Cloxacillin-Resistant *Staphylococcus aureus* in a Children's Hospital

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**SUMMARY**

A group of cultures of cloxacillin-resistant *Staphylococcus aureus* was collected over a period of 6 months. The method of determination of resistance to cloxacillin is described. The use of the disc diffusion test for cloxacillin was found to be unsatisfactory.

The incidence of cloxacillin-resistant *Staph. aureus* at the Red Cross War Memorial Children's Hospital (inpatients as well as outpatients) is slightly less than 2% of all isolates of *Staph. aureus*. Of the total non-White ward patients with penicillin-resistant *Staph. aureus* infections, 6.5% have *staphylococci* resistant to cloxacillin.

Reaction of these isolates to Fucidin, clindamycin and erythromycin is described.


During the second half of 1973 an attempt was made to investigate all cultures of penicillin-resistant *Staphylococcus aureus* isolated in the laboratory of the Red Cross War Memorial Children's Hospital. Strains resistant to methicillin and cloxacillin were sought and their incidence determined.

Annear demonstrated that the incubation of cultures of *Staph. aureus* at temperatures below 37°C accentuated the difference between sensitive and resistant strains for methicillin and to a lesser extent for other antibiotics.

Hewitt et al. examined a group of borderline cultures of which the sensitivity to methicillin had been in doubt. They performed disc sensitivity tests at 30°C and 37°C, as well as mean inhibitory concentration tests at 30°C and 37°C, and found that by the use of tests at 30°C they were able to divide their group of borderline cultures into clear-cut sensitive and insensitive groups. Their conclusions were that (a) disc diffusion tests for cloxacillin were unsatisfactory; (b) disc diffusion tests for methicillin at 30°C were satisfactory; and (c) the mean inhibitory concentration (MIC) test at 37°C for methicillin read at 48 hours gave equivalent results to the MIC test at 30°C for methicillin read at 18 hours, and both these gave equivalent results to the MIC test at 30°C for cloxacillin read at 48 hours.

Hallander et al. also investigated sensitivity testing with methicillin at 30°C and 37°C and found that incubation at the lower temperature gave more accurate results.

It thus becomes evident that if a strain of *Staph. aureus* can be demonstrated to be resistant to methicillin, it can be inferred that it will also be resistant to cloxacillin.

**METHODS**

**Disc Diffusion Test**

Methicillin 10-μg blotting paper discs, 6 mm in diameter, were prepared and dried *in vacuo* at 4°C. Fresh batches of discs were prepared every 3-4 weeks and stored with a desiccant at 4°C. Cloxacillin 5-μg blotting paper discs 6 mm in diameter were supplied by Beecham Research Laboratories together with a supply of methicillin. The disc diffusion tests were set up with Oxoid Blood Agar Base No. 2 and zone sizes were compared with those of an Oxford *Staphylococcus* used as a control with each batch. Cultures were incubated for 18 hours. The zone diameters stated are the total inhibitory zone diameters minus the diameters of the discs.

**Mean Inhibitory Concentration Tests**

**Plate method:** Nutrient agar plates (Oxoid Blood Agar Base No. 2) were prepared containing the appropriate concentration of methicillin. These were spot-inoculated with 0.02 ml (c. 10⁵ organisms) of a fresh broth culture of each organism being tested. Controls with Oxford *Staphylococcus* were set up with each batch of tests.

**Tube method:** Rows of tubes containing 1-ml quantities of broth (Oxoid Nutrient Broth No. 2) and progressively doubling concentrations of antibiotic were set up. These were inoculated with 0.02-ml quantities of the broth cultures of test organisms as described above. Oxford *Staphylococcus* controls were set up with each batch. Sets of tubes containing either methicillin or cloxacillin were incubated at 30°C and 37°C. They were read after both 18 hours and 48 hours' incubation.

**Laboratory Investigations**

As the cultures of penicillin-resistant *Staph. aureus* were isolated they were subcultured and disc diffusion tests for methicillin at 30°C were carried out. If zones of 5 mm or less were obtained, the test was repeated to exclude the possibility of an unsatisfactory disc, which did on occasion occur. In the event of an organism showing resistance to methicillin by the disc technique on two occasions, the culture was retained and, when a batch of such cultures...
<table>
<thead>
<tr>
<th>Specimen No.</th>
<th>Oxford staph.</th>
<th>S. A. MEDISE TYDSKrif</th>
<th>Number resistant</th>
<th>Number doubtful</th>
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<tbody>
<tr>
<td>13136</td>
<td>+ 17 20</td>
<td>+ 17 20</td>
<td>32</td>
<td>3</td>
</tr>
<tr>
<td>9463</td>
<td>+ 10</td>
<td>+ 11</td>
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<td>26006</td>
<td>+ 13</td>
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**Mean inhibitory concentration tests**

<table>
<thead>
<tr>
<th>Specimen No.</th>
<th>甲氧西林 (10 μg 盘)</th>
<th>甲氧西林 (30 ℃)</th>
<th>甲氧西林 (37 ℃)</th>
<th>甲氧西林 (30 ℃)</th>
<th>甲氧西林 (37 ℃)</th>
<th>甲氧西林 (30 ℃)</th>
<th>甲氧西林 (37 ℃)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxford staph.</td>
<td>+ 17 20</td>
<td>+ 11 16</td>
<td>29</td>
<td>29</td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
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</table>

*Colonies within the zone of inhibition.*
was obtained, they were submitted to disc diffusion tests with methicillin and cloxacillin, at both 30°C and 37°C, as well as MIC tests consisting of plate and tube methods for methicillin at 30°C, tube method for methicillin at 37°C, as well as tube method for cloxacillin at both 30°C and 37°C.

RESULTS AND DISCUSSION

Table I demonstrates the well-marked resistance of all the cultures to methicillin on disc sensitivity testing at 30°C and also shows the temperature-dependent difference in zone size of between 0 and 13 mm with methicillin. This pattern of difference in zone diameter was lacking with cloxacillin, for which the disc sensitivity tests were found to be unsatisfactory.

MIC tests with methicillin at 30°C after 18 hours' incubation as well as those at 37°C after 48 hours and those with cloxacillin at 30°C after 48 hours, all showed unequivocal evidence of resistance in all cultures.

Table II gives information regarding the type of specimen from which each culture was obtained, as well as brief clinical data. These patients are divided into three groups: (i) acute staphylococcal infections — 2 cases with pneumonia and empyema; (ii) patients contracting a hospital infection — 13 cases including neonatal tetanus, postoperative surgical cases, a burn case and a chronic enteritis case developing impetigo — illustrating how difficult it is to control hospital infection, especially in a children’s hospital; (iii) 2 chronic debilitated patients probably admitted with cloxacillin-resistant staphylococci — including a case of kwashiorkor with otitis media and a case of pneumonia and possible pulmonary TB with otitis media. From 9 of the patients more than one specimen each was sent to the laboratory.

Incidence of Cloxacillin-Resistant Staphylococcus aureus

An analysis of cultures of penicillin-resistant Staph. aureus obtained is shown in Fig. 1, while an analysis of the patients producing resistant cultures is given in Fig. 2.
During the period of investigation penicillin-sensitive strains consisted of over 40% of the isolates of *Staph. aureus* in outpatients. If this is taken into account, the isolates of cloxacillin-resistant *Staph. aureus* probably consisted of slightly less than 2% of the total isolates of *Staph. aureus* in the laboratory.

In the UK Dyke et al. found an incidence of methicillin-resistant *Staph. aureus* of 0.06% during 1960, and this rose to 0.37% in 1964. In the UK, Parker and Hewitt found an incidence of 4.11% during 1969. Much higher figures have been reported from various countries in Europe.*

The advisability of retaining penicillinase-resistant penicillin for penicillin-resistant *Staph. aureus* infections is stressed, in the hope that the incidence of cloxacillin-resistant strains in the general population will increase as slowly as possible. The treatment of infections for an adequate period of time is also important.

**Sensitivity to Other Antibiotics**

Disc tests for sensitivity to other antibiotics, including Fucidin, clindamycin and erythromycin, were carried out on each of the 32 cultures resistant to cloxacillin. The following results were obtained:

No. 13136 This strain from the UK was sensitive to erythromycin, Fucidin and clindamycin.

No. 11164 This strain from the UK was sensitive to Fucidin but resistant to erythromycin and clindamycin.

No. 25540 This strain was sensitive to each of the three antibiotics.

The following are of particular interest:

- Both cultures, from the same patient, showed sensitivity to Fucidin and clindamycin.
- All the other cultures showed resistance to erythromycin and sensitivity to Fucidin and clindamycin.

Fucidin and clindamycin should not be widely used for staphylococcal infections, but should be reserved for selected cases, otherwise it is likely that a strain of *Staph. aureus* resistant to these antibiotics may replace the sensitive strains.

We wish to thank Dr M. T. Parker, Director of the Cross-Infection Reference Laboratory, Colindale, London, for providing cultures of methicillin-resistant staphylococci; and Beecham Research Laboratories Ltd for supplying methicillin and cloxacillin discs.

**REFERENCES**