GROWTH OF STAPHYLOCOCCI ON MERCURIC CHLORIDE AGAR

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ABSTRACT

SMITH, P. B. (Communicable Disease Center, Atlanta, Ga.). Growth of staphylococci on mercuric chloride agar. J. Bacteriol. 84:1016–1019. 1962.—An attempt has been made to corroborate a report that “epidemic” strains of staphylococci are more resistant to mercuric salts than are “nonepidemic” strains. A comparison of mercury resistance with coagulase production and phage type or pattern of 493 staphylococcal strains revealed that nearly half of the coagulase-negative strains tested were mercury-resistant, whereas many “epidemic” strains of certain phage types were sensitive to mercuric salts. Results obtained are compared with those from a previous investigation. The mercury resistance of some strains was found to vary significantly depending on the basal medium used; this could not be correlated with mercapto-group content of the medium. The data indicate that a medium containing mercuric chloride would have little practical value at the present time for the average laboratory engaged in isolating staphylococci.

Persistent attempts by numerous investigators to establish a distinct correlation between an in vitro test and real or potential pathogenicity of staphylococci have usually been disappointing, but have not diminished particularly in frequency. Mannitol fermentation, orange-yellow pigmentation, production of α-hemolysin, phosphatase production, the ability to render egg-yolk medium opaque, and coagulase production are only some of the criteria which have been proposed (Elek, 1959). Of these, coagulase production has been accepted most widely as the best indicator of potential pathogenicity.

Among the pathogenic staphylococci, there are certain phage types which seem to be transmitted more easily from one host to another, and thus have been called “epidemic” strains, i.e., those with phage patterns 80/81, 52/52A/80/81, 6/7/47/53/54/75, etc. Moore (1960) suggested that such strains can be selectively isolated because of their resistance to mercuric chloride, as determined by inoculating them onto agar surfaces containing this compound. In laboratory tests with approximately 500 strains, he found that such a medium would select those staphylococci “that tend to give rise to outbreaks of staphylococcal infection in hospitals,” since they were more resistant to mercuric salts than were “nonepidemic” strains. We repeated Moore’s procedures, with slight modifications, and present here the results obtained as well as a comparison with his data.

MATERIALS AND METHODS

The basal media used for mercury-resistance tests were Trypticase Soy Agar and Trypticase Soy Broth (BBL). A stock solution of mercuric chloride was prepared in distilled water (1:275) and stored without sterilization in a refrigerator. Melted agar was cooled to 45°C, and mercuric chloride solution added to give a final concentration of 1:27.500 (1:100 of stock solution). Plates were poured, dried overnight at 37°C, refrigerated, and used within 3 days. Resistance to mercuric chloride was tested by placing one loopful of a broth culture (18 to 24 hr) on the agar surface, incubating the plate overnight at 37°C, and examining for growth.

The 493 strains used were selected from those submitted to this laboratory for phage typing, and were from all varieties of human sources, as well as air, fomites, and environments. Each strain was transferred to broth, incubated for 18 to 24 hr at 37°C, and tested for coagulase by the standard tube test, using either dehydrated rabbit plasma (Difco) or sterile human plasma diluted 1:3. Strains which were coagulase-positive after 3 hr of incubation at 37°C were phage typed by standard procedures (Blair and Williams, 1961) using phages 29, 52, 52A, 79, 80, 3A, 3B, 3C, 55, 71, 6, 7, 42E, 47, 53, 54, 75, 77, 42D, 187, 83A, and 81, at the Routine Test Dilution.

The influence of the basal medium on mercury
resistance was tested by preparing each of the following media with and without mercuric chloride: Trypticase Soy Agar (BBL); Neopeptone agar, Tryptone Glucose Extract Agar, Penassay Seed Agar, Proteose No. 3 Agar, and Heart Infusion Agar (Difco); and a peptone agar [2% peptone (Difco), 0.5% NaCl, and 1% agar (Difco)]. The last-named medium was equivalent to Difco’s peptone agar, except that Difco rather than Oxo products were employed.

Mercapto groups were determined by Anson’s ferricyanide method as modified by Katyal and Gorin (1959).

RESULTS

Table 1 shows the results obtained as they relate to coagulase production and phage type. It is apparent that, of the various groups of strains listed, only in group I was there a majority (71%) which would grow on the mercuric chloride medium. Approximately 90% of all other typable strains were mercury-sensitive. It was especially notable that about half (49%) of the coagulase-negative strains examined were also mercury-resistant.

A comparison of these results with those obtained by Moore (1960) is given in Table 2. Fairly good agreement between the two sets of data was achieved with strains of phage groups I, II, and “IV, mixed and miscellaneous,” but group III and nontypable strains gave significantly different results. A comparison of results with coagulase-negative strains is precluded, since Moore reported no specific data on them.

Since most “epidemic” staphylococcal strains belong to groups I and III, these results were examined more closely. Tables 1 and 2 show that only 7% of the group III strains in this study

<table>
<thead>
<tr>
<th>TABLE 1. Mercury resistance of staphylococci</th>
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<tr>
<td>Phage group</td>
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<tr>
<td>I</td>
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<tr>
<td>II</td>
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<tr>
<td>III</td>
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<tr>
<td>IV, mixed and misc.</td>
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<td>Nontypable</td>
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<td>(Coagulase-negative)</td>
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<td>Totals</td>
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<th>TABLE 2. Comparison of data on mercury resistance of staphylococci</th>
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<tr>
<td>Phage group</td>
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</tr>
<tr>
<td>I</td>
</tr>
<tr>
<td>II</td>
</tr>
<tr>
<td>III</td>
</tr>
<tr>
<td>IV, mixed and misc.</td>
</tr>
<tr>
<td>Nontypable</td>
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<tr>
<td>(Coagulase-negative)</td>
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</tbody>
</table>

* First numeral refers to data from the Communicable Disease Center, the second to Moore’s (1960) data.
† Indicates data not available.

<table>
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<th>TABLE 3. Comparison of data on mercury resistance of phage group I staphylococcus strains</th>
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<tbody>
<tr>
<td>Phage pattern</td>
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<td>---------------</td>
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<tr>
<td>80 or 80/81</td>
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<tr>
<td>52/52A/80 or 52/52A/80/81</td>
</tr>
<tr>
<td>Others with phage 80</td>
</tr>
<tr>
<td>Others without phage 80</td>
</tr>
</tbody>
</table>

* First numeral refers to data from the Communicable Disease Center, the second to Moore’s (1960) data.

were mercury-resistant, in contrast to Moore’s 40%. A more detailed comparison of these results would, therefore, be pointless. On the other hand, the apparently good correlation between the present data and Moore’s, with group I strains (71% and 76% resistant, respectively), allows further inspection. Table 3 gives somewhat more detailed results with group I strains, and a comparison with Moore’s data. In general, the correlation between “epidemic” strains of group I and mercury resistance was less than Moore obtained, but was still good. The major difference in results involved strains which were lysed by phage 80 but were not phage types 80, 80/81, 52/52A/80, or 52/52A/80/81. The significance of this difference is questionable, however, because of the few strains in this category.

Because mercuric chloride was used close to
its limiting concentration, and our medium was different from Moore’s, the effect of various media on mercury resistance was tested. Each of the media listed in Materials and Methods was prepared with added mercuric chloride, and agar plates were then inoculated with each of 42 strains of *Staphylococcus aureus*. The same media without additional mercuric chloride served as controls. Most of the strains used were coagulase-positive, and either phage group III or nontypable, since significant differences in results in the two studies were most marked in these two groups.

All strains grew well on control plates, but inhibition of growth ranged from 95.2%, on Heart Infusion Agar and Tryptone Glucose Extract Agar, to 78.6%, on Trypticase Soy Agar (Table 4).

Determination of mercapto groups in each of these media, and comparison of the percentages of mercapto groups with mercury resistance did not permit any general correlation (Table 4). For example, Tryptone Glucose Extract Agar and Proteose No. 3 Agar contained similar amounts of mercapto groups, but two strains grew on the former medium and nine on the latter.

The receipt of samples of peptone (Oxo) and agar (Davis) from B. Moore permitted comparison of mercapto-group content with the peptone agar prepared from Difco products. Interestingly, Moore’s peptone agar, as prepared from these ingredients, contained approximately twice the mercapto groups as did the Difco peptone agar, and therefore should permit more strains to grow since the concentration of active mercury ions would be reduced. Re-examination of Table 2 shows this to apply to phage group III and nontypable strains, but not to other groups of strains.

To enhance pigment production, Moore (1960) also used the basal peptone medium supplemented with milk. We did not add milk to Trypticase Soy Agar, but did notice that the pigment of mercury-resistant cultures varied from white to deep orange-yellow. Pigment production could not be associated entirely with either coagulase production or phage type; e.g., all mercury-resistant group I colonies were orange-yellow, but so were some strains in all other classifications, including nontypable and coagulase-negative.

**DISCUSSION**

The technique of isolating “epidemic” strains of staphylococci by utilizing their greater resistance to mercuric salts was proposed as a potentially valuable screening procedure in laboratories lacking ready access to phage typing (Moore, 1960). For a technique to be so used, it should (i) inhibit the numerous coagulase-negative saprophytic staphylococci which are normally present on the skin and in the environment, (ii) inhibit most coagulase-positive staphylococci which are seldom involved in epidemics, and (iii) select primarily those staphylococcal strains which are highly communicable and pathogenic. The data presented herein support only the second point.

In his original article, Moore (1960) stated that only occasionally are coagulase-negative skin staphylococci resistant to mercuric chloride. The data in Table 1 indicate that nearly half of the coagulase-negative strains, which were from various sources, were mercury-resistant, a far greater percentage than is implied in Moore’s statement. An exact comparison is not possible, however, because no specific data are given in the earlier publication. If our results are representative, then it becomes necessary to perform coagulase tests on all colonies picked from the agar surface (a procedure now in use), with the probability that many of them will be coagulase-negative.

It should be pointed out that some liberties were taken in grouping phage patterns into groups I, II, etc. These were limited primarily to group I strains which were lysed by phage S1. This phage

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**TABLE 4. Influence of medium on mercury resistance of 42 Staphylococcus aureus strains**

<table>
<thead>
<tr>
<th>Medium</th>
<th>No. of mercury-resistant strains</th>
<th>Mercapto groups*</th>
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<tbody>
<tr>
<td>Heart Infusion Agar</td>
<td>2</td>
<td>0.050</td>
</tr>
<tr>
<td>Tryptone Glucose Extract</td>
<td>2</td>
<td>0.050</td>
</tr>
<tr>
<td>Agar</td>
<td>2</td>
<td>0.050</td>
</tr>
<tr>
<td>Neopeptone agar</td>
<td>4</td>
<td>0.065</td>
</tr>
<tr>
<td>Peptone agar (Difco)</td>
<td>6</td>
<td>0.016</td>
</tr>
<tr>
<td>Trypticase Soy Agar</td>
<td>9</td>
<td>0.027</td>
</tr>
<tr>
<td>Penassay Seed Agar</td>
<td>9</td>
<td>0.047</td>
</tr>
<tr>
<td>Proteose No. 3 Agar</td>
<td>9</td>
<td>0.046</td>
</tr>
<tr>
<td>Peptone agar (Oxo)</td>
<td>Not done</td>
<td>0.036</td>
</tr>
</tbody>
</table>

* Average of two or three determinations.
is classified as "miscellaneous" (Blair and Williams, 1961), but where its reaction on a
culture clearly indicated that the strain was of
the "80/81 complex," then the pattern was placed
in group I. In other instances where phage 81
was part of a pattern which included group III
phages (e.g., 6/42E/54/83A/81), the pattern was
classified as "mixed." Phage 81 was not employed
in the investigation by Moore (1960).

Although the majority (71%) of the phage
group I strains, and 84 to 89% of the "80/81
complex" strains, were mercury-resistant (Tables
1 and 3), only a few of the "epidemic" strains of
phage group III or "mixed" phage types would
grow on such a medium. The latter strains are
less notorious than those of the "80/81 complex,"
but are frequent and persistent causes of hospital
outbreaks. In our laboratory, only 12 of 167
strains in these two groups were mercury-resis-
tant; i.e., 93% of them failed to grow. These
results differ from those of Moore, who found
40% of the group III strains to be mercury-
resistant.

Some of the discrepancies in the two studies
seem to be due to the use of different media.
Variation in mercapto-group content of the media
might explain some of the differences, since this
would affect the number of free mercury ions.
However, the poor correlation shown between
mercapto groups and mercury resistance indicates
that other mechanisms must be involved also.
At the present time, it seems that more complete
evaluations of this technique are required before
it can be of practical value in different labora-
tories.

Although there are some good reasons, as are
presented by Moore (1960), for desiring a cultural
procedure which would select "epidemic" strains,
there are also good arguments against it. Staphy-
lococcus outbreaks caused by strains of phage
types 187 or 3A/3B, for example, might be
entirely missed if a mercury-containing medium
were used for primary isolation, yet both have
been involved in hospital infections and food
poisonings. The potential pathogenicity of any
coagulase-positive staphylococcus strain must be
recognized whether or not it is phage-typable and
regardless of specific phage type or pattern.

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