Effect of Borate on the Growth of Coagulase-Positive and Coagulase-Negative Staphylococci

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Received for publication 30 September 1969

Of 235 strains of coagulase-positive Staphylococcus aureus studied, 221 or 94% were inhibited by 1.5 × 10⁻⁸ M sodium borate, whereas only 6 of 57 (10.5%) coagulase-negative strains were inhibited by the same borate concentration. Four of the six coagulase-negatives were food poisoning strains and one was a hospital pathogen. Borate sensitivity was found to correlate well with lysozyme and α-toxin production by coagulase-positive strains.

Since the production of coagulase by Staphylococcus aureus was first associated with virulent and potentially virulent strains, numerous other biochemical properties of pathogenic staphylococci have been described, including: production of α-toxin, lysozyme, deoxyribonuclease, egg yolk precipitation, pigment, and utilization of mannitol. In addition, the sensitivity or resistance of coagulase-positive staphylococci to mercuric chloride and polymyxin has been described. The present report describes the sensitivity of coagulase-positive staphylococci to borate and compares borate sensitivity to several other characteristics of these and coagulase-negative strains.

MATERIALS AND METHODS

Of the 235 strains of coagulase-positive staphylococci employed in this study, 194 were hospital strains (obtained from James E. Greer of Henry Ford Hospital, Detroit, Mich.), 22 were obtained from the arms and nares of healthy adults, 11 were well-known food-poisoning strains, and 8 were of food origin. The 57 coagulase-negative strains consisted of five food-poisoning strains (obtained from M. S. Bergdoll, Food Research Institute, University of Wisconsin) and 5 hospital pathogens; the remainder were obtained from the arms and nares of healthy adults.

Sensitivity to borate was determined by plating on 4% NZ-Amine Type NAK medium (Sheffield Chemical, Norwich, N.Y.) to which was added 0.0005% thiamine, 0.001% niacin, and 1.5% agar. This medium has been shown to support the production of enterotoxins (2) and was found to be an excellent growth medium for staphylococci. All other media employed were made up according to manufacturers' directions. Stock solutions of 0.10 M Na₂B₄O₇, 0.20 M Na₈B₁₀O₁₆·10H₂O, and 0.12 M H₂BO₃ were prepared separately in distilled water. The decahydrate salt was solubilized by adding 1 g of salt to 1.2 ml of glycerine followed by addition of distilled water. The anhydrous salt was solubilized overnight at room temperature. All forms of borate were added to the media prior to sterilization and adjusted to pH 7.0 to 7.2 with HCl. Borate-containing media were streaked with cultures grown in NZ-Amine broth and incubated at 37 C for 24 hr. The other biochemical reactions were determined as previously described (1).

RESULTS

The effect of 1.5 × 10⁻⁸ M borate on the growth of the 235 coagulase-positive and 57 coagulase-negative strains of staphylococci is presented in Table 1. Of the coagulase-positive strains, 94% or 221 were partially or completely inhibited, whereas only 10.5% or 6 of the coagulase-negative strains were inhibited. Levels of borate on the order of 1.0 × 10⁻⁸ M or less allowed the growth of most coagulase-positive strains, whereas levels in excess of 2.0 × 10⁻⁸ inhibited both coagulase-positive and coagulase-negative strains. Boric acid was also inhibitory, but levels of 2.4 × 10⁻⁸ M and above were required to inhibit coagulase-positive strains.

The relationship of borate sensitivity to the production of α-toxin, lysozyme, egg yolk precipitation, and pigmentation among these strains is presented in Table 2. Of the coagulase-positive strains, 94% were inhibited by borate, 95.3% produced lysozyme, 95.7% produced α-toxin, 92.8% were pigmented, and 87.7% precipitated egg yolk. Of the coagulase-negative strains, 10.5% were resistant to 1.5 × 10⁻⁸ M borate, and 8.8, 12.3, and 45.6%, respectively, were lysozyme-positive, egg yolk-positive, and produced α-toxin; 21% of these strains were pigmented.

In an attempt to characterize the 14 coagulase-positive strains that grew in the presence of 1.5 × 10⁻⁸ M borate, these organisms were found to be...
TABLE 1. Relative percentage of coagulase-positive and coagulase-negative strains of staphylococci inhibited by 1.5 × 10^{-8} M borate

<table>
<thead>
<tr>
<th>Coagulase type</th>
<th>No. of strains</th>
<th>No. inhibited</th>
<th>Per cent inhibited</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-positive</td>
<td>235</td>
<td>221^a</td>
<td>94.04</td>
</tr>
<tr>
<td>Coagulase-negative</td>
<td>57</td>
<td>6^a</td>
<td>10.52</td>
</tr>
</tbody>
</table>

^a Six were completely inhibited; eight were partially inhibited.

^b Four food-poisoning strains, one hospital pathogen, and one phage-propagating strain 73.

TABLE 2. Comparison of borate inhibition to several other characteristics of coagulase-positive and coagulase-negative staphylococci

<table>
<thead>
<tr>
<th>Organisms</th>
<th>No. of strains</th>
<th>Borate Inhibited</th>
<th>Lysosome-positive</th>
<th>α-Toxin-positive</th>
<th>Pigmented</th>
<th>EY-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-positive</td>
<td>235</td>
<td>94.0</td>
<td>95.3</td>
<td>95.7</td>
<td>92.8</td>
<td>87.7</td>
</tr>
<tr>
<td>Coagulase-negative</td>
<td>57</td>
<td>10.5</td>
<td>8.8</td>
<td>45.6</td>
<td>21.1</td>
<td>12.3</td>
</tr>
</tbody>
</table>

^a EY = precipitated egg yolk.

TABLE 3. Some characteristics of the borate-resistant coagulase-positive strains and borate-sensitive coagulase-negative strains

<table>
<thead>
<tr>
<th>Strains</th>
<th>No. α-Toxin</th>
<th>Lysosome</th>
<th>Egg yolk</th>
<th>Pigmented</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-positive</td>
<td>13</td>
<td>11</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>Coagulase-negative</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

essentially the same as the sensitive strains (Table 3). All but one of these were from hospital patients. With the possible exceptions of α-toxin and pigmentation, the coagulase-negative strains that did not grow in the presence of 1.5 × 10^{-8} M borate were essentially the same as the other coagulase-negative strains (Table 3). As is noted in Table 1, four of the coagulase-negative strains that were sensitive to borate were food-poisoning strains, one was a hospital pathogen, and one was phage-propagating strain 73.

With respect to the sensitivity of other organisms to the effective level of borate, the following nine species were inhibited: Bacillus cereus, B. subtilis, Micrococcus rubens, M. l lysodeikticus, M. radiodurans, Mycobacterium smegmati, Neisseria catarrhalis, Pseudomonas aeruginosa, and Sarcina lutea. The following 10 organisms were found to be resistant to this level of borate: Aerobacter aerogenes, Alcaligenes metalcaligenes, Lactobacillus casei, Leuconostoc species, Proteus mirabilis, Pseudomonas species, Salmonella typhi, S. typhimurium, S. newington, and Streptococcus faecalis. In all cases of inhibition, the effect was found to be bacteriostatic rather than bactericidal, and the inhibition was in effect well beyond the 24-hr incubation period.

The effective level of borate also inhibited coagulase-positive staphylococci when added to nutrient and Brain Heart Infusion agars. Lower levels were required to inhibit in mannitol-saltagar than in the other media.

By employing NZ-Amine agar, borate was found to inhibit coagulase-positive strains over the pH range 5.4 to 8.5, with inhibition being greater at the higher pH levels than at around 6.0.

DISCUSSION

The sensitivity of coagulase-positive staphylococci to levels of borate on the order of 1.5 × 10^{-8} M correlates well with α-toxin and lysozyme production and better than the egg yolk reaction. Five of the 10 coagulase-negative pathogenic strains employed in this study were sensitive to borate, along with most of the coagulase-positive strains, suggesting that borate sensitivity might aid in the better detection of these strains. Of the 16 food-poisoning strains employed, of which five were coagulase-negative, all but one were borate-sensitive.

Although boric acid has been employed for a long time as a mild antiseptic, results of this study suggest that the salts are more effective than the acid, especially against coagulase-positive staphylococci. Studies on the mechanism of borate inhibition are in progress.

ACKNOWLEDGMENTS

This investigation was supported by research grant FD-00168 from the Consumer Protection and Environmental Health Service, Food & Drug Administration, U.S. Public Health Service.

I thank Eddie Betel, Lois E. Mustonen, and Karen I. Virley for technical assistance.

LITERATURE CITED