Role of coagulase-negative staphylococci and micrococci in ocular disease

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SUMMARY Forty-nine strains of coagulase-negative staphylococci and 26 strains of micrococci isolated from human eyes were classified by the Baird-Parker system of 1966. The staphylococci belonged to subgroups II, III, IV, and V with one strain each in subgroups III and IV. However, when biotyped by Baird-Parker’s system of 1974, less than 50% (23/49) of strains were typable and they belonged to biotypes 1 and 2. With our proposed modified classification all strains could be classified into biotypes 1, 2, and 3. All the 26 strains of micrococci belonged to subgroup VII.

Staphylococci isolated from diverse sources produced, qualitatively and quantitatively, different corneal pathology in rabbits when compared to those isolated from healthy conjunctiva. This bore no relation to the present system of subtyping based on biochemical reactions. Micrococci were incapable of producing experimental corneal lesions in the rabbit.

Coagulase-negative staphylococci and micrococci are being increasingly accepted as human pathogens.1 In ocular infections, Staphylococcus epidermidis has been recognised as a pathogen in corneal ulcers2 and several postoperative infections including endophthalmitis.3-5 It has also been held to be responsible for cases of blepharoconjunctivitis,6 and it is known to be pathogenic for the eye of the rabbit.6,7 Similar information concerning micrococci is lacking. The purpose of this communication is to biotype and to establish the ocular pathogenicity in the rabbit of a collection of staphylococci and micrococci from the human eye.

Material and methods

Material was collected and processed as previously described.8 Preoperative conjunctival swabs were collected from patients who had come mostly for cataract extraction. From corneal ulcer cases swabs were invariably collected from the ulcerated area. Postoperative infections included surgical wounds, endophthalmitis, and infections of corneal grafts, uvea, and socket.

Swabs were streaked immediately on sheep blood agar medium. Strains were classified according to Baird-Parker,9,10 carbohydrate reactions being observed on solid media in Petri dishes. Phosphatase production was determined by both the Petri-dish and tube methods.11 Acetoin production was tested using Barrit’s method, and the sensitivity of these organisms to penicillin, tetracycline, chloramphenicol, streptomycin, erythromycin, framycetin, gentamicin, cloxacillin, and novobiocin was tested by the disc diffusion technique. The results were interpreted as recommended by Bauer et al.12

EXPERIMENTAL STUDY

Twenty rabbits, in four groups, were inoculated with strains of subgroup V staphylococci and micrococci. Group 1, consisting of six rabbits, were given intraocular injections of Staph. epidermidis isolated from healthy conjunctival sacs. Three strains were tested, two eyes per strain, and the remaining six eyes were controls. Groups 2 and 3 also comprised six rabbits each. In group 2, three strains of Staph. epidermidis isolated from cases of corneal ulcer were tested, and in group 3, three strains isolated from postoperative endophthalmitis, suture abscess, and uveitis were used. In group 4, three strains of micrococci, one each from a normal sac, a corneal ulcer, and a case of endophthalmitis, were studied on three eyes of two rabbits. The fourth eye was kept as a control, sterile saline being injected intracorneally. Staphylococci and micrococci, suspended in saline containing approximately 5 x 10^12 and 11 x 10^12 viable organisms/ml, were injected in doses of 0.01-0.02 ml.

Ocular examination was carried out every 24
hours, and 50% of the eyes were enucleated immediately after regression of the lesions. The corneas were removed aseptically. One half was cultured, and the other half was examined histologically after staining with haematoxylin and eosin.

Results

Of 49 strains of catalase-positive, coagulase-negative staphylococci, 25 (51.0%) belonged to subgroup V, 22 (44.9%) to subgroup II, and one strain each to subgroups III and IV of Baird-Parker.\(^9\) In corneal ulcers, subgroup II strains (9/11) predominated, whereas in postoperative infections subgroup V strains (16/24) were more numerous. Nearly 50% of strains could not be biotyped by the latest classification proposed by Baird-Parker.\(^10\) Most of our strains produced acid aerobically from lactose and maltose, but all the strains were classified into types 1, 2, and 3 if these two tests were ignored (Table 1). The proposed modified scheme for biotyping is as follows:

<table>
<thead>
<tr>
<th>Clinical category</th>
<th>S. epidermidis biotype I</th>
<th>S. epidermidis biotype II</th>
<th>S. epidermidis biotype III</th>
<th>S. epidermidis biotype IV</th>
<th>S. epidermidis biotype V</th>
<th>S. epidermidis biotype VI</th>
<th>Modified classification biotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative ulcer</td>
<td>6</td>
<td>9</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Postoperative infection</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Contracted socket</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Postoperative uveitis</td>
<td>2</td>
<td>1</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>1</td>
<td>25</td>
<td>22*</td>
<td>1</td>
<td>26</td>
<td>2</td>
</tr>
</tbody>
</table>

*Remaining 26 did not fit into classical biotypes.

Correlation of clinical categories with different types of Staph. epidermidis is shown in Table 1. Most strains were sensitive to gentamicin (26) followed by framycetin (25), chloramphenicol (19), erythromycin (18), streptomycin (12), and tetracycline (8). Fewest strains (7) were sensitive to penicillin. The resistant strains were sensitive to cloxacillin. Antibiotic sensitivities did not appear to be related in any way to particular staphylococcal subgroups or to any clinical category. Of 29 strains tested for sensitivity to 30 μg of novobiocin, five were resistant. All 26 strains of micrococci belonged to type VII (Table 2). Most of them were sensitive to gentamicin, tetracycline, and erythromycin (25) followed by chloramphenicol (23), framycetin (19), and streptomycin (15). Only five were sensitive to penicillin. Of 25 strains tested for sensitivity to 30 μg of novobiocin, only one strain was resistant. Sensitivity patterns were not related to clinical categories.

EXPERIMENTAL STUDY

In group 1, all three strains produced superficial central corneal ulcers by the third day (Fig. 1), which healed with a leucomatous opacity in 15 days. In tissue sections, epithelium was deficient in the central part of the cornea, and only the anterior stroma was diffusely infiltrated (Fig. 2). In group 2, the pathology involved deeper layers of the cornea,
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Fig. 1 Rabbit eye showing a small central corneal ulcer six days after intracorneal injection of Staph. epidermidis (group 1).

leading to intracorneal abscess formation with hypopyon (Fig. 3). The lesions healed in 27 days with vascularisation. Histopathology revealed involvement of the anterior and deeper layers of the cornea (Fig. 4). In group 3, the strains produced diffuse involvement of the cornea. Posterior corneal abscesses and profuse vascularisation were prominent features (Fig. 5). Histologically, the anterior layers were less involved than the posterior cornea. The retrocorneal region showed an acute inflammatory exudate (Fig. 6). Cultured corneal buttons yielded a pure growth of Staph. epidermidis of the same subtypes. In group 4, the strains failed to produce any corneal pathology.

Control eyes became clear within 24 hours.

Fig. 2 Corneal button showing superficial ulceration, epithelium being deficient in the centre. Anterior stroma showing diffuse infiltration with acute inflammatory cells. Deeper layers are relatively unaffected. Haematoxylin and eosin x 80.

Discussion

In the year 1976-77, of 88 strains of coagulase-negative staphylococci typed, 45 were subgroup II, 27 subgroup V, and the remaining 16 subgroup IV. Of 49 such strains typed in the present study, subgroup V strains are more numerous than subgroup II. Similarly, postoperative infections (16) were mainly due to subgroup V compared with seven caused by subgroup II strains, whereas in the previous study postoperative infections were more commonly due to subgroup II strains. However, those isolated from corneal ulcers were subgroup II, in contrast to the previous finding of subgroup V strains. This is because the phosphatase test was done by the tube method in this study. With the Petri-dish method used in the earlier study, a sizeable number of strains produced doubtful results. In the present study also, the Petri-dish method produced
false results in 10 strains (20%) when verified by the tube method. The importance of this test was further appreciated when 26 strains, which could not be typed by the recent classification of Baird-Parker,10 were typable if acid production from lactose and maltose was ignored, as shown by the proposed classification. We, therefore, recommend that the phosphatase test should be done by the tube method when classifying staphylococci or micrococci. The present study shows the importance of hospital strains of subgroup V/3 biotype in postoperative infection though they may also be produced by the preoperative flora.

Resistance to penicillin has dropped from 92% in our previous study to 86%. There are certain other differences. The number of strains resistant to gentamicin and framycetin has risen from 7·0% and 11·0% to 47·0% and 49·0%. There have been marginal increases in resistance to other antibiotics (Table 3). Micrococci showed a similar pattern of resistance to penicillin (80·8%), but only 4% strains were resistant to gentamicin, erythromycin, and tetracycline. Mitchell13 tested 30 strains of micrococci from the urinary tract and found all of them resistant to penicillin, while Corse and Williams14 reported 28% of their collection to be resistant. All our strains belonged to subgroup VII, and the source was the human conjunctival sac. As in the case of staphylococci, the resistance patterns of micrococci do not seem to be related to the type of lesion.

It is interesting to note that strains belonging to the same biotype produced varying pathology in the rabbit cornea. Those recovered from normal eyes produced minimal superficial epithelial changes. Those from corneal ulcers produced deeper involvement, and those from postoperative infections...
Table 3 Antibiotic resistance patterns (%) of staphylococci and micrococci isolated from ocular tissues

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Staphylococci</th>
<th>Micrococci</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>85.7</td>
<td>88.8</td>
</tr>
<tr>
<td>(92.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>61.0</td>
<td>11.5</td>
</tr>
<tr>
<td>(75.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>47.0</td>
<td>4.0</td>
</tr>
<tr>
<td>(7.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Framycetin</td>
<td>49.0</td>
<td>27.0</td>
</tr>
<tr>
<td>(11.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>63.3</td>
<td>4.0</td>
</tr>
<tr>
<td>(59.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptomycin</td>
<td>75.5</td>
<td>42.0</td>
</tr>
<tr>
<td>(62.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>84.0</td>
<td>4.0</td>
</tr>
<tr>
<td>(83.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figures in parentheses denote percentages in our previous study.6

Fig. 7 A correlation of the histopathological picture obtained in the different groups.

produced diffuse involvement of the cornea in the form of posterior corneal abscess (Fig. 7). It would be interesting to see if strains of subgroup II/biotype I recovered from different conditions would show similar results. This is necessary before hypothesising that the present classification of staphylococci based on certain biochemical features is inadequate. Valenton and Okumoto6 reported that strains from subjects without disease were not dermonecrotic in rabbits, while those isolated from patients with disease were. The studies of our clinical colleagues have shown that corneal ulcers or postoperative infections due to Staph. epidermidis responded to the timely application of appropriate antibiotics.15 In a number of cases, visual acuity was restored.

Micrococci failed to produce disease in rabbits after the intracorneal injection of almost double the number of organisms that produced staphylococcal lesions.

References


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