

## Comparative bacteriology of chronic blepharitis\*

JOEL M. DOUGHERTY AND JAMES P. McCULLEY

From the Department of Ophthalmology, University of Texas Southwestern Medical School, USA

**SUMMARY** One hundred and fifteen patients with chronic blepharitis were compared with 47 normal controls. Six clinically distinct groups of blepharitis were observed: staphylococcal; seborrhoeic, alone, with associated staphylococcal superinfection, meibomian seborrhoea, or secondary inflammation of the meibomian glands; and meibomian keratoconjunctivitis (MKC). *Staphylococcus aureus* was isolated in appreciable frequency from the staphylococcal and the mixed staphylococcal/seborrhoeic groups in contrast to the normal and non-staphylococcal groups. Coagulase-negative *Staphylococcus* spp., *Propionibacterium acnes*, and cornyneform bacteria were the most commonly isolated bacteria from the lid for all groups. Cultures of material expressed from the meibomian glands yielded similar organisms but in reduced frequency. Testing of antibiotic susceptibility revealed *Staph aureus* to be, usually sensitive to most commonly used ophthalmic antimicrobials except sulphonamides.

Chronic blepharitis is a common and often difficult problem for both the physician and the patient. Much confusion has surrounded its aetiology, which often renders treatment futile or frustrating at best. Three types of chronic blepharitis have been generally recognised: staphylococcal, seborrhoeic, and a mixture of both types. Much emphasis has been, and still is, placed on the role of *Staphylococcus aureus* as an aetiological agent. Staphylococcal infection of the lids alone or in combination with seborrhoeic dermatitis has been considered the commonest cause of blepharitis.<sup>1</sup> Conjunctivitis in these patients has been attributed to direct staphylococcal infection,<sup>2</sup> allergic response,<sup>3</sup> or reaction to exotoxin.<sup>2,4,5</sup> Superficial punctate corneal epitheliopathy, when present, has been attributed largely to staphylococcal exotoxin.<sup>5</sup> In meibomianitis it was believed that *Staph. aureus*, although not culturable, was present deep within the meibomian glands and hence inaccessible to the microbiologists' probings.<sup>6,7</sup> In fact *Staph. aureus* cannot be cultured from many cases of clinical staphylococcal blepharoconjunctivitis, and it has been proposed that toxin-producing strains of coagulase-negative *Staphylococcus* spp. might be the primary culprits when *Staph. aureus* cannot be found.<sup>8</sup> Indeed the ability of *Staph. epidermidis* to cause

human infection has been reported, but not in blepharitis.<sup>9-11</sup> *Propionibacterium acnes* has been the focus of attention from time to time for dermatologists involved in acne research.<sup>12,13</sup> Although it appears that *P. acnes* represents one of the main components of normal skin and ocular flora,<sup>14</sup> the role of this organism in the chronic blepharitides has not been thoroughly studied.

In general good scientific data are lacking to support a pathogenic role of any single organism in all types of blepharitis. Much of the supporting evidence for the present views of blepharitis was obtained in the 1930s and 1940s. More recent clinical impressions have raised serious doubts about their continued validity.

The objective of this study was to delineate thoroughly and evaluate the lid and conjunctival flora in patients with chronic blepharitis and to compare the results with those from an age- and sex-matched population of normal individuals free of lid disease. We specifically wanted to study the role of *Staph. aureus* and *P. acnes* as it relates to the aetiology of chronic blepharitis. We have previously reported some results of a preliminary bacteriological survey.<sup>15</sup> We have greatly expanded this series and report here the detailed findings of the entire study.

### Materials and methods

#### SELECTION OF PATIENTS

Patients with chronic blepharitis were given a careful

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Correspondence to Joel M. Dougherty, Department of Ophthalmology, University of Texas Health Center Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75235, USA.

ophthalmic and dermatological examination. They had symptoms for at least six months and had received no therapy for at least two weeks. Signs and symptoms were graded and recorded as previously described.<sup>15 16</sup> Normal persons were matched for age and sex to the patient groups, were examined in the same manner as the patients, and were selected only after it was determined that they were free of ocular disease. Samples were obtained from all individuals as outlined in the following section.

#### BACTERIOLOGICAL EVALUATION

Specimens from all patients and controls were cultured by standard techniques and before the instillation of topical anaesthetics. Calcium alginate swabs moistened with brain-heart infusion broth (Baltimore Biological Laboratories, BBL) were passed along both the superior and inferior lash line for lid cultures, and the inferior cul-de-sac for conjunctival culture. This was done bilaterally, providing four cultures per individual. Meibomian secretions were collected from 21 patients and 14 normal controls to provide a fifth culture. The lid margin was wiped with a sterile swab, and the secretions were collected with a sterile platinum spatula after the glands had been expressed by compressing the lid between a sterile lid conformer and a sterile swab.

Each swab was rolled on to a brucella agar (BBL) plate supplemented with a 5% sheep blood, and a chocolate agar plate for aerobic culture. Each swab was also rolled on to another brucella blood agar plate supplemented with yeast extract, vitamin K, and haemin for anaerobic culture. The swab was then broken off into a tube of thioglycollate 135C (BBL) supplemented with vitamin K, haemin, and 0.1% Tween 80. Meibomian expressions were cultured in a similar fashion except that the spatula was used to inoculate the thioglycollate broth after plate inoculation.

The plates were immediately streaked for isolation and the anaerobic cultures were placed into GasPak Jars (BBL). All cultures were incubated at 35°C. Aerobic cultures were incubated under 5% carbon dioxide and were examined at two, five, and seven days. Anaerobic cultures were examined after seven days' incubation. Thioglycollate broths were incubated for seven days and then subcultured to dual sets of brucella blood agar plates for aerobic and anaerobic incubation as described above. The relative quantity and morphology of each different type of aerobic colony was recorded. Each isolate was then subcultured on to brucella blood agar or chocolate agar for use in biochemical testing for identification and antimicrobial susceptibility testing. The relative quantity and morphology of each anaerobic isolate was also recorded, and each isolate was subcultured

on to chopped meat glucose (Scott) and supplemented thioglycollate 135C for use in further testing as described for aerobes. Subculture to tubed anaerobic media was facilitated by the use of a VPI anaerobic inoculator and anaerobic grade carbon dioxide.

In general, attempts were made to identify all different colony types to genus (and if possible species) level by means of standard media, reagents, and methods as established by the American Society for Microbiology.<sup>17</sup> Specifically, use was also made of the Minitek miniaturised identification system (BBL) for identification of fermenting and non-fermenting Gram-negative rods, and anaerobes (in conjunction with gas-liquid chromatography of metabolic end products).

We determined the sensitivities to commonly given ophthalmic antibiotics and sulphonamides for all rapidly growing aerobic isolates by the Barry agar overlay method,<sup>18</sup> using commercially available antimicrobial discs and Mueller Hinton Agar (BBL). We used the broth disc method of Wilkins and Thiel<sup>19</sup> for *P. acnes* (the only anaerobic isolate used in susceptibility testing), using commercially available discs and prerduced anaerobically sterilised brain-heart infusion broth (Scott).

#### Results

##### PATIENT CLASSIFICATION

Patients could be grouped into one of six distinct groups based on clinical evaluation alone (Table 1) as described in detail elsewhere.<sup>15</sup> Staphylococcal blepharitis was characterised by relatively inflamed lids, with collarettes and signs and symptoms that waxed and waned. Seborrhoeic blepharitis was characterised by minimal to moderate inflammation with oily crusting, and tended to be chronic. Mixed staphylococcal/seborrhoeic blepharitis was characterised by relatively inflamed lids, with crusting of mixed characteristics, and tended to be chronic with significant exacerbations. Meibomian seborrhoea was characterised by excessive secretions with few other clinical signs. Secondary meibomianitis showed characteristics of seborrhoeic blepharitis with spotty inflammation of the meibomian glands. Primary meibomianitis (MKC) was characterised by inflammation centered round all the meibomian glands,

Table 1 Clinical classification of chronic blepharitis

Staphylococcal
Seborrhoeic
alone
with associated staphylococcal superinfection
with meibomian seborrhoea
with spotty meibomianitis
Primary meibomianitis (MKC)

with concomitant gland plugging, and was generally associated with keratoconjunctivitis. MKC was frequently associated with acne rosacea and seborrhoeic dermatitis. Seborrhoeic blepharitis of all types was associated with seborrhoeic dermatitis. The staphylococcal group was normal dermatologically.

#### BACTERIOLOGY

Bacterial cultures were obtained on all normal controls and 90 patients with chronic blepharitis. Table 2 lists the frequency of the most commonly isolated organisms and *Staph. aureus*. Coagulase-negative *Staphylococcus* spp., lipophilic *Corynebacterium* spp., and *Propionibacterium acnes* were the most frequently isolated organisms from all individuals.

The frequency of isolation from the conjunctivae was reduced, but the relative distribution of organisms among the blepharitis groups and normal persons was similar to that for the lids. No significant differences in the frequency of isolation of these organisms were observed among the groups of patients and the normal persons. *Staph. aureus* was isolated in appreciably higher frequency from the staphylococcal group and significantly higher frequency ( $p < 0.01$ ) from the mixed staphylococcal/seborrhoeic group than from the other groups and normal persons.

Other aerobic organisms isolated relatively infrequently included *Streptococcus* spp., *Micrococcus* spp., *Neisseria* spp., *Branhamella* sp., non-lipophilic *Corynebacterium* spp., *Bacillus* spp., and Gram-negative fermentative and non-fermentative rods. (*Proteus* spp. were the most frequently isolated Gram-negative fermentative rods.) No significant differences were observed among the groups.

*P. acnes* was the most frequently isolated anaerobe

from all groups and normal persons. Other less frequently isolated anaerobes included other *Propionibacterium* spp., *Peptococcus* spp. and *Peptostreptococcus* spp. *Veillonella* sp., *Fusobacterium* spp., *Bacteroides* spp., *Eubacterium* spp., *Lactobacillus* spp., and *Clostridium* spp. were isolated very infrequently. No significant differences were observed among groups.

Meibomian expression cultures yielded flora similar to the lids but in markedly reduced frequency. Coagulase-negative staphylococci were the predominant aerobes and *P. acnes* was the predominant anaerobe. Overall 48% of meibomian expression cultures were sterile, and positive cultures reflected the lid flora. In no case was an organism isolated from meibomian expressions that was not also isolated from the lids of the same individual. No significant differences among groups of patients and normal persons were observed.

As would be expected, lid cultures were less frequently sterile than conjunctival cultures for all groups and normal persons. This pattern was consistent among all groups and normal persons, with no significant differences being observed. The same pattern was also true for anaerobic cultures. The use of thioglycollate broth improved the overall recovery rate in aerobic conjunctival cultures by 50%. In general the conjunctival flora mimicked the lid flora in each individual.

The results of antimicrobial susceptibility testing are given in Table 3. *Staph. aureus* showed a relatively high frequency of resistance to sulphonamides but low frequency of resistance to tetracycline, erythromycin, and bacitracin. Uniform sensitivity was observed to neomycin, gentamicin, and chloramphenicol. This pattern did not vary significantly among isolates from different groups or normal

Table 2 Frequency of most commonly isolated lid bacteria (including *Staph aureus*)\*

	Normal	Staph.	Seborrhoeic	Mixed staph./ seborrhoeic	Meibomian seborrhoeic	Secondary meibomianitis	MKC
<i>Staph. aureus</i>	4/47†	8/20	1/14	8/11	2/14	3/13	1/13
<i>Staphylococcus</i> spp.	45/47	19/20	13/14	11/11	14/14	13/13	13/13
<i>Corynebacterium</i> spp.	30/47	7/20	4/14	3/11	9/14	9/13	9/13
<i>P. acnes</i>	41/47	18/20	14/14	11/11	13/14	13/13	11/13

\*For other organisms cultured from lids and conjunctivae in reduced frequency with non-significant differences between groups see text.

†Numbers indicate patients with organism/patients cultured.

Table 3 Antimicrobial susceptibilities of *Staph. aureus* and coagulase-negative staphylococci

	Te	E	Sulpha	B	N	GM	C
<i>Staph. aureus</i>	39/42*	41/42	21/42	40/42	42/42	42/42	42/42
<i>Staphylococcus</i> spp.	519/773	685/773	228/773	743/773	745/773	773/773	764/773

Te=tetracycline; E=erythromycin; sulpha=sulphonamides; B=bacitracin; N=neomycin; GM=gentamicin; C=chloramphenicol.

\*Numbers indicate sensitive isolates/total isolates.

persons. Coagulase-negative *Staphylococcus* spp. showed a similar pattern of resistance. Overall, 70% of the isolates were resistant to sulphonamides, 33% to tetracycline, 11% to erythromycin, and general sensitivity was noted for other antibiotics. The only significant difference was observed in the MKC staphylococcal isolates. 75% of these isolates were resistant to tetracycline ( $p < 0.05$ ). Almost nine out of every 10 persons had one staphylococcal isolate resistant to sulphonamides, and six out of every 10 had an isolate resistant to tetracycline. Multiple antibiotic resistance (resistance to two or more antibiotics) was observed in 25% of the staphylococcal isolates from normal persons and patients. However, the resistance patterns (antibiograms) were similar among the blepharitis groups and normal persons. *P. acnes* was routinely sensitive to tetracycline, erythromycin, and chloramphenicol—the only antibiotics tested against these isolates.

### Discussion

Several significant findings have emerged from this study. First it does not appear that blepharitis can be categorised so simplistically as it has been in the past. We have found that at least six distinct entities exist that can be clinically distinguished. *Staph. aureus* does not seem to be a contributing factor in any but two of these classes—staphylococcal and mixed staphylococcal/seborrhoeic blepharitis. In all the other groups there is a striking absence of a single bacterial species which could be considered a strict pathogen. Even in the most severe cases of MKC, the frequency of isolation of *Staph. aureus* from the lids, conjunctivae, and the meibomian glands was less than, or equal to, the frequency of isolation in normal individuals. Half of all *Staph. aureus* isolates are resistant to sulphonamides *in vitro*. There have been several reports of sulphonamide-resistant *Staph. aureus* in the past decade,<sup>20–22</sup> and in this organism it is not new. As early as 1941 it was shown that sulphonamides might be ineffective in the control of staphylococcal conjunctivitis.<sup>23</sup> Many other early clinical trials reported high rates of success with these drugs, but often adequate controls were not used, sulphonamide preparations (and vehicles) varied considerably among investigators, and *in-vitro* susceptibility testing of the causative organisms was rarely done.<sup>24–26</sup> In fact it was not until the 1960s that a reliable and satisfactory method for *in-vitro* testing of sulphonamide sensitivity was developed.<sup>27</sup> In view of this frequent resistance it would appear that sulphonamides should not be considered the drug of choice in treating *Staph. aureus*-induced blepharitis.

The types of bacteria isolated from all the groups of

patients and normal persons are similar to those isolated in other studies of the normal and infected conjunctiva.<sup>28–31</sup> However, the overall frequency of isolation is higher in this study. Only 2% of all lid cultures and 19% of all conjunctival cultures were sterile. Specifically, coagulase-negative staphylococci, *P. acnes*, and coryneform organisms were isolated in much higher frequency than in previous studies. Our higher frequency of isolation and lower number of sterile cultures is probably a result of the manner in which specimens were processed: direct inoculation of plated media, immediate streaking for isolation and incubation, the use of several enriched media, and the use of thioglycollate broth. A 50% improvement in the recovery rate of aerobic conjunctival cultures underscores the utility of using a liquid back-up system.

It is questionable whether coagulase-negative *Staphylococcus* spp. play a significant role in blepharitis. At least the role is not obvious, since these species are isolated in such high frequency from all groups and normal persons. Several different colony types of coagulase-negative staphylococci were isolated from each individual. No significant differences in proportions of the different colony types were seen among the groups of patients or normal persons, but we did not attempt to speciate the isolates further. However, we did categorise the isolates based on antibiograms in an effort to observe whether certain biotypes were more frequently associated with one group of blepharitis or another. Except for a high frequency of tetracycline resistance in the MKC isolates no significant differences were observed. The significance of tetracycline resistance is not clear. It might possibly represent a response to prolonged treatment, or it might perhaps be truly reflective of a subpopulation of staphylococci unique to this group. When we compared the percentage of multiple resistant staphylococcal isolates among the groups, we did not observe any significant patterns.

*P. acnes* apparently is not a primary etiological agent in chronic blepharitis, since it, like the staphylococci, appears to be a part of the normal flora. Whether there exist subpopulations of *P. acnes* that predominantly colonise one particular blepharitis group or another could not be determined from this study. Different variants (colonial morphology) are distributed among all groups and normals. The same could also be said for coryneform organisms, which, while not isolated in as great a frequency as the staphylococci and *P. acnes*, were present in a majority of individuals without favouring any particular group.

In the light of this study it does not appear that bacteria have a primary role in the pathogenesis of most chronic blepharitides (except staphylococcal and mixed staphylococcal/seborrhoeic). We do not,

however, rule out a secondary role given some predisposing factor such as an abnormality of the meibomian secretion or a generalised sebaceous malfunction. In any event, *Staph. aureus* is not the last word in chronic blepharitis, and it may be prudent to think again about this common irritating disease.

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