Antibiotic resistance of coagulase-negative staphylococci and micrococci

JEAN CORSE AND R. E. O. WILLIAMS

From the Wright-Fleming Institute, St. Mary’s Hospital Medical School, London

SYNOPSIS

Five hundred and seventy strains of Gram-positive, catalase-positive, coagulase-negative coci from various sources were classified on the basis of their ability to grow anaerobically and to ferment mannitol. Five groups were distinguished. The frequency of strains classified in the five groups varied with the source.

Only 26% of the strains were sensitive to all the nine antibiotics used in the tests. About half the strains from the lesions of hospital patients were resistant to penicillin, streptomycin, and tetracycline, and about 10% of them were resistant to cloxacillin.

In general the frequency of antibiotic resistance and the ‘width’ of the resistance spectrum were greater in strains from hospital sources than in those from outside hospital, and they were also greater among strains regarded as falling in the genus Staphylococcus than in those classed as Micrococcus.

During recent years increasing interest has been shown in the coagulase-negative staphylococci and micrococci that are isolated from man. It is now known that there are circumstances in which at least some of these strains can be pathogenic for man (Quinn, Cox, and Fisher, 1965, Wilson and Stuart, 1965); it has been found that the strains isolated from hospital patients and from the hospital environment are commonly resistant to many antibiotics (Kjellander, Klein, and Finland, 1963, Kryński, Borowski, Becla, Galifski, Niemiro, Szymańska-Malottke, and Wroczynski, 1963), and some progress has been made in their classification (Baird-Parker, 1963, 1965a, 1965b). It seemed of some interest to try to bring these three lines of study in relation to one another, so we made a collection of strains from hospital patients, healthy carriers outside hospital, and air both in hospital and outside. The strains have been examined for a few physiological reactions and for antibiotic sensitivity.

**TABLE I**

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital patients, pathological lesions</td>
<td>48</td>
</tr>
<tr>
<td>Urinary infections</td>
<td>47</td>
</tr>
<tr>
<td>Wounds, pus</td>
<td>66</td>
</tr>
<tr>
<td>Peritoneal dialysis fluid</td>
<td>47</td>
</tr>
<tr>
<td>Sputum, eye swabs, miscellaneeons from patients</td>
<td>36</td>
</tr>
<tr>
<td>Hospital patients, nose</td>
<td></td>
</tr>
<tr>
<td>skin</td>
<td>50</td>
</tr>
<tr>
<td>Hospital patients, nose</td>
<td></td>
</tr>
<tr>
<td>skin</td>
<td>11</td>
</tr>
<tr>
<td>Healthy persons outside hospital, nose</td>
<td></td>
</tr>
<tr>
<td>skin</td>
<td>62</td>
</tr>
<tr>
<td>Air, hospital wards</td>
<td>59</td>
</tr>
<tr>
<td>Air, public post offices</td>
<td>97</td>
</tr>
<tr>
<td>Total</td>
<td>197</td>
</tr>
</tbody>
</table>

probably to be responsible for an infective process. The strains from healthy carriers were given to us by Dr W. C. Noble who isolated them from schoolboys and Royal Air Force personnel. The air strains were from colonies selected by a random procedure from sedimentation plates exposed either in the hospital wards or in two local post offices.

**MATERIAL**

A total of 570 strains were studied (Table I). Of these 197 were isolated in the Diagnostic Bacteriological Laboratory here from specimens sent from pathological lesions; most of the strains had been present in abundant or pure culture and in many cases were considered

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**METHODS**

All the strains were examined microscopically in a Gram-stained film, and were tested for catalase production on an agar slope culture and for coagulase production by incubation overnight in broth containing 10% human plasma. All were catalase-positive, coagulase-negative, Gram-positive cocci arranged in pairs or clusters.

The strains were stab-inoculated into tubes containing
tryptone 1%, yeast extract 0·5%, K₂HPO₄ 0·5%, NaCl 0·5%, agar 1·5%, bromocresol purple 0·0015%, and man- 
nitol 1·0%, at pH 7·0 to 7·2 and incubated at 37°C for six 
days. These tubes were used to distinguish the facultative 
aerobes from the obligate aerobes, and to determine the 
ability of the strains to ferment mannitol aerobically or 
an aerobically. Preliminary investigation showed that 
all the strains that were able to grow anaerobically 
also fermented glucose anaerobically and for this 
reason a separate glucose-ager stab culture was omitted.

Phosphatase production was tested by inoculation on 
to Oxoid nutrient agar containing 5% horse serum and 
0·01% phenolphthalein diphosphate and incubated for 
18 to 20 hours at 37°C. The plates were exposed to 
ammonia vapour and the reaction was read as ‘strong 
positive (+++)’ if a deep pink colour developed im-
mediately, ‘weak positive (+)’ if the colony turned pale 
pink, and ‘negative (−)’ if it remained white.

Antibiotic sensitivity was tested with discs (Mast 
Laboratories or Oxoid Ltd), nutrient agar plates being 
inoculated with a diluted overnight broth culture to give 
a just-confluent lawn. Paper discs containing one 
of nine antibiotics (see Table IV) were then placed on the 
surface of the plate and the plates held at room tem-
perature for one to one-and-a-half hours. After overnight 
incubation at 37°C the diameter of the inhibition zone 
was measured. One hundred and thirty-three strains 
were also tested by tube-dilution test for sensitivity to 
cloxacillin; 0·02 ml of a diluted overnight broth culture 
was inoculated into 1·0 ml volumes of series of two-fold 
falling dilutions of cloxacillin in nutrient broth. The 
diluting machine developed by Trotman (1967) proved 
invaluable for this purpose.

Penicillinase production was tested in the first place 
by adding a solution containing 1 unit of penicillin G to 
1·0 ml of an overnight broth culture of the strain under 
test; after one hour at room temperature the fluid was 
pipetted into Heatley cylinders placed on a plate of 
nutrient agar containing a 5% concentration of a broth 
culture of Sarcina lutea (NCTC 8340). The plates were 
allowed to diffuse for one hour at room temperature 
before incubation at 30°C for 18 to 24 hours. Strains 
were considered to produce penicillinase when there 
was growth of the S. lutea up to the edge of the cylinder.

A small number of strains were tested for penicillinase 
production by an iodometric method based on that 
described by Perret (1954). Overnight cultures grown on 
nutrient agar containing 0·08% of sodium starch 
yglcylate (BDH) were flooded with a solution con-
taining 100 units/ml penicillin G. After one hour this 
was replaced by a 0·01 N solution of iodine. Any degree 
of clearing around or under the colony was taken to 
indicate penicillinase production.

Deoxyribonuclease production was tested by stab 
inoculating the bacteria into a plate of medium con-
taining Difco proteose-peptone 2%, sodium chloride 
0·5%, Difco Bacto agar 1·5%, to which was added a 
Seitz-filtered solution of sodium deoxyribonuclease 
(BDH) to a final concentration of 0·2%. After overnight 
incubation at 37°C the plate was flooded with N hydro-
chloric acid; a clear zone around the colony was taken to 
indicate deoxyribonuclease production.

DEFINITIONS

The set of diagnostic tests used does not enable us to 
allocate the strains precisely to the detailed subdivisions 
described by Baird-Parker (1963, 1965a, 1965b), but 
we can allocate them to five groups that correspond 
broadly with his classification. We use the term ‘Staphylococcus’ to refer to the facultatively anaerobic 
strains. This group has three subdivisions based on 
mannitol fermentation. The strains that fermented 
mannitol anaerobically are very similar indeed to 
S. aureus but all were coagulase-negative and only one 
of the 16 was a strong phosphatase producer; they are 
conveniently termed S. albus. Strains that fermented 
mannitol aerobically but not anaerobically appear to 
conform with Baird-Parker’s (1965a) S. epidermidis, 
group VI, while those that fail to ferment mannitol but 
can still grow anaerobically fall into his S. epidermidis, 
groups II to V.

The strains that proved to be obligate aerobes are 
referred to the genus Micrococcus, those that fermented 
mannitol being allocated to groups 3 to 6 (Baird-Parker, 
1963), while those that did not ferment mannitol are 
classed as Micrococcus groups 1 and 2.

RESULTS

CHARACTERISTICS OF THE S. ALBUS STRAINS In view 
of the similarity between the 16 strains referred to 
as S. albus and coagulase-positive S. aureus, we 
examined the 13 of these strains that were still 
available by a series of further tests; none of the 
13 produced deoxyribonuclease or haemolysin 
resembling the α-lysine of S. aureus, and none was 
sensitive to any of the standard set of S. aureus 
typing phages.

PHOSPHATASE-PRODUCTION Only 19 of the 570 
strains gave a strong phosphatase reaction (Table II). 
It is reactions of this order that are used in the 
Barber and Kuper (1951) method for recognition of 
Staphylococcus aureus and it is apparent that false 
positives would have been rare among these strains. 
However, 73% of the S. epidermidis II to V strains 
gave a weak positive reaction. Baird-Parker’s (1963) 
method for detecting phosphatase production 
involves incubation for three to five days at 30°C 
and it is probable that our ‘weak positive’ reactions 
correspond to his ‘positive’ reactions. He found 
phosphatase production to be characteristic of his 
S. epidermidis groups II and III; he also found it 
characteristic of his Micrococcus group 6, but in 
our material ‘weak’ phosphatase reactions were 
much commoner among the Micrococcus strains 
that did not ferment mannitol.

’SPECIES’ DISTRIBUTION OF STRAINS FROM VARIOUS 
SOURCES The distribution of the different varieties
Jean Corse and R. E. O. Williams

TABLE II
PHOSPHATASE REACTIONS

<table>
<thead>
<tr>
<th></th>
<th>No. of Strains</th>
<th>Strong</th>
<th>Weak</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus albus</td>
<td>16</td>
<td>1</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Staphylococcus epidermidis VI</td>
<td>86</td>
<td>2</td>
<td>2</td>
<td>82</td>
</tr>
<tr>
<td>Staphylococcus epidermidis II-V</td>
<td>253</td>
<td>15</td>
<td>184</td>
<td>54</td>
</tr>
<tr>
<td>Micrococcus 1, 2</td>
<td>141</td>
<td>0</td>
<td>35</td>
<td>106</td>
</tr>
<tr>
<td>Micrococcus 3-6</td>
<td>74</td>
<td>1</td>
<td>2</td>
<td>71</td>
</tr>
<tr>
<td>Total</td>
<td>570</td>
<td>19</td>
<td>227</td>
<td>324</td>
</tr>
</tbody>
</table>

of cocci in the material from different sources is shown in Table III and Figure 1. Almost all the 16 (88%) S. albus strains came from pathological lesions compared with 35% of the S. epidermidis strains and 30% of the Micrococcus strains. On the other hand, only about 7% of all the lesion strains were S. albus, over half of them being S. epidermidis and one third being Micrococcus. Among carrier strains there were very few S. albus and a large proportion of S. epidermidis. Nearly two-thirds of the air strains fell into the Micrococcus group.

RESULTS OF DISC TESTS FOR ANTIBIOTIC RESISTANCE
The distributions of zone diameter observed in the disc tests with the nine antibiotics are shown in Figure 2. The shape of the distribution curves varies with the different antibiotics: tetracycline, chloramphenicol, streptomycin, erythromycin, and novobiocin showed clear bimodal distributions making the distinction of 'resistant' and 'sensitive' strains quite simple (Bauer, Perry, and Kirby, 1959). With the penicillins, kanamycin, and neomycin there were large numbers of strains showing zones of a size intermediate between those of fully resistant and obviously sensitive strains.

For the purpose of further analysis the strains were classified as sensitive, resistant, or, in the case of cloxacillin, partially resistant, on the basis of the zone diameters in the disc tests, as shown in Figure 2. The overall frequency of resistant strains is given in Table IV.

After the main part of the study had been completed 82 strains were tested for sensitivity to cephaloridine, using discs containing 5 mg of the drug. No strain showed an inhibition zone of less than 18 mm and all but nine of the strains had zones greater than 24 mm. No strain was, therefore, classified as resistant. Eighty-nine strains were similarly tested with Oxoid discs containing 10 µg gentamycin or 2 µg lincomycin. No resistant strains were found.

TUBE-DILUTION TESTS WITH CLOXACILLIN FOR ANTIBIOTIC RESISTANCE Particular interest attaches to the resistance to cloxacillin, so 133 strains were retested to determine the minimal inhibitory

TABLE III
FREQUENCY OF VARIOUS 'SPECIES' FROM DIFFERENT SOURCES

<table>
<thead>
<tr>
<th>Pathological lesions</th>
<th>Staphylococcus albus</th>
<th>Staphylococcus epidermidis</th>
<th>Micrococcus I-V</th>
<th>Micrococcus 3-6</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urines</td>
<td>4</td>
<td>10</td>
<td>21</td>
<td>9</td>
<td>48</td>
</tr>
<tr>
<td>Wounds</td>
<td>7</td>
<td>12</td>
<td>29</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Dialysis fluid</td>
<td>0</td>
<td>7</td>
<td>17</td>
<td>17</td>
<td>47</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>3</td>
<td>8</td>
<td>14</td>
<td>9</td>
<td>36</td>
</tr>
<tr>
<td>Subtotal</td>
<td>14</td>
<td>37</td>
<td>81</td>
<td>48</td>
<td>197</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Carrier sites</th>
<th>Staphylococcus albus</th>
<th>Staphylococcus epidermidis</th>
<th>Micrococcus I-V</th>
<th>Micrococcus 3-6</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital patients</td>
<td>1</td>
<td>3</td>
<td>51</td>
<td>6</td>
<td>61</td>
</tr>
<tr>
<td>Community</td>
<td>1</td>
<td>6</td>
<td>83</td>
<td>22</td>
<td>121</td>
</tr>
<tr>
<td>Subtotal</td>
<td>2</td>
<td>9</td>
<td>134</td>
<td>28</td>
<td>182</td>
</tr>
<tr>
<td>Air</td>
<td>0</td>
<td>26</td>
<td>15</td>
<td>32</td>
<td>97</td>
</tr>
<tr>
<td>Hospital wards</td>
<td>0</td>
<td>14</td>
<td>23</td>
<td>33</td>
<td>94</td>
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<tr>
<td>Post offices</td>
<td>0</td>
<td>40</td>
<td>38</td>
<td>65</td>
<td>191</td>
</tr>
<tr>
<td>Subtotal</td>
<td>16</td>
<td>86</td>
<td>253</td>
<td>141</td>
<td>570</td>
</tr>
</tbody>
</table>
Antibiotic resistance of coagulase-negative staphylococci and micrococci

![Image](http://jcp.bmj.com/)

**TABLE IV**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Percentage of Strains Classed as Resistant</th>
<th>Antibiotic Content of Disc (μg)</th>
<th>Maximum Zone Diameter Classed as 'Resistant' (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin G</td>
<td>59.6</td>
<td>(1 unit)</td>
<td>0.6</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>8.8</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>30.7</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>14.0</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>44.2</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>8.4</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Neomycin</td>
<td>22.8</td>
<td>10</td>
<td>19</td>
</tr>
<tr>
<td>Novobiocin</td>
<td>11.9</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>20.0</td>
<td>30</td>
<td>18</td>
</tr>
<tr>
<td>Cephaloridine</td>
<td>0</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>0</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>0</td>
<td>2</td>
<td>14</td>
</tr>
</tbody>
</table>

183 strains tested

*Discs obtained from Mast Laboratories Ltd, except for gentamycin and lincomycin which were obtained from Oxoid Ltd.

Concentrations (MIC) in a tube-dilution test; the results are shown in Figure 3. There was a rather broad scatter of MIC corresponding to each inhibition zone diameter but the trend was quite clear and the results showed that the narrow inhibition zones undoubtedly reflected a high MIC. Seven strains had an MIC of 16 μg/ml or more. All but one of the strains that showed inhibition zones of 22 mm or more had an MIC of 0.5 μg/ml or

![Image](http://jcp.bmj.com/)

**FIG. 1.** 'Species' distribution among strains from different sources. U = urine; W = wounds; M = miscellaneous pathological specimens; PDF = peritoneal dialysis fluid; HC = carrier sites in hospital patients; HA = hospital air; CC = carrier sites in healthy persons outside hospital; CA = air outside hospital. For definition of 'species', see text.

![Image](http://jcp.bmj.com/)

**FIG. 2.** Distribution of zone diameters in disc sensitivity tests. R10 includes strains showing no inhibition zone. Arrow indicates minimum zone diameter taken as boundary between 'sensitivity' and 'resistance', or in the case of cloxacillin, 'partial resistance'.

*Discs obtained from Mast Laboratories Ltd, except for gentamycin and lincomycin which were obtained from Oxoid Ltd.*
less. There are clearly, by both methods of testing, a number of strains of intermediate sensitivity.

ANTIBIOTIC RESISTANCE IN RELATION TO SOURCE AND 'SPECIES' The *S. albus* strains, which were almost all from lesions, were all resistant to one or more antibiotics and 19% of them were resistant to cloxacillin (Table V). Among the other 'species' the strains from hospital sources were in all cases more frequently resistant than those from extra-hospital sources (Fig. 4). In general the *Micrococcus* strains were less often resistant than the *Staphylococcus* strains. Complete resistance to cloxacillin was very rare in strains isolated from carriers or air but it was notable that strains partially resistant to cloxacillin were common among all varieties and from all sources.

In contrast to the experience of Alder, Brown, and Mitchell (1966) resistance to novobiocin was observed among strains from all the 'species', being commoner in those classed as *S. epidermidis VI* (22.1%) and *Micrococcus* 3 to 6 (21.6%) than in the others.

PENICILLINASE PRODUCTION The results of the tests for penicillinase are set out in Table VI. In general there was good correlation of penicillin resistance with penicillinase production though there were some anomalous results. Twenty-three strains produced penicillinase although they had appeared sensitive in disc tests. Repetition of the disc sensitivity tests on these strains confirmed the original result in all cases. It was possible to retest only seven of these strains by the iodometric method but all were penicillinase-positive. They were not strains with borderline inhibition zones. Of 10

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**TABLE V**

ANTIBIOTIC SENSITIVITY IN THE VARIOUS 'SPECIES'¹

<table>
<thead>
<tr>
<th>Antibiotic Resistance</th>
<th>Staphylococcus</th>
<th><em>Micrococcus</em></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>albus</em></td>
<td><em>epidermidis VI</em></td>
<td><em>epidermidis other</em></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>10</td>
<td>29</td>
</tr>
<tr>
<td>Penicillin G only</td>
<td>0</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>Penicillin G, streptomycin, and tetracycline</td>
<td>88</td>
<td>52</td>
<td>22</td>
</tr>
<tr>
<td>Pen., strep., tet., and cloxacillin</td>
<td>19</td>
<td>35</td>
<td>2</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>19</td>
<td>35</td>
<td>5</td>
</tr>
<tr>
<td>Cloxacillin, partial resistance</td>
<td>63</td>
<td>30</td>
<td>12</td>
</tr>
<tr>
<td>No. of strains</td>
<td>16</td>
<td>86</td>
<td>253</td>
</tr>
</tbody>
</table>

¹The figures give the total percentage of strains with the stated resistance pattern, so the columns do not total 100%
Antibiotic resistance of coagulase-negative staphylococci and micrococci

resistant strains that did not appear to produce penicillinase by the Sarcina lutea test only one was available for retesting and it was shown to be a weak penicillinase producer by the iodometric test.

DISCUSSION

The coagulase-negative staphylococci have been neglected by medical bacteriologists in the past, doubtless largely because the very great majority of pyogenic lesions are associated with infection by coagulase-positive strains, and partly because of the lack of any accepted criteria for distinguishing the seemingly numerous varieties of coagulase-negative cocci. It is fortunate that Baird-Parker's (1963, 1965a, 1965b) taxonomic studies have appeared at a time when it is becoming evident that some coagulase-negative cocci, at least, can cause disease in man. The present paper reports some preliminary studies aimed to discover whether Baird-Parker's broad categories appeared to be of use in investigating cocci of medical interest.

It is notoriously difficult to judge whether an infecting organism is, in some particular case,
striking findings classed as Micrococcus. The strains called Staphylococcus albus were practically all isolated from pathological specimens; there was a great predominance of S. epidermidis groups II to V in strains from carrier sites, and over half the air strains were classed as Micrococcus. It is probable that a more detailed study might well sharpen these differences. Roberts (1967) reported Micrococcus group 3 strains to be particularly common in bladder urine; our data are not strictly comparable with this but do not seem to confirm his observations.

It is now well known that coagulase-negative cocci isolated from hospital patients are often resistant to antibiotics, and that the antibiotic resistance patterns are often, in comparison to those seen with S. aureus, very bizarre. The most striking findings in our study are the high frequency of resistance to penicillin, streptomycin, and tetracycline among strains from all hospital sources, and the finding of 51 strains (9%) that were resistant to cloxacillin. These strains were all from hospital sources and were commonest in two of the five taxonomic groups—S. albus and S. epidermidis, group VI. In view of the apparently close metabolic similarity between many of these strains and S. aureus, it is very surprising that the frequency of cloxacillin-resistant S. aureus is still no more than 1 to 2% (Parker, M. T., personal communication, 1967). Genetic exchange between S. aureus and S. epidermidis must, happily, be rather rare.

It was also interesting to note that partial resistance to cloxacillin was observed in a moderate proportion of strains of all species and, though this is not shown in Fig. 4, from all sources. Evidently the coagulase-negative staphylococci exhibit two different forms of cloxacillin resistance.

REFERENCES
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