Urinary infection with coagulase-negative staphylococci in a teaching hospital

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SUMMARY In the eight-month period of study of all urine samples processed in our routine laboratory, only 85 out of 12 152 specimens yielded a bacteriologically significant growth of either Staphylococcus epidermidis or micrococci. Their growth on MacConkey medium was strictly comparable to that on cysteine lactose electrolyte-deficient (CLED) media. Most micrococci isolated were from urine samples of non-hospitalised women patients, were resistant to a novobiocin (5 μg) disc, and belonged to Baird Parker type 3. Staph. epidermidis came mainly from postoperative surgical inpatients. Their antibiotic sensitivity patterns are variable whereas micrococci are fully sensitive to all urinary antibiotics. We agree that the use of a novobiocin (5 μg) disc for provisional identification of micrococci and Staph. epidermidis is simple and practical for a busy routine diagnostic laboratory. The use of more extensive systems to biotype these organisms in a routine laboratory is not practical and not relevant to patient management.

The undoubted role of catalase-positive, coagulase-negative Gram-positive cocci, Staphylococcus epidermidis, and Micrococcus species in causing urinary tract infection has gradually become accepted in recent years. These organisms did not appear to occur commonly in our routine practice, though other workers have reported an incidence ranging from complete absence (Arneil et al., 1970) to 30% of isolates (Gillespie et al., 1978). We therefore decided to collect and identify all these organisms occurring in urine samples reaching our diagnostic laboratory from inside and outside the hospital in order to establish their frequency and to assess the suitability of our methods for isolation and identification.

Material and methods

Urine samples received in the routine laboratory are cultured without delay or stored for up to 3 hours at 2-4°C on a CHEMLAB Bench Cooler. The well-mixed unspun urine is streaked onto a MacConkey plate (Oxoid CM76) using a calibrated loop delivering 0.003 ml of urine. For the purposes of this investigation duplicate cultures were prepared on plates of Cysteine Lactose Electrolyte Deficient (CLED) Agar, (Oxoid CM301). These were prepared in an exactly comparable manner to the McConkey plates, four specimens being cultured on one plate. A growth of 300 colonies or more of an organism is considered bacteriologically significant. Those yielding 30 colonies or less are discarded.

A wet preparation of the spun deposit (centrifuged for 10 minutes at 2000 rpm) is examined microscopically for formed elements, leucocytes, red blood cells, and bacteria. Those samples which have got pus cells, bacteria, or red cells require primary sensitivity tests, to sulphafurazole (200 μg), trimethoprim (2.5 μg), ampicillin (25 μg), nitrofurantoin (50 μg), and cephaloridine (25 μg). These tests are carried out on lysed blood agar plates ((Iso-Sensitest) Oxoid CM471 with 5% horse blood lysed with saponin) using Stokes' method and Escherichia coli NCTC 10418 as the control organism. After overnight incubation at 37°C all the cultures on CLED medium were read by one of us (TLS), and the results were compared with those of the routine cultures set up at the same time on MacConkey medium. Cultures yielding a bacteriologically significant growth of Gram-positive cocci, as defined above, were tested for coagulase production by the tube method of Gillespie (1943); catalase production was tested by the slide method.
Catalase-positive, coagulase-negative strains were biotyped using the methods of Baird-Parker (1963). In addition, strains were tested for sensitivity to a 5 µg novobiocin disc (Mitchell, 1968) as a diagnostic sensitivity test. These tests were carried out in parallel on Mueller-Hinton agar (Oxoid CM337), Wellcotest sensitivity test agar (Wellcome Reagents Limited, CM53), and Iso-Sensitest agar (Oxoid CM471) without added blood. Sensitive organisms gave a zone of inhibition measuring 8 mm or more from the edge of the disc.

Results

During the eight-month period October 1977-May 1978, our diagnostic laboratory processed 12,152 urine specimens. They were received from hospital ward patients, from those attending outpatient clinics, and from general practitioners. Of these specimens, 1186 gave significant bacteriuria due to all kinds of bacteria. Only 85 samples yielded a bacteriologically significant growth of Gram-positive, coagulase-negative, catalase-positive cocci (Table 1). Of these 85 samples, 17 were from non-hospitalised patients, all but one of them being female. The rest of the specimens were from hospitalised patients, that is, inpatients and outpatients (Table 2). Cultures on MacConkey agar and CLED medium gave strictly comparable results. Each of the 85 strains was recovered on both media, giving approximately equal numbers of colonies of the same size.

Of the 85 strains tested, 15 only were found to be micrococcus type 3, now re-classified as Staphylococcus saprophyticus biotype 3 on the basis of guanine and cytosine content of DNA (Buchanan and Gibbons, 1974). All these isolates were resistant to a 5 µg novobiocin disc.

No other micrococcus biotypes were encountered

Table 1 Urine specimens, October 1977-May 1978

| Total number processed in the laboratory | 12,152 |
| Significant bacteriuria due to all kinds of bacteria | 1186 (9.76%) |
| Gram-positive cocci out of all significant bacteriuria | 85 (7.16%) |

Table 2 Staphylococcal urinary infection in three groups of patients

<table>
<thead>
<tr>
<th>Staph. epidermidis</th>
<th>Hospital inpatients</th>
<th>Hospital outpatients</th>
<th>Non-hospitalised* patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Staph. albus)</td>
<td>Male 40</td>
<td>Male 4</td>
<td>Male 1</td>
</tr>
<tr>
<td></td>
<td>Female 16</td>
<td>Female 3</td>
<td>Female 6</td>
</tr>
<tr>
<td></td>
<td>56</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Staph. saprophyticus</td>
<td>Male 2</td>
<td>Male 0</td>
<td>Male 0</td>
</tr>
<tr>
<td>(Micrococcus type 3)</td>
<td>Female 3</td>
<td>Female 0</td>
<td>Female 10</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Totals</td>
<td>61</td>
<td>7</td>
<td>17 85</td>
</tr>
</tbody>
</table>

*General practitioner patients and patients attending casualty or antenatal clinic.
certain biotypes with specific types of infection. Care has to be taken in their correct performance. In particular, we had difficulty in interpreting some oxidation-fermentation tests when using the medium as described by Baird-Parker. Clear results were obtained when the concentration of the indicator-bromocresol purple was reduced to 0.002%, and soft paraffin was used to seal the anaerobic tubes in preference to sterile liquid paraffin (Chalmers, 1972). In contrast, novobiocin (5 μg) resistance is a simple test for provisional identification of micrococci (Mitchell, 1968). It gave comparable results irrespective of the medium used, provided lysed blood was not incorporated. Its addition had the effect of stimulating growth and giving reduced zones with some staphylococcal isolates.

In spite of the fact that not all biotypes of micrococi are novobiocin-resistant (Peed et al., 1977), it appears still to be a useful screening test for routine laboratory use (Sellin et al., 1975) to differentiate Staph. epidermidis and Staph. saprophyticus. Mitchell (1968) isolated four biotypes of Staph. epidermidis—II, IV, V, and VI—from urines, as did Peed et al. (1977). The same four types were isolated in this study. The only difference between our observations and those of others was that biotype VI was found to be commoner than biotype V in our series. These biotypes were isolated from hospital patients only in our study. Most of them came from surgical wards, and surgical intervention in and around the urinary tract seems to predispose to infection with these organisms. Most of the patients involved were males, unlike the micrococcal infections that occurred in females.

Although the numbers are small, it appears that at present micrococcus type 3 remains a fully antibiotic-sensitive organism. When it occurs as a cause of primary urinary infection it is likely to respond to any of the commonly prescribed urinary antibacterial antibiotics.

Staph. epidermidis associated with postoperative urinary tract infection, however, has a very variable sensitivity pattern. This, plus the fact that these infections occur in relation to urological surgery and the possible failure to establish urinary drainage, make these infections more difficult to manage.

Reviewing the sensitivity patterns of all our isolates (Table 3), it is surprising that so many strains of both micrococi and Staph. epidermidis are fully sensitive in vitro to sulphonamides and equally surprising that so many of the staphylococci are resistant to trimethoprim. This may reflect heavy usage of cotrimoxazole in hospital patients. In view of the complicated nature of many of the postoperative staphylococcal infections, use of sulphonamides alone would hardly recommend itself as a treatment likely to succeed. The fact that half our strains were resistant to ampicillin is not now surprising, and all strains are, of course, resistant to nalidixic acid. In view of the relative resistance to mecillinam, the place of this antibiotic in treating staphylococcal urinary infections must remain in doubt in spite of the high urinary levels attained. This means that the oral treatment of first choice for such infections would have to be an oral cephalosporin, with nitrofurantoin as a possible alternative.

We thank all the technical staff working in the urine section for their co-operation, Harry Edwards and his staff for preparation of media, and Miss A. Kirk for typing this manuscript.

Table 3  Sensitivity patterns of micrococi type 3 and other coagulase-negative staphylococci from urine

<table>
<thead>
<tr>
<th>Organism</th>
<th>Sulphonamide</th>
<th>Trimethoprim</th>
<th>Ampicillin</th>
<th>Nitrofurantoin</th>
<th>Cephaloridine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus</td>
<td>63/69</td>
<td>37/69</td>
<td>37/66*</td>
<td>65/67*</td>
<td>61/64*</td>
</tr>
<tr>
<td>Micrococcus type 3</td>
<td>14/14</td>
<td>14/14</td>
<td>14/14</td>
<td>14/14</td>
<td>14/14</td>
</tr>
</tbody>
</table>

*Low total numbers due to failure to perform test.

References


Gillespie, E. H. (1943). The routine use of the coagulase

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