Staphylococcus Epidermidis Biotype 4: Epidemiological Conclusions from Five Different Typing Methods

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ABSTRACT

There is presently no accepted method for marking individual strains of Staphylococcus epidermidis. Consequently, five parameters for distinguishing such strains were examined and compared for their epidemiological efficacy: biotyping, serotyping, proteinase grouping, aminopeptidase profiles and antibiograms. Both biotyping and proteinase grouping were of limited use in identifying a particular strain, although they were helpful in initially categorizing such strains. Antibiograms were least useful because of similarities in susceptibility patterns among isolates. Serotyping and aminopeptidase profiles provided the best means of identifying an individual strain for epidemiological use. The applicability of these typing methods was demonstrated during a one year epidemiological study at a chronic disease hospital.

Introduction

The emergence of coagulase-negative Staphylococcus epidermidis as the etiological agent of a variety of pyogenic and urinary tract infections and their ability to colonize artificial internal prostheses resulting in bacteremia has been well documented. Although their role as etiological agents in less severe infections is still under investigation, the frequent isolation of antibiotic resistant strains of S. epidermidis, especially from pediatric, geriatric and chronic disease centers (where both opportunistic and nosocomial infections are common), poses a serious therapeutic threat. The unusually high incidence of S. epidermidis biotype 4 at Goldwater Memorial Hospital (GMH) initially prompted this study. Since there are no useful methods for marking individual strains of S. epidermidis for epidemiological study, five parameters for distinguishing such strains were examined and compared for their efficacy in epidemiological studies: biotyping, proteinase typing, serotyping, aminopeptidase profiles and antibiograms.
Materials and Methods

Cultures

A total of 365 isolates* of *S. epidermidis* (coagulase-negative) was obtained over a 12 month period from 10 selected wards through the clinical microbiology laboratory of New York University Medical Center—Goldwater Memorial Hospital, Roosevelt Island, NY. All isolates were initially biotyped by the method of Baird-Parker.† Further information on these strains is reported elsewhere.24,25,26

Incidence, Frequency and Distribution of *S. epidermidis*

A computer system‡ was especially programmed for collecting and analyzing information on: (1) the incidence, frequency, and distribution of different biotypes of *S. epidermidis* from selected wards in Goldwater Memorial Hospital (GMH) over a 12-month period; (2) the number of different *S. epidermidis* biotypes isolated each month; (3) their incidence and frequency on different wards; and (4) the number isolated from various body sites. These data were compiled to determine the incidence and frequency of various biotypes of *S. epidermidis* on different wards and body sites to enhance the value of the epidemiologic investigation.

Antibiograms

Antibiotic susceptibilities were performed according to the method of Bauer et al,12 using the following antibiotic discs: ampicillin (10 μg), cephalothin (30 μg), clindamycin (2 μg), oxacillin (1 μg), chloramphenicol (30 μg), gentamicin (10 μg), kanamycin (30 μg), tetracycline (30 μg), erythromycin (15 μg) and penicillin (10 units) (BBL).

The susceptibility or resistance of the bacteria to the antibiotics was determined from the diameter of the inhibitory zones, based on the interpretative tables recommended by the Food and Drug Administration.4,5

The antibiograms of strains of *S. epidermidis* isolated during a 12 month period from selected wards at GMH were introduced into a computer‡ especially programmed to provide information on a monthly basis, on: (1) the sensitivity patterns of all isolates of *S. epidermidis*; (2) the sensitivity of these *S. epidermidis* isolates with respect to the body site from which they were isolated; and (3) the sensitivity of the *S. epidermidis* isolates from GMH in comparison to isolates from institutions using the same method and computerized on a national basis.

Protease Subtyping

All isolates of biotype 4 were subtyped as protease-positive (a) or protease-negative (b) by the method of Sandvik.21 Hence, within each Baird-Parker biotype, there were two subtypes: i.e., sub-biotypes 1a and 1b and sub-biotype 4a and 4b.

Protease Detection and Differentiation

All of the protease-positive isolates of biotype 4 were tested by enzymosero-logical reactions25 against known protease group antisera (i.e., B, C, D, E, F, G).22

Immunology

All biotype 4 isolates were serotyped, as previously reported.24

Aminopeptidase Profiles

All isolates of biotype 4 were assayed for their aminopeptidase enzyme profiles,8,27 as previously reported.26
Results

Incidence, Frequency and Distribution of S. epidermidis

The number of isolates in each biotype, based on the Baird-Parker biotyping scheme,¹ is shown in table I. Of the 365 isolates studied, 200 (54.34 percent) were biotype 4, 137 (38 percent) were biotype 1, eight (2.18 percent) were biotype 3, four (1.09 percent) were biotype 2 and 16 (4.35 percent) were non-typeable according to the scheme.

The number of S. epidermidis isolates from different body sites is shown in table II. S. epidermidis was most frequently isolated from urine and wound-abscess cultures: 260 (70.7 percent) from urines, 67 (18.2 percent) from wound- abscess cultures, 10 (2.8 percent) from sputa, 5 (1.4 percent) from throats, 1 (0.3 percent) from blood and 22 (6.8 percent) from miscellaneous specimens.

S. epidermidis biotype 4 was the most frequently isolated biotype and of 200 isolates, urine was the clinical source from which it was most often isolated (184), followed by wound- abscesses (11) and miscellaneous specimens (5) (table II). S. epidermidis biotype 1 was most frequently isolated from urine (70), wound- abscesses (47) and miscellaneous specimens (9), followed by sputa (8), throats (2) and blood (1). S. epidermidis biotype 2 and 3 were not frequently isolates in this study, and 16 isolates of S. epidermidis were not biotypeable (table II).

The wards from which S. epidermidis was most frequently isolated were: B21-22 (68), B41-42 (66), D21 (47), D-42 (41), C21-22 (39), C-41 (26), D11-12 (24), D-22 (20), A21-22 (19) and C-12 (15). The wards from which S. epidermidis biotype 4 was most frequently isolated were: B21-22 (52), B41-42 (44), D21 (28), C21-22 (19), C-41 (15), D11-12 (12), C-12 (11), D-42 (10), D-22 (5) and A21-22 (4). The B and C wards care for chronically ill, debilitated (neuro-muscular and pulmonary diseases), bedridden patients, most of whom have indwelling Foley Catheters and have chronic urinary infections. The A wards care for geriatric patients and D wards care for general medical and intensive care units. The months of July, September, October and December had the greatest number of isolates of S. epidermidis, and January, February, March, April and June had the least number of S. epidermidis isolates.

Antibiograms

Over the 12-month period, the isolates of S. epidermidis showed the following sensitivity to various antibiotics: 34 percent (of 365 isolates) were sensitive to ampicillin; 99 percent (of 365 isolates) to cephalothin; 92 percent (of 364 isolates) to chloramphenicol; 88 percent (of 353 isolates) to clindamycin; 79 percent (of 363 isolates) to erythromycin; 98 percent (of 364 isolates) to gentamicin; 87 percent (of 358 isolates) to kanamycin; 84 percent (of

<table>
<thead>
<tr>
<th>Substrate or Test</th>
<th>Biotype 1</th>
<th>Biotype 2</th>
<th>Biotype 3</th>
<th>Biotype 4</th>
<th>Non- Typeable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetoin production</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>d</td>
</tr>
<tr>
<td>Phosphatase production</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>d</td>
</tr>
<tr>
<td>Production of acid aerobically from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>d</td>
<td>-</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>-</td>
<td>d</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td>Proteinase– Positive (a)</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteinase– Negative (b)</td>
<td>57</td>
<td>169</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total isolates</td>
<td>365</td>
<td>137</td>
<td>(38.0%)</td>
<td>(1.1%)</td>
<td>(2.2%)</td>
</tr>
</tbody>
</table>

+ = Positive reaction; - = Negative reaction; d = Different biochemical reactions depending upon isolate.
365 isolates) to oxacillin; 30 percent (of 365 isolates) to penicillin; and 42 percent (of 353 isolates) to tetracycline.

A comparison of the antibiotic sensitivities of *S. epidermidis* isolates from GMH with those contributing to a national computerized study of antibiotic sensitivity is presented in table III. Although the national data were obtained from 49,973 isolates, compared with 365 isolates from GMH, there was a remarkable similarity in antibiotic sensitivity patterns between the two groups of isolates.

The susceptibility patterns of biotypes 1 and 4 were similar with ampicillin, cephalothin, chloramphenicol, gentamicin, and penicillin G (table IV). However, there was a difference between biotypes 1 and 4 in susceptibility to clindamycin, erythromycin, kanamycin, oxacillin and tetracycline: biotype 4 was more susceptible than biotype 1 to these five antibiotics.

**Detection and Differentiation of Proteinases**

Seventeen of the 31 proteinase-positive isolates of *S. epidermidis* biotype 4 were classified by serological differentiation of their proteolytic enzymes with known specific antisera (B,C,D,E,F,G). Twenty-one of the 31 proteinase-positive isolates of *S. epidermidis* biotype 4 produced proteolytic enzymes that did not react with any of the known group antisera and were classified as NR (non-reacting). The NR proteinases of these 14 isolates of *S. epidermidis* biotype 4 had the same electrophoretic band, NR, as previously reported.

The majority of biotype 4 isolates tested had proteinases of the C type. However, 45 percent of the biotype 4 isolates produced NR proteinases, and it is probable, therefore, that type C proteinase is not necessarily characteristic of this biotype. Type C proteinase was produced by the same strain of biotype 4, as shown by the

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Disc</th>
<th>GMH No./ Percent Sensitive</th>
<th>Nat. No./ Percent Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>10 ug</td>
<td>365/34</td>
<td>31,67/36</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>30 ug</td>
<td>365/99</td>
<td>42,36/97</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30 ug</td>
<td>364/92</td>
<td>41,25/92</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>2 ug</td>
<td>351/88</td>
<td>38,61/83</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>15 ug</td>
<td>363/79</td>
<td>39,33/77</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10 ug</td>
<td>364/98</td>
<td>23,40/98</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>30 ug</td>
<td>358/87</td>
<td>28,13/80</td>
</tr>
<tr>
<td>Oxacillin/ nafcillin</td>
<td>1 ug</td>
<td>365/84</td>
<td>12,76/81</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>10 units</td>
<td>365/30</td>
<td>37,59/31</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30 ug</td>
<td>355/42</td>
<td>38,79/53</td>
</tr>
</tbody>
</table>

*With some antibiotics, the number of isolates was less than 365, since sometimes an antibiotic disc did not drop from an automatic dispenser. The results were introduced into a computer programed to a base of 365 isolates; however, the differences in the actual number of isolates were statistically insignificant.*

**Immunology**

The 200 isolates of *S. epidermidis* biotype 4 were serotyped using four antisera to thermolabile (TL) antigens and 2 antisera to thermostable (TS) antigens. When both TL and TS antigens were used in serologically categorizing the

<table>
<thead>
<tr>
<th>Clinical Source</th>
<th>Isolates in Biotype</th>
<th>Non-typeable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine (260/70.7)*</td>
<td>70 0 0 184 6</td>
<td></td>
</tr>
<tr>
<td>Wound-abscess (67/18.2)</td>
<td>47 1 3 11 5</td>
<td></td>
</tr>
<tr>
<td>Sputum (10/2.7)</td>
<td>8 1 0 0 1</td>
<td></td>
</tr>
<tr>
<td>Throat (5/1.4)</td>
<td>2 0 2 0 1</td>
<td></td>
</tr>
<tr>
<td>Blood (1/0.3)</td>
<td>1 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>Miscellaneous (22/6.8)</td>
<td>9 2 3 5 2</td>
<td></td>
</tr>
<tr>
<td>Total isolates: 365</td>
<td>137 4 8 200 16</td>
<td></td>
</tr>
</tbody>
</table>

*Numbers in parenthesis represent the number of isolates per cent of total 365 isolates.

**Table III**

**Number and Biotype of 365 Isolates of *Staphylococcus epidermidis* from Clinical Sources at Goldwater Memorial Hospital**

**Table II**

**Sensitivity to Antibiotics* of *Staphylococcus epidermidis* Isolates from Goldwater Memorial Hospital (GMH) Compared with Isolates in National Computerized Data Bank for One Year Period**
TABLE IV
Comparison of Antibiotic Susceptibility Patterns of Isolates of Staphylococcus epidermidis Biotypes 1 and 4 from Goldwater Memorial Hospital

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Disc Potency</th>
<th>Biotype 1 Percent Sensitive of 137 Isolates</th>
<th>Biotype 4 Percent Sensitive of 200 Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>10 µg</td>
<td>35</td>
<td>33</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>30 µg</td>
<td>98</td>
<td>99</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30 µg</td>
<td>92</td>
<td>94</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>2 µg</td>
<td>84</td>
<td>91</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>15 µg</td>
<td>73</td>
<td>84</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10 µg</td>
<td>98</td>
<td>98</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>30 µg</td>
<td>84</td>
<td>91</td>
</tr>
<tr>
<td>Oxacillin/Nafcillin</td>
<td>1 µg</td>
<td>76</td>
<td>90</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>10 units</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30 µg</td>
<td>39</td>
<td>45</td>
</tr>
</tbody>
</table>

S. epidermidis isolates, five different serological types were evident: Al-2, B1-2, CD1-2, CD-1 and E-1, where the letters refer to the TL antigens and the numbers refer to the TS antigens.

AMINOPEPTIDASE PROFILES

Five different profiles were obtained for S. epidermidis biotype 4 (each representing a different serotype). The overall aminopeptidase activity of S. epidermidis appeared to be low, as none of the substrates was hydrolyzed 100 percent after 24 hrs of incubation. Nevertheless, each serotype of S. epidermidis biotype 4 had a distinctive aminopeptidase profile (table VII).

All staphylococci hydrolyzed alanyl- and methionyl-beta-naphthylamides to a moderately high degree and glutamyl-beta-naphthylamide to the highest degree. Of all of the substrates used, only valyl-beta-naphthylamide was not hydrolyzed by any staphylococcus studied.

In table VII are listed five parameters for distinguishing strains of S. epidermidis biotype 4 in epidemiological studies. Proteolytic enzymes were present in 31 of the 200 isolates, which fell in one of two proteinase groups (17 isolates in C and 14 isolates in NR). Five distinct serotypes (A1-2, B1-2, CD1-2, CD-1, E1), five distinct aminopeptidase profiles and antibiograms using five common antibiotics are presented also.

Discussion

These results suggest that once a subdivision of S. epidermidis isolates to biotype is made by the Baird-Parker scheme, further division can be made on the basis of proteolytic activity (on casein agar) of the isolates. Serologic grouping of the proteolytic enzymes can also be per-

TABLE V
Derivation of Staphylococcus epidermidis Biotype 4 TS and TL Serotypes*

<table>
<thead>
<tr>
<th>Bio-Isolate Type</th>
<th>TS Preabsorbed 4a Antiser</th>
<th>4b Antiser</th>
<th>TS Further Absorbed C-D', C-D'' or E Antigen</th>
<th>TS Preabsorbed 4a Antiser with C-D' or E then C-D'' or E</th>
<th>TS Preabsorbed 4a Antiser with A, B or C-D'' or E then C-D'' or A, B or C-D''</th>
</tr>
</thead>
<tbody>
<tr>
<td>TL Isolate No.</td>
<td>Preabsorbed</td>
<td>Preabsorbed</td>
<td>Preabsorbed</td>
<td>Preabsorbed</td>
<td>Preabsorbed</td>
</tr>
<tr>
<td>4a 1 - 17 A</td>
<td>1+ to 3+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4a 18 - 31 B</td>
<td>2+ to 3+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4b 32 - 45 C-D''</td>
<td>2+ to 3+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4b 46 - 64 C-D''</td>
<td>2+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4b 65 - 200 E</td>
<td>1+ to 2+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*1+ to 3+ = agglutination of various degrees. - = no agglutination. TS = thermostable. O = not tested, since previously negative for agglutination. C-D = used instead of C and D in order to show that there were similarities between these isolates with respect to the thermolabile antigens but differences with respect to the thermostable antigens. Note: No repeat isolates were used in this study.
Decreasing Order of Beta-naphthylamide Hydrolysis by Eight Different Isolates of Staphylococci after 24 Hours of Incubation at 37°C

TABLE VI

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Decreasing Order of Hydrolysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>I</td>
<td>GLU</td>
</tr>
<tr>
<td>II</td>
<td>GLU</td>
</tr>
<tr>
<td>III</td>
<td>GLU</td>
</tr>
<tr>
<td>IV</td>
<td>GLU</td>
</tr>
<tr>
<td>V</td>
<td>GLU</td>
</tr>
<tr>
<td>VI</td>
<td>GLU</td>
</tr>
<tr>
<td>VII</td>
<td>GLU</td>
</tr>
<tr>
<td>VIII</td>
<td>GLU</td>
</tr>
</tbody>
</table>

*1 = most; 7 = least.

ALA = L-alanyl; ASP = L-alpha-aspartyl; BANA = benzyl-L-arginyl; CYS = L-cysteinyl; GLU = L-gamma-glutamyl; GLY = glycyl; ILEU = L-isoleucyl; LEU = L-leucyl; LYS = L-lysyl; MET = L-methionyl; 4MLEU = 4-methoxy-leucyl; PHE = L-phenylalanyl; and TRY = L-tryptophyl.

formed, thereby, further sub-dividing the isolates so that a more complete biochemical characterization can be obtained. The proteinase typing provides an additional tool that can augment other identification systems in epidemiological studies.

A comparison of the TL and TS antigens, as a further basis for classification, showed that formalin-killed TL antigen preparations generally provided a greater number of distinct groups of antigens than did the TS antigen preparations. The TS antigens of S. epidermidis appeared to be more widespread and were shared more frequently. Hence, TL antigens provided the better serological tool, although the combination of both TL and TS antigens provided a better serological system than either of the two alone.

S. epidermidis appeared to be considerably more heterogeneous than S. aureus and, therefore, it was not unreasonable to assume that serotyping should prove as valuable a tool in epidemiological studies of S. epidermidis as it has for S. aureus. Four distinct TL antigenic groups and two distinct TS antigenic groups were detected for biotype 4 strains, representing five different serotype patterns (A1-2, B1-2, CD-1, C-D1-2 and E1) (table V). It is

TABLE VII

Comparative Categorization of 200 Isolates of Staphylococcus epidermidis (Biotype 4) Using Five Distinctive Characteristics for Determining Epidemiological Efficacy*

<table>
<thead>
<tr>
<th>No. of Isolates</th>
<th>Bio-type</th>
<th>Proteolytic Enzymes</th>
<th>Proteinase Group</th>
<th>Serotype TL</th>
<th>Serotype TS</th>
<th>Aminopeptidase Profile Type</th>
<th>Antibiotic with Five Common Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>4</td>
<td>a</td>
<td>C (17)</td>
<td>B (17)</td>
<td>I</td>
<td>S S R S R R R</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NR (14)</td>
<td>A (12)</td>
<td>II</td>
<td>S S R S R R R</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NT (2)</td>
<td>1, 2</td>
<td>II</td>
<td>S S R S R R R</td>
<td></td>
</tr>
<tr>
<td>169</td>
<td>4</td>
<td>b</td>
<td>C-Dr (14)</td>
<td>1, 2</td>
<td>III</td>
<td>S S R S R R R</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C-D* (19)</td>
<td>1</td>
<td>IV</td>
<td>S S R S R R R</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>E (136)</td>
<td>1</td>
<td>V</td>
<td>S S R S R R R</td>
<td></td>
</tr>
</tbody>
</table>

200 Total

*TL = thermolabile; TS = thermostable; ( ) = number of isolates; NT = non-typeable; a = proteinase positive; and b = proteinase negative.

$\$CC = clindamycin; OX = oxacillin; Te = tetracycline; AM = ampicillin; E = erythromycin; S = more than 80 percent sensitive; and R = less than 50 percent sensitive.
probable that at least as many antigenic
types probably exist with other biotypes
of *S. epidermidis*, and the same serologi­
cal techniques could be applied to distin­
guish between these biotypes.

Five different profiles for *S. epider­
midis* biotype 4, one for each serotype, were established on the basis of the de­
creasing order of hydrolysis of the amino
acid-beta-naphthylamides (table VI). From this order, the relatedness of these
organisms became apparent. Profiles I
and II were most related to one another,
and both strains were biotype 4a. Profiles
III, IV and V were related and were pro­
duced by *S. epidermidis* biotype 4b. These data also showed that all the
staphylococci studied have the ability to
hydrolyze glutamyl-beta-naphthylamide.
The *S. epidermidis* isolates have in com­
mon a high capacity for the hydrolysis of
 glutamyl- and methionyl-beta-naph­
thylamides. However, despite these
similarities, distinct patterns of hydrolysis
were evident for each individual strain.

The most commonly isolated biotype of
* S. epidermidis* from man is usually re­
ported as biotype 1, as it is presumably
indigenous to the normal skin and nose
flora.20 However, in this study, conducted
primarily at GMH, the presumably non­
indigenous (i.e., low incidence) biotype 4
was the most frequently isolated biotype.
Most of the biotype 4 (Serotype E-1) strains were isolated from urine speci­
mens and, primarily, from polymicrobial
bladder infections predominantly to­
gether with *Providencia stuartii, Proteus
rettgeri* and *P. morganii.*13

Members of the tribe, *Proteeae*, were
isolated more often with *S. epidermidis*
than any other organisms from clinical
specimens (especially urines) processed
at GMH.13 This is a special hospital that
deals with chronically diseased, debili­
tated and geriatric patients, all of whom
have one or more underlying diseases, in
addition to any infectious processes
which may develop. Both opportunistic
(i.e., infections that are the result of poor
host defenses rather than of highly viru­
 lent microorganisms) and nosocomial
(hospital acquired) infections are common
at GMH. The aforementioned Proteeeae
are seldom found as primary pathogens in
a high frequency in non-hospital or acute
hospital populations, and their presence
is almost always nosocomial.13

The nosocomial character of these mi­
crobes (both *S. epidermidis* and Proteeeae)
is evident by the fact that they were iso­
lated from specimens obtainable on every
ward in GMH.13 Because of their frequent
appearance following surgery, instrumen­
tation or insertion of an indwelling
 catheter or other prosthetic devices, these
microbes could also be considered iat­
rogenic.12,13 The special character of
GMH must be noted to understand the
 ecological dynamics between the patients
and the environment.

There did not appear to be any monthly
rhythmic pattern to the isolation of *S.
epidermidis*, although, in the period from
July to December, a greater number of *S.
epidermidis* strains were isolated than in
the period from January to June. This
study covered too short a period of time
(one year) to conclude whether or not
biorhythmic processes were involved in
the epidemiology of *S. epidermidis*.

If the ward with the highest frequency
of isolation of *S. epidermidis* biotype 4 is
used as an indication of the index ward,
then the computerized data suggest that
epidemic *S. epidermidis* biotype 4 spread
initially from the B-wards (B21-22, B41-
42). If ward D-21 (Intensive Care Unit,
which receives transient patients from all
wards including B21-22 and B41-42 is
excluded from consideration, then it ap­
pears that the spread of this organism was
as follows: B wards to C then to D wards.
The A wards were not affected. These data
suggest that *S. epidermidis* biotype 4 was
generally confined to the B and C wards.

It is apparent that *S. epidermidis*, espe­
cially biotype 4b (Serotype E-1), is inde­
ependent of, compatible with or non-
 antagonist to the members of this tribe
 Proteeeae to a greater extent than the other
 biotypes of S. epidermidis used in this
 study. Because of this possible competi-
tive advantage, biotype 4 can probably
 compete with members of the tribe, Pro-
teeae, wherein neither is adversely af-
fected by the other or possibly both are
 benefited. However, the actual mecha-
nism responsible for the prevalence of
 biotype 4b is not known.

The origin of antibiotic resistance in
 staphylococci appears to be multifaceto-
 rial. In addition to plasmid-
determined resistance, many resistance
 markers appear to be chromosomal.
 Transduction of resistance, especially
 that determined by plasmids, has been
demonstrated in staphylococci and ap-
pears to be the major factor in the intro-
duction of additional resistance markers
 into epidemic strains. However, as
 biotype 4 isolates were equally or more
 sensitive to antibiotics than biotype 1, the
 greater prevalence of biotype 4 at GMH
 was not due to their greater drug resis-
tance and the exact reason for this greater
 prevalence remains unclear.

In conclusion, five primary parameters
 for distinguishing strains of S. epider-
midis biotype 4 (Serotype E-l) from other
 biotypes and which can be used in
 epidemiological studies, have been iden-
tified (table VII). The biotyping scheme of
 Baird-Parker of limited use in identi-
fying a particular strain, although it was
 helpful in initially categorizing strains of
 S. epidermidis, so that the incidence of
 any one of the biotypes could be detected.
 The ability to differentiate proteolytic
 from non-proteolytic strains provided
 another initial categorizing marker: pro-
teinase-positive strains were grouped
 with known antisera. However, many iso-
lates, although they produce proteinase,
did not react with known groups of anti-
sera and, hence, proteinase grouping was
 helpful only to a limited extent.

Furthermore, if the epidemic strain
does not produce proteinase, as was the
 case for the GMH epidemic strain, then
 proteinase grouping is of no value. Anti-
biograms were least useful for epidemi-
ological purposes because of the similar
 susceptibility patterns of the isolates of S.
 epidermidis. Both serotyping and amino-
peptidase assays provided the best means
 of identifying an individual strain for epi-
demiological purposes, although the
 aminopeptidase assay is subject to varia-
tions in reproducibility, if extreme preci-
sion is not maintained throughout the
 procedure.

It is apparent that one of the most impor-
tant criteria for the production of antisera
 against S. epidermidis is the intelligent
 selection of strains, based on biochemical
 and enzymatic evaluations rather than
 simply choosing strains at random.
 Hence, biotyping of isolates should be
 done first, followed by proteinase studies,
 and then by whatever other differential
 criteria are available and relevant, such as
 antibiograms, presence of other enzymes,
etc.

In addition, aminopeptidase profiles
 should, perhaps, be performed to aid in
 strain selection, inasmuch as each
 serotype, in these studies had a distinct
 aminopeptidase profile. The evaluation of
 aminopeptidase profiles provided
 another means for detecting different
 strains within the same species and could,
 therefore, be used advantageously in
 combination with serotyping as an epi-
demiological tool.

This study, therefore, suggests two use-
ful methods for identifying individual
 strains of S. epidermidis in an epidemi-
ological study: (1) serotyping and (2)
 aminopeptidase assay.

References

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