A screening technique for the detection of nasal carriers of antibiotic-resistant *Staphylococcus aureus*

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Most staphylococci giving rise to epidemic infection in hospital today are resistant to a number of antibiotics (Williams, Blowers, Garrod, and Shooter, 1966) and these patterns of resistance can serve as a useful discriminant in the selection of staphylococci for routine phage typing. In this laboratory all coagulase-positive staphylococci (*Staph. aureus*) resistant to penicillin, tetracycline, and streptomycin are routinely phage typed. The nasal carriage of *Staph. aureus* has been shown to bear an important relationship to the occurrence of wound infection, in some circumstances by autoinfection (Weinstein, 1959; Williams, Jevons, Shooter, Hunter, Girling, Griffiths, and Taylor, 1959; Williams, Noble, Jevons, Lidwell, Shooter, White, Thom, and Taylor, 1962; Rountree and Beard, 1968) and in other circumstances by dispersal through the ward environment to result in cross infection of other patients (Stokes, Hall, Richards, and Riley, 1963; Williams et al, 1962). The detection of nasal carriers of *Staph. aureus* resistant to multiple antibiotics either as part of a programme of routine surveillance or during an actual outbreak of infection due to these organisms results in a considerable increase in the work load on the laboratory and this suggested the need for a simple screening technique. Since none of the many existing selective media for the isolation of *Staph. aureus* (Cruikshank, 1965) was sufficiently selective for multiply drug-resistant strains, the medium reported here was developed.

**Medium and Methods**

Nutrient agar plates were prepared containing 5 μg/ml of streptomycin and 5 μg/ml tetracycline plus 0.01% phenol red and 1% mannitol to indicate the growth of *Staph. aureus* (Chapman, Berens, Nilson, and Curcio, 1938). The medium was stable for one week when stored at 4°C. Nasal swabs were inoculated first onto 5% horse blood agar, two per plate, by a member of the laboratory staff and then on to mannitol-antibiotic agar, eight swabs per plate, by one of us. A strain of *Staph. aureus*, resistant to penicillin, tetracycline, and streptomycin, was inoculated onto each mannitol-antibiotic plate as a control. Both series of plates were incubated at 37°C for 24 hours when they were examined for the presence of colonies resembling *Staph. aureus*; the mannitol-antibiotic plates were incubated for a further 24 hours and reexamined. The blood agar plates were read by the laboratory staff and the mannitol-antibiotic plates by the sister in control of infection who then referred any plates on which colonies thought to be *Staph. aureus* were growing to the laboratory for further investigation. Colonies resembling *Staph. aureus* were subcultured onto nutrient agar containing 6% lysed horse

**Table The concentration of various cysts, ova, and larvae by two methods**

<table>
<thead>
<tr>
<th></th>
<th>Direct Examination</th>
<th>Concentration (Old Method)</th>
<th>Concentration (New Method)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. histolytica</em></td>
<td>13</td>
<td>418</td>
<td>540</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>3</td>
<td>43</td>
<td>60</td>
</tr>
<tr>
<td><em>G. lamblia</em></td>
<td>35</td>
<td>177</td>
<td>929</td>
</tr>
<tr>
<td><em>Ascaris</em></td>
<td>65</td>
<td>54</td>
<td>1,187</td>
</tr>
<tr>
<td>Trichuris</td>
<td>8</td>
<td>225</td>
<td>262</td>
</tr>
<tr>
<td>Hookworm</td>
<td>27</td>
<td>817</td>
<td>1,259</td>
</tr>
<tr>
<td>Strongyloides</td>
<td>15</td>
<td>138</td>
<td>236</td>
</tr>
<tr>
<td>Schistosoma</td>
<td>5</td>
<td>11</td>
<td>76</td>
</tr>
<tr>
<td>Toxoa</td>
<td></td>
<td>32</td>
<td>433</td>
</tr>
</tbody>
</table>

*The figures for the cysts represent the mean of three counts of 25 fields each. The figures for ova are the counts per coverslip.*

Comment

The superiority of formol-ether concentration over the well known zinc sulphate flotation method was demonstrated by Ritchie, Pan, and Hunter (1952). In the latter method the heavier ova (*Schistosoma, Fasciola*) fail to rise to the surface; with formol-ether, a sedimentation technique, all parasites go to the bottom.

The increased yield of positive findings with all types of faecal parasites, the relatively clean deposit, and the enhanced visibility of the structural detail of cysts obtained by this method justify its use as a routine diagnostic procedure.

Unformed stools need to be examined by direct microscopy also for trophozoites, but formed stools without mucus or blood need only be examined by concentration (Ridley and Hawgood, 1956).

We have made many attempts further to improve the efficiency of the method by the addition of wetting or mucolytic agents but none has been a consistent success.

References


blood and their sensitivity to penicillin, streptomycin, and tetracycline was determined by the disc diffusion method, zone diameters being measured after incubation at 37°C for 18 hours. A slide coagulase test was carried out on all strains using growth from the sensitivity plates, and any strains which were slide coagulase negative were then examined by the tube coagulase method (Cruikshank, 1965).

Results

*Staph. aureus* resistant to penicillin, tetracycline, and streptomycin grew on mannitol-antibiotic agar within 24 hours as bright yellow colonies surrounded by a yellow halo. Coagulase-negative staphylococci growing on this medium were usually pale pink and produced no colour change in the medium; eight strains, however, fermented mannitol and these had the appearance of *Staph. aureus*. Apart from three strains of enterococci no other organisms were encountered in nasal swabs which resembled *Staph. aureus* growing on this medium.

Of the 926 nasal swabs examined, 201 yielded *Staph. aureus* when cultured on blood agar and 36 of these proved to be multiply antibiotic-resistant on subsequent testing. Culture of these same 926 swabs on mannitol-antibiotic agar produced 50 strains of *Staph. aureus* all of which were multiply antibiotic-resistant. Only two of the strains growing on blood agar which were resistant to multiple antibiotics were not isolated on mannitol-antibiotic agar, but 16 strains on the latter medium were not detected on blood agar (Table). The difference is statistically significant (p = 0.001).

<table>
<thead>
<tr>
<th>No. of Nasal Swabs Examined</th>
<th>No. of Swabs Yielding Staph. aureus on Blood Agar</th>
<th>No. of Swabs Yielding Staph. aureus Resistant to Tetracycline and Streptomycin</th>
<th>No. of Swabs Yielding Staph. aureus Resistant to Penicillin and Mannitol-antibiotic Agar Only</th>
<th>Blood Agar Only</th>
<th>Both Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>926</td>
<td>201</td>
<td>16</td>
<td>2</td>
<td>52</td>
<td></td>
</tr>
</tbody>
</table>

Table: Comparison of blood agar and mannitol-antibiotic agar in the isolation of *Staph. aureus* from nasal swabs

The slide coagulase test was positive with 47 (85%) of 54 multiply antibiotic-resistant strains of *Staph. pyogenes* growing on mannitol-antibiotic agar. Of the seven strains which were slide coagulase negative only one strain proved to be slide coagulase positive when grown on sensitivity agar; the remaining six strains were, however, all positive on the tube coagulase test. It is of interest that 38 of these 54 strains were sensitive to cloxacillin and that all of them were slide coagulase positive whereas of the remaining 16 strains which were resistant to cloxacillin only 10 were slide coagulase positive.

Conclusions

The use of mannitol agar containing antibiotics for the detection of nasal carriers of *Staph. aureus* resistant to multiple antibiotics had a number of advantages compared with routine methods using blood agar. In most instances carriers were identified within 24 hours compared with the 48 hours taken by routine methods. All 50 strains of *Staph. aureus* growing on mannitol-antibiotic agar proved to be resistant to penicillin, streptomycin, and tetracycline compared with 36 of 201 strains isolated on blood agar. Thus in addition to the increased sensitivity of the mannitol-antibiotic agar technique it reduced by four-fold the number of strains which required further examination after primary isolation on blood agar. Furthermore, as the mannitol-antibiotic plates were inoculated and read by the sister in control of infection, the work load imposed on the laboratory was considerably reduced.

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References


A screening technique for the detection of nasal carriers of antibiotic-resistant Staphylococcus.
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