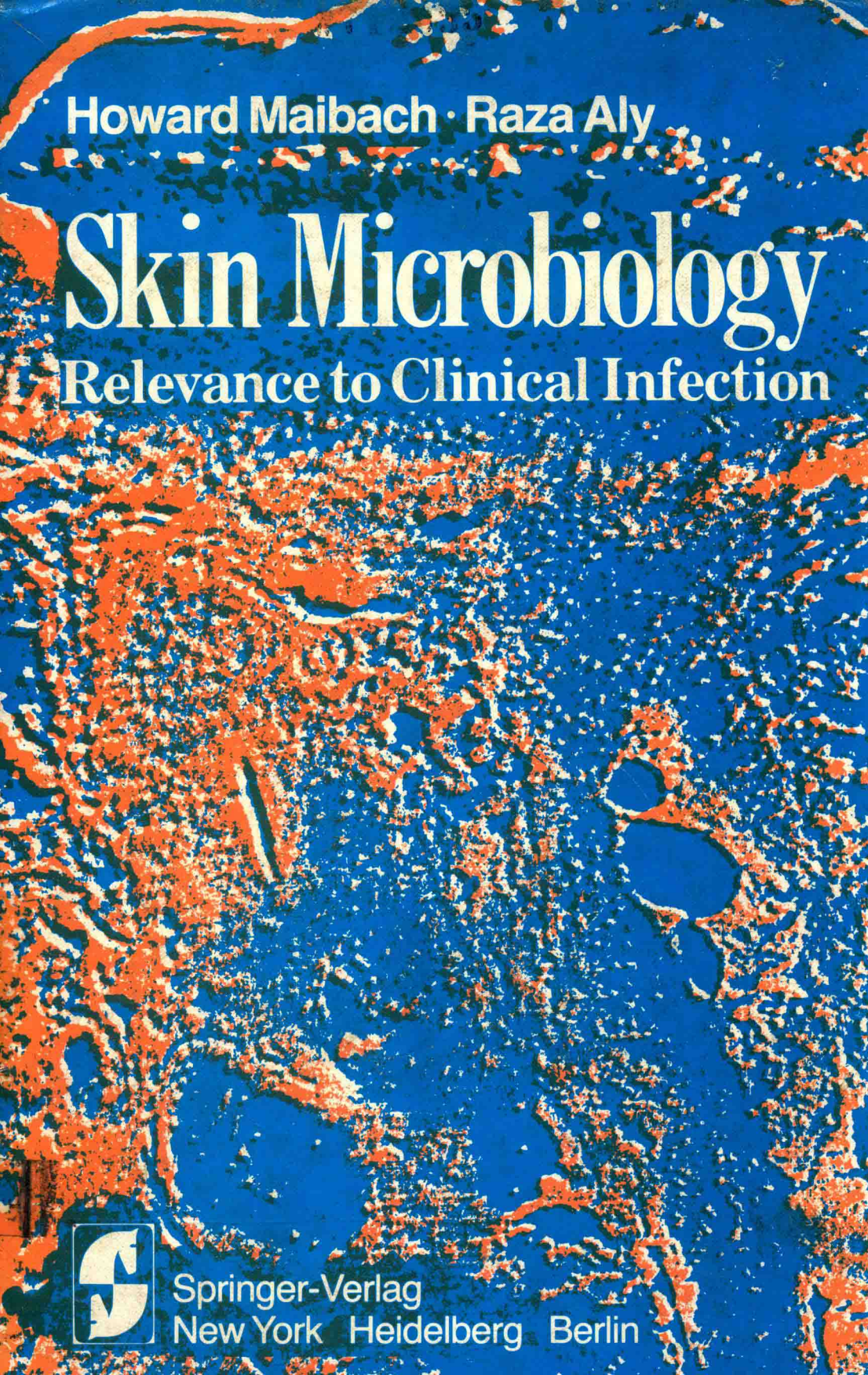
Howard Maibach · Raza Aly

Skin Microbiology

Relevance to Clinical Infection



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Edited by
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Preface

Not since the 1965 publication of *Skin Bacteria and Their Role in Infection* has our knowledge of clinical skin microbiology been reviewed and summarized. In the more than a decade and a half since that publication, we have seen a careful reevaluation of the ideas and information current in 1965 and the development of important new discoveries and information.

This volume, *Skin Microbiology: Relevance to Clinical Infection*, reviews developments in the field since 1965 and summarizes the current state of the art in thirty-six carefully prepared chapters. Emphasis is on the clinical perspective rather than straight microbiology, although we include enough of the latter to put the clinical aspects in a proper scientific context.

The authors contributing to this volume represent a cross section of authorities in the many specialty areas that contribute to our knowledge of skin microbiology. They include investigators in microbiology, infectious disease, epidemiology, surgery, pediatrics, and dermatology. Significant efforts have been made to minimize repetition and overlap in the various chapters. In some cases, however, information is deliberately repeated in order to provide for the reader a necessary frame of reference.

We hope that this volume will be of value to dermatologists, microbiologists, pediatricians, surgeons, public health workers, nurses, and others involved in the diagnosis and treatment of dermatologic problems caused by bacteria.

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Contents

PART ONE

THE CUTANEOUS FLORA AND ITS CONTROL

Chapter 1

The Identification of *Staphylococcus* and *Micrococcus* Species Isolated from Human Skin

WESLEY E. KLOOS

Staphylococci and micrococci represent major groups of bacteria inhabiting human skin. The identity of each genus and currently recognized species offers a challenge to the skin microbiologist that should be rewarding in uncovering the population structure of cutaneous microbial communities and in providing a starting point from which to explore adaptive and evolutionary mechanisms. I believe that most human *Staphylococcus* and *Micrococcus* species have been discovered and given an appropriate taxonomic status. Perhaps others will be found, especially from unique niches or specially isolated human populations. Schleifer and coworkers[20] (K. H. Schleifer, personal communication) have shown that strains identified as *Peptococcus saccharolyticus* are actually anaerobic staphylococci which might constitute a new species. Preliminary ecological studies have indicated that the human forehead may carry rather large populations of this organism.[14]

In this paper, an attempt will be made to familarize the reader with the current status of *Staphylococcus* and *Micrococcus* taxonomy including the choice of simple characters that may be tested in the routine laboratory for genus and species identification.

Separation of Staphylococci and Micrococci

Staphylococci and micrococci can be separated by several key tests easily performed in the routine laboratory. One such test proposed by Schleifer and Kloos[46] is based on the ability of staphylococci to produce acid, aerobically, from glycerol in the presence of 0.4 μg erythromycin per ml and on their susceptibility to lysostaphin at a concentration of 200 μg/ml. Curry and Borovian[9] have shown that a nitrofuran-containing medium (FTO agar) permits the growth of micrococci but not staphylococci. Most *Staphylococcus* species produce detectable growth in a semisolid thioglycolate medium described by Evans and Kloos,[16] whereas most *Micrococcus* species will not grow in this medium except near the surface.[25,47] The

species usually not conforming to this test include *S. hominis*, some strains of *S. haemolyticus*, *S. cohnii*, and *S. xylosus*, and *M. kristinae*; however, the test provides more reliable results for the separation of these genera than the standard anaerobic glucose utilization test proposed by the International Subcommittee on Staphylococci and Micrococci (1965). Seidl and Schleifer[52] introduced a serological test for separating staphylococci from micrococci based on different peptidoglycan types. Preliminary identification of these organisms can, in most instances, be accomplished on the basis of colony morphology and pigment and growth rate. Colonies of micrococci develop much slower than those of staphylococci (except for *Staphylococcus sciuri* subsp. *lentus*, which has been isolated from sheep and goats). Human *Staphylococcus* species produce detectable colonies (> 1 mm in diameter) on most nonselective plating agars, within 18 h at 34–37°C; whereas, micrococci require 36–48 h to produce detectable colonies. Most micrococci produce colonies typically more convex than staphylococcus colonies. Occasionally colonies of micrococci may be confused with those of corynebacteria, though these two groups can be resolved on the basis of cell morphology.

Micrococci and staphylococci are included currently in the family Micrococcaceae[5] representing gram-positive, catalase-positive cocci; however, major differences in their DNA base composition[1,6,25,30,33,44,47] and 16S ribosomal RNA sequences[53] indicate clearly that these organisms bear no special relationship to one another. It is expected that staphylococci and micrococci will be eventually separated and moved into other existing families.

Characterization of *Staphylococcus* Species Inhabiting Human Skin

Staphylococci are currently classified according to specific combinations of phenotypic characters[10,19,25,31,47] and DNA relatedness.[10,22,28,39,43,50] Extensive systematic and ecological studies have identified at least ten different *Staphylococcus* species living on human skin.[8,11,12,15,18,23,24,25,26,31,47,50,57,58] These species include *S. aureus*, *S. epidermidis*, *S. hominis*, *S. haemolyticus*, *S. capitis*, *S. warneri*, *S. saprophyticus*, *S. cohnii*, *S. xylosus*, and *S. simulans*. In the routine laboratory, they can be identified with reasonably good accuracy (> 80 to > 95 percent, depending upon the species) using specific combinations of selected characters that are relatively simple to test and have a high predictive value in resolving DNA homology groups.[24,27,32,55] The task of resolving at least ten different species is a relatively large one in any study. Here the investigator must determine whether or not it is important to resolve all species or only certain ones. The widely accepted Baird-Parker taxonomic schemes cannot identify most of the species inhabiting human skin, though they can identify with reasonable accuracy (> 70 percent) the more medically important species *S. aureus*

(*Staphylococcus* subgroup I), *S. epidermidis* (*S.* subgroup II or *S. epidermidis* biotype 1), and *S. saprophyticus* (*Micrococcus* subgroup 3 or *S. saprophyticus* biotype 3).[2,3,4] Some microbiologists adhere to Baird-Parker's schemes, but in doing so, must realize that they are limiting their ability to recognize the variety of widely divergent populations present on skin, some of which demonstrate niche and host preferences.

S. aureus is considered the most potentially pathogenic species and for this reason has received the most attention. Several years ago, all coagulase-positive staphylococci were identified as belonging to this species. Today, we recognize the three coagulase-positive species *S. aureus*, *S. intermedius*, and *S. hyicus;* the latter two species are found primarily on lower mammals and birds.[10,19,49] A moderate percentage of *S. hyicus* strains are coagulase-negative and were classified earlier as *Staphylococcus* subgroup III or *S. epidermidis* biotype 2 according to Baird-Parker's schemes. *S. intermedius* and *S. hyicus* are probably of minor concern to one studying the human cutaneous microflora, though they may be isolated on occasion from human skin if contact has been made with natural animal hosts (e.g. with pets or farm animals). Although these species may produce infections in a variety of animals and birds, it is not known if they are capable of producing infections in man. All three coagulase-positive species produce higher deoxyribonuclease activity than usually found with coagulase-negative species. Coagulase-positive staphylococci that produce weak, delayed acid or no acid, areobically, from maltose are probably not *S. aureus*. *S. intermedius* strains isolated from carnivora (e.g. dogs, mink, raccoons, etc.) typically produce very weak acid from maltose in 48 to 72h and *S. hyicus* strains usually fail to produce acid from this carbohydrate. *S. intermedius* and *S. hyicus* do not produce acid from D-mannitol anaerobically and do not produce acetylmethylcarbinol (acetoin), except, perhaps, in small quantity. *S. aureus* on the other hand, is usually positive for these characters. Each of the coagulase-positive species are in different, widely divergent DNA homology groups exhibiting less than 20 percent relatedness under restrictive binding conditions.[10,39,42]

The nine coagulase-negative species found living on human skin may be organized into three species groups.[26,32] The major one of human skin is the *S. epidermidis* species group composed of the species *S. epidermidis*, *S. hominis*, *S. haemolyticus*, *S. warneri*, and *S. capitis*. These species are related slightly more to one another than to species in other groups. They exhibit a DNA relatedness of about 45 ± 6 percent at non-restrictive conditions and 15 ± 3 percent at restrictive conditions.[22,28,50] *S. hominis* is more related to *S. haemolyticus*, and *S. epidermidis* is more related to *S. capitis* than to other species of this group.

S. epidermidis and *S. capitis* may be identified easily and with considerable accuracy (>95 percent). On a peptone-yeast extract-salt (P agar) medium,[30] colonies of *S. epidermidis* are small and are about 3.5 to 4 mm in diameter after incubation at 34°C for 3 days, followed by storage at room temperature for an additional 2 days. Colonies are slightly raised, rather sticky,