Staphylococcus epidermidis BV: Antibiotic Resistance Patterns, Physiological Characteristics, and Bacteriophage Susceptibility

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Staphylococcus epidermidis BV is a group of mannitol-fermenting coagulase-negative staphylococci characterized by multiple antibiotic resistance, very similar biochemical characteristics, and phage susceptibility. Clinical isolates belonging to this group are resistant to most antibiotics tested, including oxacillin, lincomycin, and novobiocin. The only antibiotic to which all tested strains are sensitive is vancomycin. Common biochemical traits of the tested S. epidermidis BV strains include fermentation of trehalose and ribose, phospho-β-glucosidase activity, growth on synthetic medium with amino acids as carbon source, and lack of deoxyribonuclease, phosphatase, lipase, and gelatinase activity. Some of these characteristics appear more frequently in mannitol-positive control strains than in mannitol-negative strains. S. epidermidis BV strains carry lysogenic phages with a host range restricted to this group. These phages allow the differentiation of individual strains.

The present classification of Staphylococcus epidermidis is primarily based on biochemical and cultural characteristics, S. epidermidis strains showing a high degree of physiological heterogeneity (12, 17). Characteristics used for the differentiation of S. epidermidis include phosphatase, lipase, gelatinase, β-glucuronidase, and deoxyribonuclease activity; fermentation of mannitol, maltose, galactose, trehalose, and mannose; growth rate; and temperature limits of growth (2, 4, 5, 17). The high degree of heterogeneity of S. epidermidis strains is also indicated by the numerical analysis of 46 characteristics made by Smith and Farkas-Himsley (17), who found that a group of clinical isolates show a continuum of properties, sharing at different degrees properties common with S. aureus. For diagnostic purposes and with a small number of relatively stable properties, Baird-Parker (2) developed a biotyping system of coagulase-negative staphylococci. By using different criteria, Bentley et al. (3, 4) described a biotyping system aimed mainly at the epidemiological analysis of clinical isolates. Bacteriophage typing of strains belonging to biotype 1 has been attempted by Van Boven et al. (20).

The present study describes a group of multiply resistant clinical isolates designated S. epidermidis BV, which appears to be homogeneous for a variety of biochemical characteristics and in its antibiotic susceptibility pattern. In the course of an investigation on extrachromosomal determinants for penicillinase activity and mannitol fermentation, which will be reported elsewhere, a mannitol-fermenting strain isolated in Bellevue Hospital, New York, N.Y. (obtained through the courtesy of B. Toharski), was found to be resistant to most of the currently used antibiotics including oxacillin, lincomycin, and novobiocin. Subsequently, similar strains were obtained from several hospitals in New York. When studied by a variety of criteria, these strains show a high degree of similarity, and lysogenic phages carried by these strains have a host range restricted to S. epidermidis BV.

A common trait of the S. epidermidis BV strains is the aerobic utilization of mannitol as a carbon source, a characteristic which appears relatively infrequently in strains described by different investigators (2, 4, 12). However, Brown et al. (5) found that over 50% of the multiply resistant S. epidermidis strains isolated from infections of bovine udders are mannitol-positive. There are, however, only few data on the relationship between mannitol fermentation and other biochemical characteristics (2, 4). A comparative study of the S. epidermidis BV strains with mannitol-positive and mannitol-negative clinical
isolates indicated that some physiological characteristics of *S. epidermidis* BV strains and multiple antibiotic resistance appear relatively more frequently in mannitol-positive strains.

**MATERIALS AND METHODS**

**Strains.** The *S. epidermidis* BV strains were isolated in six hospitals in New York, N.Y. (Table 1). Most of the strains were isolated at Bellevue Hospital and New York Veteran's Administration Hospital. The 17 strains tested were isolated from the following sources: 4 strains from blood cultures (three instances repeated positive blood cultures were obtained from the same patient); 5 from wound infections; 2 from surgical incisions; 3 from intravenous catheter tips; 2 from urine; and 1 strain from peritoneal fluid. The 36 mannitol-positive and 71 mannitol-negative control strains of *S. epidermidis* were clinical isolates from several hospitals in New York. The control strains were coagulase-negative and catalase-positive and fermented glucose anaerobically (2). Twenty-eight *S. aureus* control strains were of the phage groups I and III.

**Biochemical tests.** Oxidative and fermentative utilization of glucose and mannitol were determined as described by Baird-Parker (2). The fermentation of other carbohydrates was determined on solid media containing 0.3% casein hydrolysate (Difco), 0.3% yeast extract (Difco), 0.002% bromothymol blue, and 2% agar, brought to pH 7.3 with NaOH. Sterile solutions of carbohydrates were added to the melted medium to a final concentration of 1%. Growth on defined medium with amino acids as carbon source was tested on AOAC synthetic Staphylococcus medium (Difco) supplemented with 10 μg of thiamine per ml, 10 μg of nicotinamide per ml, 0.1 μg of biotin per ml, and 2% agar. Synthetic medium plus glucose is the same medium with 0.2% glucose. Washed cells of overnight broth cultures were suspended in 0.05 M phosphate buffer (pH 7.2) and streaked on both synthetic medium and synthetic medium plus glucose. Growth was recorded after incubation for 24 and 48 hr at 37°C. Deoxyribonuclease activity was determined by incubation for 24 hr on deoxyribonucleate test medium (BBL) and flooding with 1 N HCl. Gelatinase activity was determined by growth on beef heart infusion agar with 0.4% gelatin and flooding with acidified HgCl. Phosphatase activity was determined by the inclusion of 0.01% phenolphthalein phosphate in the phage medium of Novick and Richmond (10) containing 0.12 M tris(hydroxymethyl) aminomethane buffer, pH 7.8. Liberation of free phenolphthalein was detected by exposing the plates to ammonia vapors. Lipase activity was detected by flooding bacterial cultures grown on egg yolk medium with 10% CuSO₄ (21).

**Testing of antibiotic susceptibility.** Antibiotic susceptibility was tested by the inclusion in LB agar (20 g of tryptic (Difco), 10 g of yeast extract, 5 g of NaCl, 20 g of agar, 1,000 ml of water, brought to pH 7.0 with NaOH) of decreasing concentrations of antibiotics and inoculation with the replicating apparatus of Steers et al. (19) with an inoculum of approximatelly 10⁵ cells. Bacteria were considered resistant when the minimal inhibitory concentrations (MIC)

**Table 1. Lysogenic phages of *S. epidermidis* BV strains**

| Indicator strain | Phage group | S-1 | 2700 | 223 | 224 | 228 | 229 | 221 | 225 | 230 | 231 | 232 | 233 | 234 | 220 |
|------------------|-------------|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Blv. S-1         | A           | 0   | −    | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | (+) |
| Blv. 2700        | A           | 0   | −    | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   |
| Blv. 223         | A           | 0   | −    | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   |
| Blv. 224         | A           | 0   | −    | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   |
| Blv. 228         | A           | 0   | −    | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   |
| Blv. 229         | A           | 0   | −    | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   |
| Vet. 221         | A           | 0   | −    | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   |
| Vet. 225         | A           | 0   | −    | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   |
| Vet. 230         | A           | 0   | −    | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   |
| Vet. 231         | A           | 0   | −    | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   |
| Vet. 232         | A           | 0   | −    | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   |
| K.C. 227         | A           | 0   | −    | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   |
| Vet. 236         | B           | +   | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | 0   |
| B.I. 226         | B           | +   | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | 0   |
| B.L. 233         | B           | +   | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | 0   |
| A.E. 234         | B           | +   | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | 0   |
| Blv. 220         | C           | +   | −    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | 0   |

* Symbols: +, lysis after induction by mitomycin; −, lack of lysis; (+), strain S-1 lyed by a mutant of the lysogenic phase 225. Origin of strains tested: Blv., Bellevue Hospital; Vet., New York Veterans Administration Hospital; K.C., Kings County Hospital; B.I., Beth Israel Hospital; B.L., Bronx Lebanon Hospital; A.E., Albert Einstein School of Medicine.
were higher than 3.1 μg/ml for penicillin, tetracycline, novobiocin, oleandomycin, erythromycin, lincomycin, vancomycin, oxacillin, and cephalothin and higher than 6.2 μg/ml for streptomycin, kanamycin, neomycin, and chloramphenicol. Strains with MIC values of 6.2 to 12.5 μg/ml for tetracycline and vancomycin were considered moderately resistant (Fig. 1).

Phage typing. Typing bacteriophages of lysogenic strains were induced by growth in the presence of mitomycin (20). An inoculum of approximately 10⁶ cells was added to 10 ml of LB broth. After 2 hr of vigorous aeration, mitomycin was added to a final concentration of 1 μg/ml and CaCl₂ to a final concentration of 3.10⁻³ M. After additional incubation for 4 to 5 hr, the cultures were centrifuged at 3,000 rev/min and the supernatant fluid was cleared of bacteria by filtration through 0.45-μm membrane filters (Millipore Corp., Bedford, Mass.). Phage activity was tested by spotting 3-mm loops of undiluted filtrate and dilutions of 1:10 and 1:100 of the filtrate on the phage agar of Novick and Richmond (10). The plates were then flooded with indicator strains at a concentration of approximately 10⁸ cells/ml. The results were recorded after an incubation of 14 to 16 hr at 37 C. Confluent or near confluent lysis at dilutions of 1:10 to 1:100 was observed after induction with mitomycin.

Antiserum. Antisera were prepared by the method of Oeding (11). Rabbits were inoculated intravenously with formalinized suspensions of 5 X 10⁶ cells/ml in three weekly series of three injections. The quantity of cells was gradually increased for 0.2 to 1 ml. Agglutinations were made with suspensions of 10⁶ living bacteria and bacteria heated for 1 hr at 100 C.

RESULTS

Antibiotic susceptibility. The antibiotic susceptibility of 17 strains belonging to the group S. epidermidis BV was compared with that of 71 mannitol-negative and 36 mannitol-positive strains of S. epidermidis (Fig. 1). The strains of S. epidermidis BV (Fig. 1 C) appeared very similar in their antibiotic susceptibility. All tested strains were resistant to penicillin, oxacillin, kanamycin-neomycin, erythromycin, lincomycin, oleandomycin, streptomycin, and novobiocin. The resistance to oxacillin and kanamycin was relatively low, with MIC values of 12.5 to 25 μg/ml for oxacillin and 25 to 50 μg/ml for kanamycin. S. epidermidis BV strains showed a low degree of resistance to novobiocin (MIC, 6.2 μg/ml). Mutants with increased resistance to novobiocin were produced by all strains with high frequency. Most of the strains were resistant to tetracycline (MIC, 200 μg/ml) and cephalothin (MIC, 100 to 200 μg/ml), and seven strains were resistant to chloramphenicol. Five strains were resistant to all tested antibiotics with the exception of vancomycin. The sensitivity of the S. epidermidis BV strains to vancomycin was relatively low (MIC, 1.6 to 6.3 μg/ml). These data indicate that S. epidermidis BV is a group of strains with a very similar resistance pattern which appears to be independent of the hospital from which the strains were isolated.

Growth at 45 C for 48 hr in liquid LB medium of the strains S-1, 2700, 223, 225, and 226 resulted in the independent elimination, at different frequencies, of tetracycline resistance, oxacillin plus kanamycin resistance, and penicillinase activity, indicating the probable presence of at least three different resistance plasmids. The genetic determinant for penicillinase activity appeared to be associated with the genetic deter-
minants for mannitol fermentation, phospho-β-glucosidase activity, and phage susceptibility. A detailed description of this polyfunctional plasmid will be given elsewhere.

The strains belonging to the mannitol-negative and mannitol-positive control groups (Fig. 1A and B) generally showed a lower degree of antibiotic resistance than *S. epidermidis* BV, although 60 of the mannitol-negative and 27 of the mannitol-positive strains were resistant to at least one antibiotic and 53% of the strains to two or more antibiotics. When compared with *S. epidermidis* BV, both control groups appeared more heterogeneous in their antibiotic resistance. Only one mannitol-positive strain was resistant to novobiocin, and most of the tetracycline resistant strains belonging to both control groups showed a lower degree of resistance (MIC, 6.2 to 12.5 μg/ml) than *S. epidermidis* BV. Comparison of the mannitol-positive and mannitol-negative control groups showed that the ratio of multiply resistant strains was higher among the mannitol-positive strains. Among the 27 antibiotic-resistant mannitol-negative strains, 18 were resistant to 5 or more antibiotics, whereas among the 60 antibiotic-resistant mannitol-negative strains 28 were resistant to 5 or more antibiotics. The difference in sensitivity between mannitol-positive and mannitol-negative strains appeared most pronounced for oxacillin and cephalothin.

**Biochemical characteristics.** The tested *S. epidermidis* BV strains showed a high degree of similarity in their biochemical and cultural characteristics. All tested strains were catalase-positive, reduced nitrates, and catalyzed glucose aerobically and anaerobically, whereas mannitol was catabolized only under aerobic conditions. Some strains such as 227 and 232 yielded mannitol-negative derivatives with a relatively high frequency. The *S. epidermidis* BV strains produced hemolysis on LB agar plates with rabbit, horse, and human erythrocytes but not with sheep erythrocytes.

*S. epidermidis* BV strains were tested for a wide range of physiological characteristics showing a high degree of homogeneity. These characteristics include the catabolism of maltose, sucrose, pyruvates, lactate, succinate, and β-methyl glucoside and the hydrolysis of p-nitrophenyl-β-glucuronide and o-nitrophenyl-β-galactoside. The characteristics outlined in Fig. 2 were selected for further investigation. All tested *S. epidermidis* BV strains (Fig. 2C) fermented trehalose and ribose but not mannose. Only 3 of 17 strains fermented lactose and galactose. All strains possessed phospho-β-glucosidase A activity (16). The hydrolysis of p-nitrophenyl-β-glucoside was relatively weak [2 to 3 nmol of p-nitrophenol per min formed by 1 mg (dry weight) of cells] and was accompanied by a very limited utilization of β-methyl glucoside. The *S. epidermidis* BV strains lacked detectable phosphatase, lipase, deoxyribonuclease, and gelatinase activities. All tested strains grew on synthetic medium without glucose by using amino acids as carbon and nitrogen sources.

When compared with the two control groups, some of the characteristics of *S. epidermidis* BV appeared more frequently among mannitol-positive strains (Fig. 2B) than among mannitol-
negative strains (Fig. 2A). Among the mannitol-positive strains, 33 of 36 fermented trehalose and 27 strains fermented ribose, whereas of 71 mannitol-negative strains only 11 fermented trehalose and 4 strains fermented ribose. Most mannitol-positive strains showed a weak ribose fermentation, similar to that of \textit{S. epidermidis} BV. Mannose fermentation and growth on synthetic medium in the absence of glucose also occurred more frequently among mannitol-positive strains than among mannitol-negative strains. The ratio of strains fermenting galactose and lactose was similar in the two control groups. Both control groups contained strains possessing phosphatase, lipase, deoxyribonuclease, or gelatinase activity, enzymes which were not detected in the \textit{S. epidermidis} BV strains.

A group of \textit{S. aureus} strains were tested for fermentation of trehalose and ribose, growth on synthetic medium in the absence of glucose, and for phospho-\(\beta\)-glucosidase activity, characteristics of \textit{S. epidermidis} BV found more frequently among the mannitol-positive than the mannitol-negative control strains. The 28 tested \textit{S. aureus} strains possessed these characteristics, with the exception of one trehalose-negative strain and two strains which showed a low growth rate on synthetic medium lacking glucose. In contrast to \textit{S. epidermidis} BV, the phospho-\(\beta\)-glucosidase activity of \textit{S. aureus} was accompanied by an active fermentation of \(\beta\)-methyl glucoside (16).

\textbf{Susceptibility to bacteriophages.} Induction of lysogenic phages was made by growth in the presence of mitomycin as outlined above. After filtration, the lytic activity of each filtrate was tested with all \textit{S. epidermidis} BV strains (Table 1). With the exception of strains 234 and 236, each \textit{S. epidermidis} BV strain carried a lysogenic phage which lysed one or several strains of \textit{S. epidermidis} BV. Most of the strains were lysed by at least one of the lysogenic phages. Lysogenic phages were purified by the selection of isolated plaques on indicator strains and multiplied to titers of \(5 \times 10^8\) to \(7 \times 10^9\) phage particles per ml. Strains found resistant to the induced lysogenic phages were also resistant to concentrated phage preparations. By taking into account the host range of the lysogenic phages and the phage susceptibility patterns of the indicator strains, the 17 \textit{S. epidermidis} BV strains could be tentatively divided into three groups, designated as A, B, and C.

The strains belonging to group A carried phages which lysed one or several strains of group B, each of the strains included in group B being lysed by at least eight phages produced by strains belonging to group A. The differentiation of some of the strains of group A could be made by the host range of their lysogenic phages and their phage susceptibility. Strain S-1, for example, was lysed by phages 220 and 226 and a mutant of phage 225, and the lysogenic phage carried by this strain lysed the strains 225, 230, 236, 226, 223, 234, and 220. Strain 227 carried a phage with a similar host range as strain S-1 but was not susceptible to the phages which lysed strain S-1. Because of the identical host range of the lysogenic phages 228, 229, 230, and 231 and also of the phages 223 and 232 produced by strains of group A, only a partial differentiation of the strains belonging to this group could be achieved. Strains of group B are susceptible to most group A phages, strain 226 being an indicator for all tested phages of group A. Only strain 226 possesses a lysogenic phage which lyses strains of group A (Table 1). Strain 220 carries a lysogenic phage which lysed several strains of group A but not of group B. This property determined its inclusion in a separate group "C." It appears therefore that by taking into account both the phage susceptibility of the indicator strains and the host range of the lysogenic phages, the majority of the \textit{S. epidermidis} BV strains can be divided into two major groups with relatively limited possibilities of further differentiation inside of each group.

The \textit{S. epidermidis} BV phages appear to be specific for this group. The 71 mannitol-negative and 36 mannitol-positive control strains were tested for the presence of lysogenic phages which lyse \textit{S. epidermidis} BV; the same strains were also used as indicator strains for \textit{S. epidermidis} BV phages. In both instances, the results were consistently negative. R. E. O. Williams tested, with negative results, several \textit{S. epidermidis} BV strains belonging to phage groups A and B for susceptibility to \textit{S. epidermidis} phages from his collection. The \textit{S. epidermidis} BV strains were not susceptible to diluted and concentrated \textit{S. aureus} typing phages, and the tested indicator strains of \textit{S. aureus} were not lysed by \textit{S. epidermidis} BV phages.

\textbf{Antigenic structure.} Antisera were prepared by the method of Oeding (11) with formalinized suspensions of strains S-1, 220, 221, 223, 224, 226, 227, and 228. The homologous agglutination titers were of 1,600 to 3,200. All strains belonging to the \textit{S. epidermidis} BV group were agglutinated by the antiserum prepared with the above strains, although the agglutination titer varied from 25 to 100\% of the homologous titer. In all tested strains, the titer increased after boiling of the suspension, indicating the probable existence of a thermolabile envelope. Slide agglutination with antisera diluted 1:25 and 1:50 gave positive
results with six of the mannitol-positive control strains and five of the mannitol-negative control strains of *S. epidermidis*. Of 28 *S. aureus* strains, 4 were agglutinated by all tested *S. epidermidis* BV antisera. It appears therefore that *S. epidermidis* BV antisera are of very limited value in the differentiation of strains belonging to this group.

**DISCUSSION**

*S. epidermidis* BV strains are a homogeneous group of strains of potential clinical significance with very similar physiological characteristics and antibiotic susceptibility pattern. The apparent limitation to these strains of the host range of the *S. epidermidis* BV lysogenic phages is a further indication for the distinct taxonomic position of this group of strains. Because strains of *S. epidermidis* BV are so similar biochemically, it appears that most strains can best be differentiated by their phage susceptibility and the host range of the lysogenic phages. Strains S-1, 2700, and 220 appear identical in their antibiotic susceptibility and biochemical characteristics but can be differentiated by their phage susceptibility pattern (Table 1). The location of the patients from which these strains were isolated in different wards of the same hospital is consistent with their different phage susceptibility. With some strains, identical in phage susceptibility and the host range of their lysogenic phages, as for instance strains 228 and 230, differences in their susceptibility to chloramphenicol and in the fermentation of lactose allow their differentiation from each other.

The incidence of multiple antibiotic resistance among coagulase-negative staphylococci is relatively high (1, 6, 23), the ratio of methicillin- and oxacillin-resistant strains being much higher among *S. epidermidis* strains than among *S. aureus*. Because of the isolation of *S. epidermidis* strains from significant clinical infections (9, 13, 14, 22), their multiple resistance can represent a serious therapeutic problem. Most of the *S. epidermidis* control strains tested in this study are resistant to at least one antibiotic, and 53% are resistant to two or more antibiotics, their degree of resistance being similar to that described by other investigators (13, 22). When compared with the control strains, the *S. epidermidis* BV strains are resistant to a larger number of individual antibiotics than any of the control strains and are more homogeneous in their resistance pattern, the only significant difference in the resistance spectrum of the *S. epidermidis* BV strains being in their susceptibility to chloramphenicol and cephalothin. The similarity of the *S. epidermidis* BV strains involves not only their resistance spectrum but also MIC values for individual antibiotics and the frequency of mutation toward higher novobiocin resistance. No significant differences were found in the resistance patterns of strains isolated from different hospitals.

It seems improbable that the multiple resistance of the *S. epidermidis* BV strains is solely the result of the successive selection of mutants by antibiotic therapy. It appears more likely that, partly as the result of the extrachromosomal nature of some of the resistance determinants, the therapeutic use of one of the antibiotics, for which the resistance determinant is already present, will result in the selection of multiply resistant strains. Such a secondary colonization with multiply resistant strains of *S. epidermidis* has been described in an investigation by Bentley et al. (3); the treatment of children living in a closed community with chloramphenicol resulted in the accumulation of multiply resistant strains and the predominance of new biotypes.

Some biochemical characteristics of *S. epidermidis* BV, such as trehalose and ribose fermentation, phospho-β-glucosidase activity, and growth on synthetic medium with amino acids as carbon source, were found with a higher frequency among mannitol-positive strains than among mannitol-negative strains. These biochemical activities were also found in *S. aureus* strains. The *S. epidermidis* BV strains differ, however, from *S. aureus* by their lack of deoxyribonuclease and phosphatase activity and their inability to utilize mannitol anaerobically. Multiple antibiotic resistance is also more frequent among the mannitol-positive control strains, and among the multiply resistant strains we are currently receiving in our laboratory there is an increasing ratio of mannitol-positive strains. At present, however, because of the physiological heterogeneity of the mannitol-fermenting strains, the taxonomic value of the utilization of mannitol in the differentiation of clinical isolates is difficult to assess.

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