individual suspect, but does not prove guilt. Even the fact that so many staphylococcal products integrate neatly into the blood-clotting mechanism or can lyse red cells is not proof. Many undoubtedly non-pathogenic bacteria are known to have similar action and they have not been studied with the same care as staphylococci.

Spectacular progress has been made in this field, during the last two decades, by the purification of various toxins, which allows for far greater precision of interpretation than in the past. The precise mode of action of the staphylococcal enzymes is of great biological interest, and when, as in the case of the Panton-Valentine leucocidin, the only target is leucocytes the molecular events are of fundamental interest.

So far all this has not brought us much nearer to the understanding of virulence in chemical terms. In spite of the great number of toxins and enzymes that are identified, it is possible that virulence for man is mediated by more sophisticated and as yet unknown factors. After all, gel diffusion gives more antigen-antibody lines than can yet be accounted for by known antigens. In this respect the systematic and painstaking work on the enterotoxins is particularly impressive, and it would seem that the view held in the past that enterotoxins are formed by only a minority of strains needs to be drastically revised.

One of the most impressive areas of pure research on staphylococci is concerned with the elucidation of the chemistry and organisation of the cell wall. Gradually, the gap between chemical constituents and antigens recognised by immunological methods is being closed. What was known in the past as polysaccharide A, in precipitin reactions, is now known to have as its main component ribitol teichoic acid. Furthermore, the serological determinant is identified as a *N*-acetylglucosaminyl ribitol unit. Of equal interest is protein A, which is species-specific. This antigen was found to combine with IgG, but the union, which precipitates in a stoichometric manner giving a line in gels, is "nonspecific" in the sense that the attachment is to the Fc fragment. This is the explanation of the normally occurring "antibody" to *S. aureus* in human serum, a fact of great practical and theoretical interest. In the practical field it may cause false positive reactions in the rapid methods now used in the identification of bacteria that are based on the adsorption of specific immunoglobulins. In the theoretical field the Fc fragment is normally regarded as the nonbinding part of the antibody, and this interesting observation may prove to be of more than passing interest to immunologists.

In the purely applied field of medical bacteriology, progress has not been so spectacular. In the absence of detailed knowledge about virulence, both as a character of the individual strain and in its epidemiological sense, methods of control can go little beyond generalities. The excellent description of the clinical aspects highlights a point that is often lost sight of in the systematic studies of staphylococci—

the nature of the pathogenicity of *S. epidermidis* is fundamentally different from that of the pus-forming *S. aureus*. The former causes essentially opportunistic infections, generally in the presence of foreign bodies, while the other is a real pathogen. A search for shared virulence determinants would be futile under these circumstances. Incidentally, the much stressed statement that, in the patient, pus must be evacuated and any foreign body removed for cure is extremely important. It is also a silent admission of the fact that in spite of all our antibiotics we still do not have a full grasp of the staphylococcal problem.

Almost a hundred years have passed since staphylococci were implicated as casual factors in disease. It would indeed be rash to forecast what the future holds, but anyone reading this book will behold an entrancing panorama of modern bacteriology as a science.

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September 1972
London, England

PREFACE

When I first studied medical microbiology, I learned the long list of toxins found in bacteria and was surprised that so many of them occurred in staphylococci. There were hemolysins, leucocidins, hyaluronidases, enterotoxins, coagulases, fibrinolysins, and others. My initial impression was that here was a super-pathogen indeed. *Staphylococcus aureus* sometimes is a super-pathogen; yet why are staphylococci among the few pathogens that can be safely handled in general bacteriology? Why are these microorganisms more often innocuous and not responsible for infection? And why are most infections with this microbe focal, small, and trivial; a stye, a boil, even at times a pimple?

Our objective in writing this book is to bring together in one volume information acquired both by microbiologists and by physicians. If the subject is not covered entirely, it is because the staphylococci have been discussed in so many reports and investigations that their inclusion in one book is not possible.

The staphylococci are somewhat of an enigma. They are easy to handle in the laboratory, capable of genetic study by phage-mediated transduction and lysogeny experiments, and often sensitive to many antibiotics and therefore useful for study of their modes of action. The staphylococci have been studied as much as any Gram-positive bacterium, and yet the distinction between their interaction with man as a commensal and as a pathogen is still a mystery.

The versatility of staphylococci has stimulated research work in many diverse fields of microbiology. Their enterotoxins are important causes of many large and small food poisoning episodes. (It is doubtful whether any of us has not been affected at one time or another with staphylococcal food poisoning.) Although the enterotoxins are usually the cause of a discomfort that disappears as fast as it comes, with little damage to the victim, the frequency and number of people affected make this form of food poisoning of considerable economic importance.

In veterinary medicine staphylococci have replaced streptococci as the major cause of bovine mastitis. Dogs and other pets have been shown to carry antibiotic-resistant staphylococci of human types and to probably transmit staphylococcal disease to their owners and to veterinarians. In human medicine, although staphylococci have been notorious for skin infections since the time of Job, they have also been implicated in the disease of almost every organ of the body.

This book contains some clear-cut answers to questions about the staphylococci, such as information on the chemistry of the mucopeptide backbone of the cell wall and how it is synthesized, the efficacy and use of present-day antibiotics, and the chemistry of enterotoxins, leucocidin, coagulase, and other enzymes. In other areas we are presented with more questions than answers. Here, too, some success has been achieved in that we now know what questions to ask and how to proceed from them.

As editor, I have tried to ensure that the contributors state clearly how one goes about an epidemiological investigation, chooses the antibiotic for therapy, deter-

mines the phage type or serotype, and for what purpose. If some of these how-to sections seem elementary to the reader, I offer no apology except to say that I know of no place where this can be found in one book. More often the literature has short "materials and methods" sections with numerous references to prior papers. The references are given here too, but, wherever practical, a brief description of the method is also included.

This book is incomplete—every day during its preparation more papers appeared on the staphylococci. Some older references, no doubt, were missed, but I hope that enough of the story is here to lead the reader even to the references missed, by finding them referred to in some of the hundreds of references cited. Even if it were possible to list all the literature published before this volume, the book would still be incomplete, for life is dynamic and ever-changing, and so ever-changing are the staphylococci and their relationship to man.

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Atlanta, Georgia
May 1972

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CHAPTER ONE

Classification and Identification of Staphylococci and Their Resistance to Physical Agents

A. C. BAIRD-PARKER

I. CLASSIFICATION OF STAPHYLOCOCCI

A. Historical Introduction. One hundred years have elapsed since cocci were first observed in diseased tissues and in pus obtained from human abscesses. These organisms were called "micrococci" by Von Recklinghausen (1871), *Microsporon septicum* by Klebs (1872), and "monads" by Hueter (1872) and were classified by Billroth (1874) on the basis of their cell arrangements into "monococcos," "diplococcos," "streptococcos," and "gliacoccos." In 1880 a Scottish surgeon, Sir Alexander Ogston, presented his carefully collected data which showed conclusively for the first time that a cluster-forming coccus was the cause of certain pyogenic abscesses in man; at the same time Louis Pasteur reached similar conclusions in France (Pasteur, 1880). This pyogenic organism was named "staphylococcus" by Ogston (1882); the name, derived from the Greek nouns *staphyle* ("a bunch of grapes") and *coccus* ("a grain or berry") is very descriptive and appropriate. Ogston proposed this name for the cluster-forming cocci in order to distinguish them from Billroth's chain-forming streptococci; these he found to cause disease symptoms quite different from those due to staphylococci. Ogston showed that the subcutaneous injection into mice of pus containing staphylococci, or a culture of these organisms derived from pus, resulted in the same disease symptoms in mice as were observed in man. He found further that, if he heated the pus or treated it with phenol before injecting it into mice, neither infection nor disease resulted. Ogston marveled, as we still do today, at the variety of diseases caused by staphylococci and also at the wide variation in the resistance of man and other animals to infection by these organisms. His clear description of the disease symptoms resulting from a staphylococcus infection is a classic example of the lasting value of accurate observations and careful deduction and should be read by all persons concerned with staphylococcal infections and diseases.

Rosenbach (1884) was probably the first to isolate and grow staphylococci in pure culture and to study their characteristics in the

laboratory. He isolated from pus, organisms which he considered identical to those observed and described by Ogston and therefore adopted Ogston's name *Staphylococcus* for the genus that he erected to accommodate them. Rosenbach observed that isolates formed two types of colonies that were indistinguishable from each other except in color: one type was orange and the other white. He proposed that these organisms should be called, respectively, *Staphylococcus pyogenes aureus* (*Staphylococcus aureus*) and *Staphylococcus pyogenes albus* (*Staphylococcus albus*). In his original publication Rosenbach caused some confusion among later taxonomists by the use of both trinomials and binomials for the same organisms. In the first part of his paper he used trinomials (e.g., *Staphylococcus pyogenes aureus*), and only when referring to these organisms in a later part of the paper did he use the binomials (e.g., *Staphylococcus aureus*). The rules of bacterial nomenclature, however, clearly state that trinomials are illegitimate and that the use of a binomial accompanied by a description of the species is required for a species name to be validly published (rule 14a). Contrary to later statements by Winslow, Rothberg, and Parsons (1920), Rosenbach never used the name *Staphylococcus pyogenes* var. *aureus* or *Staphylococcus pyogenes* var. *albus*. Thus *Staphylococcus aureus* Rosenbach is accepted as the validly published name for the nomenclatural type species of the genus *Staphylococcus* (Editorial Board, 1958).

Flügge (1890) placed *Staphylococcus* in the family Coccaceae, which was later divided by Winslow and Rogers (1906) into two subfamilies, the Metacoccaceae and the Paracocacceae. Winslow and Winslow (1908) placed the orange staphylococci in the genus *Aurococcus* and the white staphylococci in the genus *Albococcus*. Both genera were placed with streptococci in the Paracoccaceae; micrococci were placed together with *Sarcina* and *Rhodococcus* in the Metacoccaceae. It was later shown by Buchanan (1911) that the subfamilial names Metacoccaceae and Paracoccaceae were invalid, and he therefore proposed that they be replaced, respectively, by the tribal names Micrococceae Trevisan and Streptococceae Trevisan. Later, when Winslow et al. (1920) restudied the genera *Aurococcus* and *Albococcus*, they concluded that the genera were not distinct and the albococci were strains of *Staphylococcus aureus* that had lost some of the original characteristics of the species through growth under unfavorable conditions outside the human body. It would appear that the main reason why these early workers placed staphylococci and micrococci into separate tribes or subfamilies was that they regarded staphylococci and streptococci to be parasitic organisms growing well anaerobically and micrococci and sarcinas to be aerobic saprophytes. This difference was accepted by the Committee of the Society of American Bacteriologists on the Characterization and Classification of Bacterial Types (1920), whose members recommended that *Staphylococcus* become a genus of the Streptococceae. The definition of the genus *Staphylococcus* was given as follows: "Parasites. Cells in groups and short chains, very rarely in packets. Generally stained by Gram. On agar, good growth of white or orange colonies. Glucose, maltose, sucrose, and often lactose fermented with the formation of moderate amounts of acid. Gelatin often liquefied very actively."

In a series of papers published between 1924 and 1928, however, Hucker concluded that micrococci and staphylococci were indistinct and therefore classified them together in the genus *Micrococcus*. Rahn (1929) also decided that staphylococci and micrococci were insufficiently distinct from each other to warrant separate generic rank. This view gained acceptance, and Bergey's *Manual of Determinative Bacteriology*, having recognized the genus *Staphylococcus* in all its editions from the first in 1923 to the fifth in 1939, relegated the staphylococci to the genus *Micrococcus* when this section of the *Manual* was revised by Hucker in the sixth (1948) edition. Also, Abd-El-Malek and Gibson (1948) concluded that staphylococci and micrococci had been indiscriminately named and that strains isolated from milk were all members of a large group of organisms, which they called the "*Staphylococcus-Micrococcus* Complex." Within this complex they were able to distinguish a "staphylococcus group," a "dairy micrococci

group," and a group intermediate between these two. Similarly, Shaw, Stitt, and Cowan (1951) were unable to find sufficient evidence for separating staphylococci from micrococci and, after concluding that the genus *Micrococcus* was invalid, placed organisms previously recognized as micrococci into the genus *Staphylococcus*; in so doing they further confused the classification of staphylococci.

In the mid-fifties there was renewed interest in the classification of staphylococci and micrococci, and a number of papers presented arguments for and against the separation of these organisms into two genera. Papers published by Van Eseltine (1955) and by Thatcher and Simon (1957) supported the view that these organisms should not be separated. However, Evans, Bradford, and Niven (1955) proposed that staphylococci should be separated from micrococci on the basis of their ability to grow anerobically and under these conditions to form acid from glucose. These findings were accepted by Breed when he revised the classification of members of the Micrococcaceae in the seventh edition of Bergey's *Manual* (1957) and were confirmed by Baird-Parker (1963).

The editorial board for the eighth edition of *Bergey's Manual* has accepted the genera *Staphylococcus*, *Micrococcus*, and *Planococcus* for inclusion in the Micrococcaceae (R. E. Buchanan, personal communication, 1971). The genus *Aerococcus* which was recognized by the International Committee on Systematic Bacteriology (ICSB), Subcommittee on the Taxonomy of Staphylococci and Micrococci, as a member of the Micrococcaceae is more correctly placed by the editorial board in the family Streptococcaceae. The main characteristics distinguishing these genera are shown in Table 1.

The separation of staphylococci from the other cluster-forming, Gram-positive, and catalase-positive cocci on the basis of their ability to produce acid anaerobically from glucose, that is, to ferment glucose, has been criticized by such authors as Klesius and Schuhardt (1968), Auletta and Kennedy (1966), Mortensen and Kocur (1967), and Gibson (1967) on the grounds that the division is not clear cut. These authors point out rightly that under certain growth conditions some Gram-positive and catalase-positive cocci are able to grow slowly anaerobically

Table 1. Some Differential Characteristics of Genera of Micrococcaceae and *Aerococcus*[a]

Characteristic	*Staphylococcus*	*Micrococcus*	*Planococcus*	*Aerococcus*
Shape: spherical	+	+	+	+
Arrangement				
Irregular clusters	+	+	−	−
Tetrads	−	V	+	+
Gram reaction	+	+	+	+
Glucose fermentation[b]	+	−	−	d
Ctyochromes	+	+	+	−
Catalases:				
Hem	+	+	+	−
Nonhem	−	−	−	V
Hydrogen peroxide formation	−	−	−	+
Motility	−	−	+	−
Yellow-brown pigment	−	−	+	−
GC content of DNA (mole %)	30–40	30–75[c]	39–52	37–41

[a] + = most (90% or more) strains positive; − = most (90% or more) strains negative; V = characters inconstant and in one strain—may sometimes be positive, sometimes negative; d = some strains positive.

[b] Growth and acid anaerobically from glucose in the standard medium proposed by the ICSB Subcommittee on the Taxonomy of Staphylococci and Micrococci (Recommendations, 1965).

[c] Except for strains of *Micrococcus saprophyticus* and some *M. lactis*, the range is 57 to 75.

and to ferment glucose slowly. These would be classified as micrococci on the basis of the standard glucose fermentation test proposed by the ICSB Subcommittee (Subcommittee 1965) and mainly belong to the species *Micrococcus saprophyticus*. When strains of this species have been examined, they have been shown to be similar to staphylococci in the mole percentage of guanine + cytosine (per cent GC) in their DNA, in the sensitivity of their cells to lysis by the enzyme lysostaphin, and in the structure and composition of their peptidoglycans. However, they differ from other staphylococci in their high resistance to novobiocin (Mitchell and Baird-Parker, 1967; Jeffries 1969), in the presence of generally different menaquinones in their cell membranes (Jeffries et al., 1968), and often in their growth requirements (Baird-Parker 1963, 1965a). *Micrococcus saprophyticus* is of particular interest to clinicians as it causes infections of the urinary tract (Torres Pereira, 1962; Mitchell, 1964, 1968; Roberts, 1967). Recent information indicates that this microorganism together with *M. lactis* strains with percent GC in their DNA of 30–40% should be transferred to the genus *Staphylococcus* (Kocur, Bergan, and Martensen, 1971; Schleifer and Kandler, 1970). Despite possible limitations, the standard method proposed by the ICSB Subcommittee for determining the fermentation of glucose by *Staphylococcus* species remains the most reliable and practical procedure for recognizing staphylococci. However, the recent medium proposed by Evans and Kloos (1972) for distinguishing staphylococci and micrococci is of considerable interest as most *M. saprophyticus* and *M. lactis* strains with percent GC in their DNA similar to that of staphylococci behave like staphylococci in this medium. Peny and Buissiere (1970) have recently reported that two other tests, the degradation of DL-alanyl-β-naphthylamide and the decarboxylation of arginine, provide useful additional means for distinguishing staphylococci from micrococci. Also Morrison, Tornabene, and Kloos (1971) report that the presence of aliphatic hydrocarbons in neutral lipids extracted from micrococci distinguishes these organisms from staphylococci.

B. Description of Members of the Genus *Staphylococcus*. Staphylococci are nonmotile, Gram-positive, and catalase-positive cocci (0.5–1.5 μ in diameter) that are able to divide in more than one plane to form irregular clusters of cells.

Cell walls contain two main components, the peptidoglycan and the teichoic acids. The peptidoglycan is quite characteristic of this genus. It is composed of a glycan made up of repeating units of β-1,4-*N*-acetylglucosamine and *N*-acetylmuramic acid residues that are linked through *N*-acetylmuramyl-L-alanine linkages to peptide subunits consisting of *N*α-(L-alanyl-D-isoglutamyl)-L-lysyl-D-alanine. The peptide subunits are cross-linked by pentapeptide bridges containing solely or mainly glycine. These bridges extend from the *N*ε-lysine residue of one peptide subunit to the C-terminal D-alanine of a neighboring peptide subunit. Attached to, or in close proximity to, the peptidoglycan are teichoic acids which, depending on the species, contain either ribitol or glycerol linked to a sugar or an amino sugar.

Staphylococci are facultative anaerobes growing best in the presence of air. For aerobic growth, they require a medium containing amino acids and growth factors; for anaerobic growth they also require uracil and a fermentable carbon source. They are typical mesophiles, growing at temperatures between 6.5 and 46°C (optimum 35–40°C) and at pH values between 4.5 and 9.3 (optimum 7.0–7.5). Most strains will grow in the presence of up to 15% sodium chloride or 40% bile. They are sensitive to lysis by lysostaphin endopeptidase, which breaks the glycyl-glycine links in the peptide bridges of the peptidoglycan; they are resistant to lysis by lysozyme.

The metabolism of staphylococci is both respiratory and fermentative, and menaquinones and cytochromes a, b_1, and O form the electron transport system; carotenoid pigments may be present. Oxygen is the universal, terminal electron acceptor, although some strains can grow anaerobically using nitrate as an electron acceptor. Fermentation of glucose under anaerobic conditions results in the production of mainly lactic acid,