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Methicillin resistance of bacteria isolated from vitreous fluid from patients undergoing vitrectomy

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PURPOSE. *The aim of this study was to compare the resistance patterns of bacteria in vitreous fluid from patients undergoing vitrectomy for diagnostic reasons, with bacteria of other nosocomial infections.*

METHODS. *Vitreous fluid samples (n=144) were obtained from 133 patients undergoing vitrectomy for endophthalmitis, and 11 for uveitis as suspected endophthalmitis. They were Gram stained and cultured. Antibiotic susceptibility tests were run on all isolates.*

RESULTS. *Gram stains were positive in 45/144 cases (31%), among which 38/45 (84%) were confirmed by a positive culture. Cultures were positive in 74/144 patients (51%) with mainly coagulase-negative* staphylococci *(n = 44) and* Staphylococcus aureus *(n = 13). In 133 patients endophthalmitis occurred after lens implantation (80 cases) and in 53 cases there was another origin (e.g. corneal transplantation, endogenous). In 26/ 80 post-lens implantation infections, culture remained negative; 32 infections occurred with coagulase-negative* staphylococci*, 10 with* Staphylococcus aureus*, 9 with* streptococci *and 3 with Gram-negative bacteria. For endophthalmitis, ophthalmologists in our institution give an intraocular injection of vancomycin and ceftazidim after vitrectomy. Among the 44 isolates of coagulase-negative* staphylococci*, 12 (27%) were resistant to methicillin. This is in contrast to other hospital-related coagulase-negative* staphylococcus *infections in general, and the resistance rate is 75% in our hospital. Only 2/13* Staphylococcus aureus *isolates were methicillin-resistant.*

CONCLUSIONS. *We conclude that isolates of coagulase-negative* staphylococci *from vitreous fluid are less resistant to methicillin than those isolated in other nosocomial infections. (Eur J Ophthalmol 2001; 11: 160-5)*

KEY WORDS. *Coagulase-negative* staphylococcus*, Endophthalmitis, Methicillin resistance, Lens implantation*

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INTRODUCTION

Coagulase-negative *staphylococci* have become the most common cause of endophthalmitis after ocular surgery (1). These micro-organisms sometimes complicate the outcome of glaucoma surgery and vitrectomy, but they mainly threaten cataract surgery, especially extracapsular extraction with artificial lens implantation (2-4). A previous study in our ophthalmology department found that coagulase-negative *staphylococci* were implicated in 66% of the endophthalmitis cases after extracapsular lens extraction with implant (5). It has since been reported that resistance of the infecting isolates to multiple antibiotics may further complicate therapy (6,7). Our current strategy for the treatment of endophthalmitis is

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vitrectomy with intravitreal injection of antibiotics covering Gram-positive and Gram-negative bacteria (8).

Vitrectomy in these cases serves a therapeutic goal on the one hand: the infecting micro-organisms are removed surgically, and a cavity is created for the antibiotics (vancomycin and ceftazidim) which are injected at the end of the operation. In addition this strategy provides the microbiology laboratory with a diagnostic specimen. The aim of our study was the isolation, identification and antibiotic susceptibility testing of bacteria from vitreous fluid samples from patients undergoing vitrectomy for endophthalmitis. We focussed in particular on the pattern of resistance of the isolates to antibiotics.

MATERIALS AND METHODS

Patients and samples. Between January 1995 and March 1999 in our Department of Ophthalmology 144 vitrectomies were performed on patients with endophthalmitis (133/144) and suspected endophthalmitis (chronic uveitis no longer responding to steroid or antimitotic treatment) (11/144): 100 cases were post-surgery, 18 were post-traumatic and 15 were endogenous. Vitrectomy is considered in postoperative cases of endophthalmitis in patients with pain, visual loss, hypopion, invisible fundus and visual acuity of finger counting or less. These criteria are somewhat broader then those of the EVS study, where light perception is set as the upper limit (9).

Vitrectomy has three purposes: provision of a good specimen for direct bacteriological examination and culture, removal of toxins and cells and creation of a space for the injection of antibiotics. When uveitis is involved, only some of the chronic cases, resistant to therapy – for instance corticoids or antimitotics – need vitrectomy. The study population consisted of 53% men and 47% women, mean age 64 years (range 24-93). All vitreous fluid samples were undiluted, taken at the beginning of the operation and included the posterior capsule. The samples were sent to the laboratory for bacteriological examination.

Microbiological isolation and identification

Gram-stained smears were used for direct microscopic examination of micro-organisms and any leukocytes in the vitreous fluid.

In accordance with the NCCLS criteria for culture technique, the specimens were plated on different nutrient agar plates, and incubated in aerobic or anaerobic atmospheres at 37°C for 24h (unless mentioned otherwise). Tryptich Soy Agar with 5% sheep blood (5% CO_2) allowed the growth of the majority of the bacteria. GCM chocolate agar (5% CO_2) was used for the growth of *Haemophilus* and other fastidious organisms. Anaerobic cultures were done using thioglycolate broth (under paraffin), amikacin blood agar and Schaedler agar with Vit K1 and 5% sheep blood (both media in anaerobic jar for 48h). Bacteria were identified with the API system (Biomérieux, France).

Antibiotic susceptibility

The Kirby-Bauer disc diffusion method was used to test the susceptibility of the isolates, in accordance with NCCLS criteria.

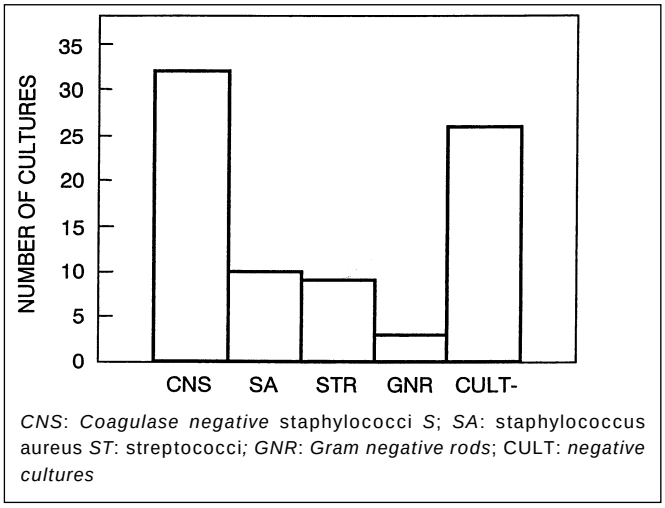
The following antibiotics were tested for *staphylococcus* isolates: ampicillin, methicillin, sulfamethoxazol + trimethoprim, doxycyclin, fucidic acid, vancomycin, rifamicin, gentamicin and quinolones.

RESULTS

Gram stains showed polymorphonuclear leukocytes and micro-organisms in 45/144 (31%) endophthalmitis cases, among which 38/45 (84%) were confirmed by a positive culture. Cultures were positive in 74/144 patients (51%) and the following bacteria were isolated: 44 coagulase-negative *staphylococcus*, 13 *Staphylococcus aureus*, 4 *Streptococcus pneumoniae*, 4 *Enterococcus*, 3 *Proteus vulgaris*, 3 *Propionibacterium species* and 1 *Haemophilus influenzae*.

Culture was positive in 3/11 patients with uveitis (27%). Isolates were identified as 1 coagulase-negative staphylococcus, 1 *Proteus vulgaris* and 1 *Propionibacterium species*.

In 133 patients with endophthalmitis, the infection was a result of lens implantation in 80 (60%) and a variety of other etiologies in 53 cases (40%) (post-operative – e.g. corneal transplantation – in another 15%, post-traumatic in 13% and endogenous in 12%). Only 3/15 endogenous endophthalmitis cases had a positive culture. In the 80 post-lens implantation in-



CNS: *Coagulase negative* staphylococci *S*; *SA*: staphylococcus aureus *ST*: streptococci*; GNR*: *Gram negative rods*; CULT: *negative cultures*

Fig. 1 - *Bacteria isolated from vitrectomy samples after lens implantation. Culture results from 80 endophthalmitis cases revealed the following etiologic agents: coagulase-negative* staphylococcus *(CNS),* Staphylococcus aureus *(SA),* Streptococcus pneumoniae *(STR) and Gram-negative rods (GNR). In 33% of the cases culture remained negative (CULT-).*

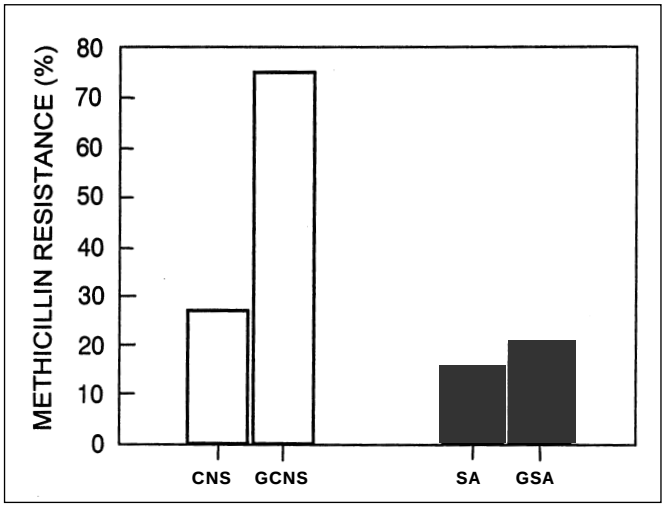


Fig. 2 - *Methicillin resistance of* staphylococci *isolated from vitrectomy samples from vitrectomy patients. The percentage of resistant coagulase-negative* staphylococci *(CNS) and* Staphylococcus aureus *(SA) is compared with the general percentage of resistant CNS (GCNS) and SA (GSA) in our hospital.*

fections, culture remained negative in 26 (33%) cases-culture was positive in 54 (47%). Isolates were identified as coagulase-negative *staphylococcus* in 32 cases (40%), *Staphylococcus aureus* in 10 (13%), *Streptococcus* in 9 (11%) and Gram-negative rods in 3 (4%) (Fig. 1).

Seventy-two percent (32/44) of the coagulase-negative *staphylococcus endophthalmitis* infections occurred after lens implantation.

Table I shows the antibiotic resistance rate of the *staphylococcus* isolates. Among the 44 isolates of coagulase-negative *staphylococci* only 12 (27%) were resistant to methicillin, in contrast to other hospital-related coagulase-negative *staphylococcus* infections where the resistance rate in our hospital is 75%. Two of 13 *Staphylococcus aureus* isolates were resistant to methicillin (Fig. 2).

In comparison with previous findings in our hospital (10), we found that about 40/80 (50%) of eyes with endophthalmitis after lens implantation are functionally lost. The best functional results in post-lens implantation infections are obtained when coagulase-negative *staphylococci* are involved (25/32 cases; 78%).

TABLE I - ANTIBIOTIC SUSCEPTIBILITY OF *STAPHYLOCOCCI* IN VITREOUS FLUID

	Coagulase-negative *staphylococcus*	***Staphylococcus aureus***
Antibiotics tested	n=44	n=13
Ampicillin	36 (81%) R	8 (61%) R
Methicillin	9 (20%) R	2 (15%) R
Sulfamethoxazol+trimethoprim	12 (27%) R	0 (0%) R
Doxycyclin	4 (9%) R, 1 (2%) I	2 (15%) R
Fucidic Acid	3 (7%) R, 2(4%) I	0 (0%) R
Vancomycin	0 (0%) R	0 (0%) R
Rifamicin	4 (9%) R, 1 (2%) I	0 (0%) R
Gentamicin	6 (14%) R, 1 (2%) I	0 (0%) R
Quinolones	9 (20%) R	0 (0%) R

R: resistant, I: intermediate

DISCUSSION

Our study allowed the isolation of a variety of micro-organisms from vitreous fluid samples from patients undergoing vitrectomy for endophthalmitis. In accordance with previous studies in our (5) and other (2-4) Ophthalmology Departments, the findings confirm that coagulase-negative *staphylococci* are the most common causal agents of endophthalmitis after lens implantation: coagulase-negative *staphylococci* could be isolated in 32/80 cases. In general, infections with these germs are frequently associated with foreign bodies, such as catheters (11), prostheses (12,13), pacemakers (14), vascular grafts (15,16), hemodialysis shunts (17) and breast implants (18).

Factors produced by coagulase negative *staphylococci* that contribute to the pathogenesis of foreign body infections are being investigated. *Staphylococcus epidermidis* produces an exopolysaccharide (19) that may be involved more in persistence of the organism on the foreign body by thwarting host defenses and impairing antimicrobial killing than in initial adherence (20). Also, surface adherins have been identified that promote the initial interaction between *Staphylococcus epidermidis* and the foreign body. The best studied, PS/A, is a polysaccharide surface antigen found on almost all *Staphylococcus epidermidis* isolates from clinical specimens (21-23).

Other surface polysaccharides (24, 25), proteins (26) and a hemagglutinin (27-29) have also been associated with adherence to polymer surfaces of *Staphylococcus epidermidis* isolates recovered from infections. The clinical importance of this micro-organism/foreign body adhesion is illustrated by comparison of surgical lens extraction techniques with or without implantation, that are complicated respectively with a high and low frequency of *Staphylococcus epidermidis* infections (5).

Antibiotic susceptibility testing revealed an unexpectedly low resistance rate to methicillin and other antibiotics. Only 12/44 (27%) of the coagulase-negative *staphylococci* and 2/13 (17%) of the *Staphylococcus aureus* isolates were resistant to methicillin. This is in contrast with data on nosocomial infections with coagulase-negative *staphylococci* in general from our own hospital and from the literature (30), where resistance rates reach 75% and more than 80% respectively. For *Staphylococcus aureus* the resistance rate in our and other (31) hospitals is about 25%.

Resistance-promoting factors are plasmid-mediated conjugative resistance transfer between *Staphylococcus epidermidis* and *Staphylococcus aureus* (32, 33) and selection pressure due to widespread antibiotic use in hospitals (34, 35). These mechanisms mean that the resistance pattern of the coagulase-negative staphylococci reservoir in hospital patients and staff is different from the one in non-hospitalised patients.

The methicillin-resistance of coagulase-negative *staphylococci* and of *Staphylococcus aureus* from conjunctivae of non-hospital resident preoperative patients appears to be not higher than 0.3% and 1.3% respectively (36), although infecting organisms after cataract surgery do not always match with the previous resident conjunctival flora (37). Since endophthalmitis after lens implantation can be considered as a sequel of surgical persistence of environmental micro-organisms, our data suggest that hospital-acquired methicillin-resistant *staphylococci* are only partly responsible for these infections.

When culture shows infection with coagulase-negative *staphylococcus*, our data suggest that vancomycin can be safely replaced. In the absence of microbiological data, clinical “prediction” is not reliable enough to guide antibiotic therapy and broad-spectrum therapy is still recommended (10).

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