Use of Mueller-Hinton Agar to Determine Novobiocin Susceptibility of Coagulase-Negative Staphylococci

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A group of 254 isolates of coagulase-negative staphylococci were tested in parallel for novobiocin susceptibility by using P agar and Mueller-Hinton agar. Zones of inhibition of 16 mm or less around a 5-µg novobiocin disk on Mueller-Hinton agar indicated novobiocin resistance, as demonstrated by Staphylococcus saprophyticus.

Coagulase-negative staphylococci (C-NS), once regarded as contaminants and nonpathogens, are now the subject of growing interest. Members of this group have been implicated as the etiological agents of prosthetic valve endocarditis (6), urinary tract infections in young women (3), and possibly even as a cause of nongonococcal urethritis in men (2). Kloos and Schleifer have developed a simplified schema for routine identification of human Staphylococcus species (5). An important characteristic in this schema is the determination of susceptibility or resistance to 1.6 µg of the antibiotic novobiocin per ml. Of the three human C-NS species (S. saprophyticus, S. cohnii, and S. xylosus) which are resistant to novobiocin, S. saprophyticus appears most prominent, primarily as a urinary pathogen of young, sexually active females (3). Anderson (1) has concluded that novobiocin-resistant urinary isolates may be reported as "presumptive S. saprophyticus" by laboratories which do not choose to perform rigorous identification of C-NS. Kloos' simplified method for determining novobiocin resistance involves the use of a 5-µg novobiocin disk and P agar as the test medium. Isolates which are inhibited "from 1-5 mm from the edge of the disk," are classified as novobiocin resistant (5). A difficulty with general use of this procedure is that P agar is not commercially available in either prepared or dehydrated form. The purpose of this study was to determine whether Mueller-Hinton agar, which is widely used by clinical laboratories, could be employed to determine the novobiocin susceptibility of C-NS.

A total of 254 clinical isolates of C-NS were identified as to species according to the most recent methods of Kloos (4). The species and the number of isolates included in the study group were S. epidermidis (111), S. hominis (50), S. saprophyticus (31), S. simulans (27), S. haemolyticus (23), S. warneri (10), and S. capitis (2). Four to five well-isolated colonies of each isolate grown on plates of Trypticase soy agar with 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md.) were inoculated into 5 ml of tryptic soy broth (Difco Laboratories, Detroit, Mich.). The broth cultures were incubated at 35°C for 2 to 5 h, until a slightly visible turbidity appeared. The turbidity of the broth cultures was then adjusted with sterile broth to obtain a density visually comparable to that of a 0.5 McFarland opacity standard. This inoculum suspension was simultaneously inoculated onto both P agar plates and Mueller-Hinton agar plates (both in 100- by 15-mm plastic plates, prepared to yield an agar bed 3 to 4 mm in depth). Three to five minutes after swabbing of the plates to obtain confluent growth, a 5-µg novobiocin disk (BBL) was applied to each plate. The plates were then inverted and placed in an incubator at 35°C. After 16 to 18 h of incubation, the plates were examined and the diameter of the zone of complete inhibition was measured to the nearest whole millimeter with vernier calipers. A zone diameter of 16 mm appeared to represent a breakpoint between novobiocin-susceptible and -resistant C-NS (Table 1). Isolates having a zone diameter of greater than 16 mm were considered susceptible (S. epidermidis, S. simulans, S. haemolyticus, S. hominis, S. warneri, and S. capitis), whereas those with a diameter of 16 mm or less were considered resistant (S. saprophyticus). The use of a 16-mm breakpoint is equivalent to Kloos' criteria of classifying strains as resistant if inhibition is not greater than 5 mm from the edge of a 6-mm disk. Out of 254 isolates studied, only 1 isolate (S. simulans) was classified as susceptible to novobiocin on P agar (19 mm) but resistant on Mueller-Hinton agar (16 mm).

A total of 222 isolates were susceptible to
TABLE 1. Mean novobiocin zone diameters of C-NS on P agar and Mueller-Hinton agar

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Zone diam(^a)</th>
<th>P agar</th>
<th>Mueller-Hinton agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Novobiocin-susceptible staphylococci (&gt;16 mm)</td>
<td>28.6 ± 4.1</td>
<td>29.0 ± 3.6</td>
<td></td>
</tr>
<tr>
<td>Novobiocin-resistant staphylococci (≤16 mm)</td>
<td>7.5 ± 3.0</td>
<td>10.1 ± 1.9</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Mean zone size ± 1 standard deviation.

novobiocin on both P agar and Mueller-Hinton agar by the same breakpoint criteria, whereas 31 isolates (all S. saprophyticus) were novobiocin resistant on both media, for a 99.6% agreement. The correlation coefficient of zone diameters as determined on both media was 0.93.

These data indicate a high degree of agreement between results obtained from novobiocin susceptibility testing of C-NS on P agar and Mueller-Hinton agar. Thus, Mueller-Hinton agar is a suitable alternative medium for determining the susceptibility of C-NS to novobiocin. Based on these data, we propose using Mueller-Hinton agar, a 5-μg novobiocin disk, and a breakpoint of 16 mm to determine novobiocin susceptibility of C-NS. Unlike P agar, Mueller-Hinton agar is easily prepared, commercially available, and in current use in many clinical laboratories for antimicrobial susceptibility testing. Thus, a single Mueller-Hinton agar plate may be used concurrently for testing susceptibility to novobiocin and other antimicrobics.

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LITERATURE CITED