Susceptibility of *Staphylococcus aureus* and Group A, B, C, and G Streptococci to Free Fatty Acids

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The susceptibility of 242 strains of *Staphylococcus aureus* and 117 strains of streptococci of groups A, B, C, and G to decanoic, dodecanoic, octadeca-9,12-dienoic, and octadeca-9,12,15-trienoic acids was estimated by determination of minimal inhibitory concentrations. *S. aureus* strains appeared to be generally less susceptible to all four fatty acids than streptococcal strains of all groups. Dodecanoic acid was the most inhibitory fatty acid against both staphylococci and streptococci. Both saturated fatty acids used were more active than the unsaturated acids. Among the unsaturated acids, octadeca-9,12,15-trienoic acid appeared to be more inhibitory for *Staphylococcus* and *Streptococcus* strains than octadeca-9,12-dienoic acid. No differences in susceptibility to fatty acids among staphylococcal and streptococcal strains isolated from skin, throat, or other sites were found.

Staphylococcus aureus and *Streptococcus pyogenes* are the most common skin pathogens. In spite of this they are unable to colonize the human skin for long periods of time (7). It was shown in in vivo experiments that some unknown factors most probably related to fatty acids of the skin sebum were responsible for elimination of these bacteria from the skin surface after their artificial application onto the skin (1-3, 10). However, except for one study based on a few isolates of group A and other streptococci and *S. aureus* (6), no other data on the direct in vitro susceptibility of many different strains of these bacteria to free fatty acids are available.

The aim of this study was to evaluate the in vitro susceptibility of *S. pyogenes* and other streptococcal strains and *S. aureus* strains isolated from different sources to several selected free fatty acids identical to those occurring in the lipids of the human skin surface.

MATERIALS AND METHODS

Bacterial strains. Two hundred forty-two strains of *S. aureus* were randomly selected from a large collection of these bacteria isolated from different human infections in Germany. Their taxonomic position was proven on the basis of catalase and coagulase production and also colony and cell morphology (5). They were further characterized by phage typing, using the International Phage Set, and by susceptibility patterns to 28 different antibiotics.

Forty-seven *S. pyogenes* strains were isolated from skin lesions and 40 were isolated from the throats of patients in Germany and Poland. Also, 10 strains of group B streptococci isolated from various non-respiratory sites, 10 strains of group C (2 from skin lesions, 2 reference strains obtained from R. C. Lancefield, and 6 from other sources), and 10 strains of group G isolated from non-respiratory clinical sources were included in the study. Altogether 117 streptococcal strains had been collected at our laboratories in Cologne and Warsaw for this study.

Culture media. Staphylococcal strains were maintained at 4°C on tryptic soy agar slants, and streptococcal strains were kept in a frozen or lyophilized state before the susceptibility testing.

Fatty acids. Decanoic, dodecanoic, octadeca-9,12-dienoic, and octadeca-9,12,15-trienoic acids were commercial products of Nu-Chec-Prep., Elysian, Minn. These four fatty acids were selected for this study since they appeared to be most active against a variety of gram-positive bacteria in our previous study (4).

Susceptibility testing. The susceptibility test was performed by an agar dilution method as described elsewhere (8). In this study Mueller-Hinton agar (Oxoid) for staphylococcal strains and Todd-Hewitt broth (Difco) solidified with 1.2% agar (Oxoid no. 4) for streptococcal strains were used as test media. Both media had been checked before for the absence of interference with free fatty acids and the solvent. Bacterial inocula were obtained by diluting 24-h cultures of staphylococci grown in antibiotic medium no. 3 (Difco) 1:100 with the same broth or from 1:10 dilutions of 24-h cultures of streptococci in Todd-Hewitt broth (Oxoid). They contained about 5×10^4 colony-forming units of staphylococci per ml and about

10⁶ colony-forming units of streptococci per ml. Fatty acids were dissolved in *n*-hexane-ethanol (Baker) (1:1, vol/vol) immediately before preparation of plates. Twofold dilutions in the solvent were added to measured volumes of the test media cooled to 48°C, mixed, and poured into petri dishes. Test plates inoculated with a spot technique (10 µl/drop) were incubated for 24 h at 37°C, and the results were read immediately thereafter. The lowest concentration of fatty acid that resulted in complete inhibition of growth was taken as the minimal inhibitory concentration. Growth controls of the strains tested on Mueller-Hinton agar and Todd-Hewitt agar alone or on these media containing the solvent were included with each assay.

RESULTS

The results of the susceptibility testing of *S. aureus* strains are summarized in Table 1. As can be seen from the table, dodecanoic acid appeared to be the most active compound. Nearly all (99.5%) staphylococcal strains were uniformly susceptible to 0.39 mmol of this acid per liter.

Most of the strains were susceptible to 1.56 mmol of decanoic acid per liter, but some proportion of the strains (26.5%) was susceptible to 0.78 mmol of this acid per liter and even lower concentrations. The susceptibility of *S. aureus* strains to unsaturated acids was not as uniform since the range of susceptibility varied from 12.5 to 1.56 mmol/liter for octadeca-9,12-dienoic acid and from 3.12 to 0.19 mmol/liter for octadeca-9,12,15-trienoic acid. In general, octadeca-

9,12,15-trienoic acid inhibited most of the strains tested at lower concentrations than octadeca-9,12-dienoic acid.

No correlation between the origin of the strains, their phage, and antibiotic susceptibility patterns and minimal inhibitory concentrations for individual strains was found.

Streptococcal strains of serological groups A, B, C, and G from different sources generally showed a uniform pattern of resistance to all fatty acids tested (Table 2). All strains were susceptible to decanoic acid at a concentration of 0.78 mmol/liter and to octadeca-9,12,15-trienoic acid at the same concentration. Three strains were susceptible to 0.39 mmol of dodecanoic acid per liter, whereas the rest were more susceptible. The same phenomenon was found for the susceptibility of the streptococcal strains to octadeca-9,12-dienoic acid, which inhibited 108 strains at a concentration of 1.56 mmol/liter; only nine strains were resistant to this concentration. As in the case of the staphylococcal strains, dodecanoic acid was the most inhibitory, whereas octadeca-9,12-dienoic acid was the least effective.

No correlation was found between the origin (skin or throat), serological group and type, and antibiotic susceptibility patterns of the strains and their susceptibility to the fatty acids used.

It can be deduced from both tables that the streptococcal strains tested were generally more

TABLE 1. Susceptibility of 242 *S. aureus* strains to free fatty acids

Fatty acid	No. of strains (%) at minimal inhibitory concentration (mmol/liter) of:								
	25.0	12.5	6.25	3.12	1.56	0.78	0.39	0.19	0.09
Decanoic (capric), C _{10:0}	0	0	0	0	178 (73.5)	56 (23.1)	8 (3.4)	0	0
Dodecanoic (lauric), C _{12:0}	0	0	0	0	0	0	241 (99.5)	1 (0.5)	0
Octadeca-9,12-dienoic (<i>cis</i> -linoleic)	0	3 (1.2)	126 (52.0)	109 (45.0)	4 (1.6)	0	0	0	0
Octadeca-9,12,15-trienoic (<i>cis</i> -linolenic)	0	0	0	3 (1.2)	3 (1.2)	5 (2.0)	185 (76.4)	46 (19.0)	0

TABLE 2. Susceptibility of 117 *Streptococcus* group A, B, C, and G strains to free fatty acids

Fatty acid	No. of strains (%) at minimal inhibitory concentration (mmol/liter) of:								
	25.0	12.5	6.25	3.12	1.56	0.78	0.39	0.19	0.09
Decanoic	0	0	0	0	0	117 (100)	0	0	0
Dodecanoic	0	0	0	0	0	0	3 (2.6)	113 (96.5)	1 (0.9)
Octadeca-9,12-dienoic	0	0	0	9 (7.7)	108 (92.3)	0	0	0	0
Octadeca-9,12,15-trienoic	0	0	0	0	0	117 (100)	0	0	0

susceptible to all fatty acids studied than were the staphylococcal strains.

DISCUSSION

The selected two saturated and two unsaturated free fatty acids represent the most inhibitory substances of both homology orders for different bacteria (4). In contrast to Kabara et al. (6), who tested only individual representatives of several bacterial species, in our studies octadeca-9,12,15-trienoic acid was more inhibitory for the bacteria tested than octadeca-9,12-dienoic acid. The same order of antibacterial activity has been shown by Kodicek (9) for several gram-positive bacteria and also by us for skin anaerobic coryneforms (8).

The range of susceptibility of staphylococcal strains to four selected fatty acids found in this study was comparable to that estimated in our previous studies (4, 8). Also, all streptococcal strains used in our study were susceptible to fatty acids at concentrations comparable to those previously described by Kabara et al. (6).

Both staphylococci and streptococci were less resistant to dodecanoic and decanoic acids than typical skin residents such as *Propionibacterium acnes* and related bacteria (8) and *Staphylococcus epidermidis* (4). Staphylococci but not streptococci appeared to be nearly as resistant as *P. acnes* to octadeca-9,12,15-trienoic acid. These observations are in keeping with the in vivo experiments of Ricketts and co-workers (10) and Aly et al. (1, 2), who found that both *S. aureus* and *S. pyogenes* strains are eliminated from the human skin in a shorter period of time than bacteria belonging to the normal flora of the human skin.

It is interesting that skin isolates of both *S. aureus* and *S. pyogenes* were not more resistant to the fatty acids tested than those isolated from other sources. This may indicate that so-called

skin strains of the pyogenic cocci are not better adapted in this respect to survival on human skin surfaces than non-skin pathogenic strains of staphylococci and streptococci.

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