BACTERIAL RESISTANCE AFTER SHORT-TERM EXPOSURE TO ANTIBIOTICS

BY *Thomas O. Wood, MD, Harold Dickson, PhD* (BY INVITATION), *Vickie A. Nix* (BY INVITATION), AND *Danielle Hamilton* (BY INVITATION)

ABSTRACT

Purpose: To determine if there is a difference in antibiotic sensitivity to coagulase-negative *Staphylococcus* (CNS) cultured from the host versus the donor cornea at the time of corneal transplantation. Then to apply this knowledge to preoperative preparation of patients undergoing eye surgery.

Method: A total of 923 donor corneas stored in Optisol and 895 host corneas with no preoperative antibiotic exposure were cultured. Forty-two CNS positive cultures grew from the donor corneas and 40 from the host corneas ($P = .5$).

Results: There was an increase in resistance in the bacteria cultured from the donor compared with the host. The most striking changes occurred in host versus donor to: ciprofloxacin 27.5% ($P = .0033$); gentamicin 27% ($P = .0113$); tobramycin 31.6% ($P = .059$). The combination of polymixin, bacitracin, and neomycin (P/B/N) was significantly better than ciprofloxacin, gentamicin, and tobramycin or the combination of ciprofloxacin, gentamicin, and tobramycin (C/G/T) ($P = .0007$).

Conclusion: The combination of C/G/T exhibited the highest change to resistant bacteria. P/B/N was the most effective commercially available preparation. These results should be considered when making the decision about which preoperative antibiotic to use, if any.

Trans Am Ophthalmol Soc 2002;100:137-142

INTRODUCTION

Resistance to antibiotics following short-term exposure has been recognized since 1952, when Starr and Reynolds[1] demonstrated that bacteria in turkey intestines exhibited 100% resistance to streptomycin after 3 days of exposure to it. Gentamicin-resistant bacteria cultured from the donor is a frequent cause of endophthalmitis following corneal transplant.[2-6] In the ophthalmic literature, it has often been assumed that resistant bacteria are present prior to antibiotic treatment. The purpose of this study was to determine if there is a change in resistance patterns to coagulase-negative *Staphylococcus* (CNS) after constant exposure to antibiotics (gentamicin and streptomycin in Optisol) compared with bacteria from the host cornea that have had no preoperative antibiotic exposure.

METHODS

We performed cultures on 923 donor corneas and 895 host corneas. There were fewer host corneas because many with endothelial dystrophy and keratoconus were used in research. In the donor corneas, the time from death to preservation averaged 3.62 hours and the time from preservation to corneal transplantation averaged 2.4 days.

Surgery was begun by preparing the eye with a 5% solution of povidone-iodine. The donor cornea was then placed in a solution of vancomycin, 1 mg per milliliter of balanced salt solution (BSS), prior to transplantation.[5] The cornea from the host was also placed in the vancomycin solution. The donor cornea was safely secured in the recipient eye, and then the donor rim and host button, after about 30 minutes in the vancomycin solution, were transferred to chocolate agar transport media and taken to the laboratory. In the laboratory the corneas were ground separately and placed on blood, MacConkey, and chocolate agars, and enriched thioglyocolate broth. The cultures were read daily for 7 days. Cultures were reported as follows: *rare*, when growth occured in the first quadrant only; *few*, when growth occurred in the first and second quadrants; *several*, when growth occurred in the first three quadrants; and *many*, when there was growth on all four quadrants.

The 923 donor corneas had 42, and the host corneas 40, positive cultures for CNS or *Staphylococcus epidermidis* ($P>.05$, Tables I and II). Sensitivities were obtained to bacitracin, ciprofloxacin, gentamicin, neomycin, streptomycin, vancomycin, tobramycin, and polymyxin (Tables III and IV).

RESULTS

The number of resistant staphylococci from the donor and host corneas was compared. Resistant strains for this study included those reported as resistant and with

From the University of Tennessee, Baptist Memorial Hospital, Memphis, Tennessee.

TABLE I: BACTERIAL RESISTANCE TO *STAPHYLOCOCCUS* IN DONOR CORNEAS

DONOR CORNEAS	BACITRACIN	CIPROFLOXACIN	GENTAMICIN	NEOMYCIN	STREPTOMYCIN	TOBRAMYCIN	VANCOMYCIN	POLYMYXIN
S. epidermidis-rare	S	R	S	S	S	R	S	R
CNS- rare	S	R	R	R	S	R	S	S
CNS- rare		S						
CNS- rare	S	R		S		S	S	S
CNS- rare	S	I	R	I	S	R	S	R
CNS- rare	S	R		R		R	S	I
S. epidermidis-rare		S					S	
CNS- rare	S	S		S		S	S	R
CNS- rare	S	S	S	S	R	S	S	S
CNS- rare	S	S	S	S	S	S	S	S
CNS- rare	S	S	S	S	S	S	S	S
CNS- rare	S	S	S	S	S	S	S	I
CNS-type I rare	S	S	S	S	S	S	S	S
CNS-type II rare	S	S	S	S	S	S	S	S
CNS-type III rare	S	S	S	S	S	S	S	I
CNS-rare	I	S	S	S	S	S	S	S
CNS-rare	S	S	S	S	S	S	S	S
CNS-rare	S	S	S	S	S	S	S	I
CNS-type I rare	S	S	R	S	S	S	S	I
CNS-type II rare	S	S	S	S	S	S	S	S
CNS-rare	S	S	S	S	S	S	S	R
CNS-rare	S	S	S	S	S	S	S	I
CNS-rare	S	S	S	S	S	S	S	S
CNS-rare	S	R	R	R	S	R	S	R
CNS-rare	S	S	S	S	S	R	S	S
CNS-CFU	S	S	S	S	S	S	S	S
CNS-rare	S	S	S	S	S	S	S	S
CNS-rare	S	S	S	S	S	S	S	I
CNS-rare	R	R	I	R	R	R	S	I
CNS-rare	S	I	R	I	S	R	S	S
CNS-rare	S	R	I	I	S	R	S	I
CNS-type I rare	S	S	S	S	S	S	S	R
CNS-type II rare	R	S	S	R	S	S	S	S
CNS-rare	S	S	S	S	S	S	S	R
CNS-rare	S	R	I	R	R	R	S	I
CNS-rare	S		S	S	S	R	S	R
CNS-Rare	S	S	R	I	S	R	S	R
CNS-rare	R	S	S	S	S	S	S	R
CNS-rare	R	R	I	S	S	S	S	I
CNS-rare	S	S	S	S	S	S	S	S
CNS-rare	S	S	S	S	S			
CNS-rare			S	R	S		S	
Rim resistance/total	5R/39	11R/40	10R/37	11R/40	3R/37	12R/38	0R/40	21R/38

I, intermediate; CFU, colony forming units; R, resistant; S, sensitive; CNS-, coagulase-negative *Staphylococcus*.

intermediate sensitivity. There were no significant changes in resistance patterns in donors versus host to bacitracin ($P = .24$), neomycin ($P = 0.2$), streptomycin ($P = 0.5$), tobramycin ($P = .059$), vancomycin ($P > .05$), or the combination of polymixin, bacitracin, and neomycin (P/B/N) ($P = .5$). There was, however, a significant increase in resistance to ciprofloxacin ($P = .0033$) and gentamicin ($P = .0113$) (Table III).

Comparing resistant bacteria, without including those with intermediate sensitivities—ciprofloxacin ($P = .01$), gentamicin ($P = .07$), tobramycin ($P = .025$)—reveals that ciprofloxacin and tobramycin have significant increases in resistance and gentamicin is almost significant (Table IV). The combination of P/B/N was the most effective commercially available topical preparation; in the bacteria cultured from the donor, only 1 of 38 was resistant to all of the antibiotics. The combination of P/B/N was significantly more effective against *Staphylococcus* than ciprofloxacin ($P = .0029$), gentamicin ($P = .003$), tobramycin ($P = .003$) and polymyxin ($P = .0025$). The total resistant strains to the combination of ciprofloxacin, and tobramycin (C/G/T) (33 of 112) revealed that P/B/N (1 of 38) was significantly

TABLE II: BACTERIAL RESISTANCE TO *STAPHYLOCOCCUS* IN HOST CORNEAS

HOST CORNEAS	BACITRACIN	CIPROFLOXACIN	GENTAMICIN	NEOMYCIN	STREPTOMYCIN	TOBRAMYCIN	VANCOMYCIN	POLYMYXIN
S. epidermidis-several	S	S		S		S	S	I
CNS- few	S	S		R		R	S	I
CNS- rare							S	
CNS- rare	S	S	S	S	S	S	S	R
CNS- rare							S	
CNS- rare	S	S		S		R	S	R
CNS- rare							S	
CNS- rare	S	S		S		S	S	R
CNS- rare	R	S		S		S	S	I
CNS- rare		S					S	
CNS- rare							S	
CNS- rare		S					S	
CNS- rare	R	S		R		S	S	S
CNS- rare		S					S	
CNS- few								
CNS- rare							S	
CNS- type I rare							S	
CNS-type II rare							S	
CNS- rare	R	S	S	S	S	S	S	I
CNS- rare	S	S	S	S	S	S	S	S
CNS- rare								
CNS- rare	S	S	S	S	S	S	S	S
CNS- rare	S	S	S	S	S	S	S	S
CNS- rare	S	S	S	S	S	S	S	S
CNS- rare	R	S	S	R	S	S	S	I
CNS- rare	R	S	S	S	S	S	S	S
CNS- CFU	S	S	S	S	S	S	S	S
CNS- rare								
CNS- rare							S	
CNS- rare	I	S	S	S	S	S	S	S
CNS- rare	S	S	S	S	S	S	S	I
CNS- rare e	S	S	S	S	S	S	S	S
CNS- rare	S	S	S	S	S	S	S	S
CNS-type I rare	S	S	S	S	S	S	S	I
CNS- type II rare	S	S	S	S	S	S	S	S
CNS- rare	S	S	S	S	S	S	S	S
CNS- rare	S	S	S	S	S	S	S	S
CNS- type I rare	S	S	S	S	S	S	S	S
CNS- type II rare	S	S	S	S	S	S	S	S
CNS- rare	S	S	S	R	R	I	S	R
Host resistance/total	6R/26	0R/29	0R/20	4R/26	1R/20	3R/26	0R/37	11R/26

I, intermediate; CFU, colony forming units; R, resistant; S, sensitive ; CNS-, coagulase-negative *Staphylococcus.*

more effective (P = .0025). There was no significant difference when P/B/N was compared to bacitracin (P = .101), neomycin (P = .056), streptomycin (P = 0.29), or vancomycin (P = .49) (Table V).

DISCUSSION

The most common bacteria cultured in endophthalmitis following intraocular surgery were *S epidermidis* and other coagulase-negative staphylococci.[6,7] The bacterial flora cultured from the ocular surface is decreased following the preoperative use of topical antibiotics; there is, however, no proof that this correlates to a decreased incidence of endophthalmitis.[7,8] Fluoroquinolone-resistant *Staphylococcus* has been demonstrated in corneal wound infections following cataract surgery in eyes treated preoperatively for 1 to 3 days with ciprofloxacin.[9] Our study indicates that there is a change in resistance patterns to fluoroquinolone and aminoglycosides in CNS exposed for about 2.5 days to gentamicin and streptomycin. Interestingly, the resistance changes occurred mainly in gentamicin, ciprofloxacin, and tobramycin.

Four mechanisms may produce bacterial resistance:

- Mutation, such as gyrase gene mutation, producing quinolone resistance
- Transduction, which is transfer by bacteriophage of

TABLE III: HOST VERSUS RIM RESISTANCE

Bacitracin			Ciprofloxacin		
Host	6/26	(*P* = .24)	Host	0/29	(*P* = .0033)
Donor rim	5/39		Donor rim	11/40	
Gentamicin			Neomycin		
Host	0/20	(*P* = .0113)	Host	4/26	(*P* = .50)
Donor rim	10/37		Donor rim	11/40	
Streptomycin			Tobramycin		
Host	1/20	(*P* = .50)	Host	3/26	(*P* = .059)
Donor rim	3/37		Donor rim	12/38	
Vancomycin			Polymyxin		
Host	0/36	(*P* >.05)	Host	11/26	(*P* = .2)
Donor rim	0/40		Donor rim	21/38	
P/B/N					
Host	1/26	(*P* = .5)			
Donor rim	1/38				

P/B/N, polymixin, bacitracin, and neomycin.

DNA with resistance gene(s) from one bacterium to another (important in *Staphylococcus aureus* resistance)

- Transformation, when DNA that is free in the environment is taken up by the bacterium followed by homologous recombination (important in penicillin-resistant pneumococci)
- Conjugation, when DNA is transferred from one bacterium to another by direct contact. This mechanism is important in spreading DNA that produces multiple antibiotic resistance and is a frequent mechanism in the transfer of multiresistance genes from gram-negative to gram-positive organisms.[10]

Hoiby and colleagues[11] reported the development of resistance to ciprofloxacin in *S epidermidis* (CNS) cultured from axillary sweat, a relatively closed system. The resistant isolates occurred in a mean time of 2.7 days (range, 1-7 days). The CNS also exhibited the development of multiresistance to methicillin, erythromycin, sulfonamides, trimethoprim, and gentamicin. Cultures of specimens from the nose, a relatively open system, took an average of 18 days to develop resistant strains.

Occlusion causes rapid bacterial growth on the skin, providing an environment for increasing frequency of mutation and thereby increasing the chances of bacterial resistance. Corneal storage solutions are closed systems with no bacterial, antibiotic, or fluid turnover.

The conditions in corneal storage would be similar to the relatively closed environments of occluded skin, the axillae, and the intestinal tract.[1,11] Corneal storage solution may also be an ideal environment for transduction (phage), transformation (uptake of free DNA), and/or conjugation (DNA transfer by direct exchange) to produce resistance in CNS. This study and others, such as the 1952 report of Starr and Reynolds,[1] indicate that in a conducive environment, resistance as well as cross-resistance can develop following short-term antibiotic exposure.[1,8,9,11-13] The conjunctival cul-de-sac may provide a similar relatively closed environment. The low resistance rate in CNS cultured from the host corneas may reflect the fact that the cornea is an "open system" similar to the nose. Can this information help us decide if prophylactic antibiotics are indicated or contraindicated prior to surgery? If indicated, P/B/N appears to be the best commercially available preparation, combined with a povidone-iodine preparation.[8,12-14]

TABLE IV: RESISTANCE PATTERNS WITHOUT INCLUDING INTERMEDIATE SENSITIVITIES

Bacitracin			Ciprofloxacin		
Host	5/26	(*P* = .025)	Host	0/29	(*P* = .01)
Donor rim	4/39		Donor rim	9/40	
Gentamicin			Neomycin		
Host	0/20	(*P* = .07)	Host	4/26	(*P* = .50)
Donor rim	6/37		Donor rim	7/40	
Streptomycin			Tobramycin		
Host	1/20	(*P* = .50)	Host	2/26	(*P* = .025)
Donor rim	3/37		Donor rim	12/38	
Polymyxin			Vancomycin		
Host	4/26	(*P* = .23)	Host	0/36	(*P* >.05)
Donor rim	10/38		Donor rim	0/40	

TABLE V: COMPARISON OF P/B/N TO SINGE ANTIBIOTIC RESISTANCE TO *STAPHYLOCOCCUS* CULTURED FROM DONOR RIM

P/B/N	1/38		P/B/N	1/38	
vs		(*P* = .101)	vs		(*P* = .0029)
Bacitracin	5/39		Ciprofloxacin	11/39	
P/B/N	1/38		P/B/N	1/38	
vs		(*P* = .003)	vs		(*P* = .0561)
Gentamicin	10/35		Neomycin	11/40	
P/B/N	1/38		P/B/N	1/38	
vs		(*P* = .27)	vs		(*P* = .003)
Streptomycin	3/37		Tobramycin	12/38	
P/B/N	1/38		P/B/N	1/38	
vs		(*P* = .49)	vs		(*P* = .0025)
Vancomycin	0/40		Polymyxin	21/38	
C/G/T	33/112				
vs		(*P* = .0007)			
P/B/N	1/38				

C/G/T, ciprofloxacin, gentamicin, and tobramycin; P/B/N, polymixin, bacitracin, and neomycin.

REFERENCES

1. Starr MP, Reynolds DM. Poisonous poultry. *Sci Am* 1952 Jan:38.
2. Leveille AS, McMullan FD, Cavanaugh HD. Endophthalmitis following penetrating keratoplasty. *Ophthalmology* 1983:90(1);38-39.
3. Baer JC, Nirankari VS, Glaros DS. Streptococcal endophthalmitis from contaminated donor corneas after keratoplasty. Clinical and laboratory investigations. *Arch Ophthalmol* 1988:106(4); 517-520.
4. Poole TG, Insler BS, Insler MS. Contamination of donor cornea by gentamicin-resistant organisms. *Am J Ophthalmol* 1984:97(5);560-564.
5. Wood TO, Mohay J, McLaughlin BJ. Vancomycin in corneal transplantation. *Trans Am Ophthalmol Soc* 1993;91:391-396; discussion 396-400
6. Han DP, Wisniewski SR, Wilson LA, et al. Spectrum and susceptibilities of microbiologic isolates in the Endophthalmitis Vitrectomy Study. *Am J Ophthalmol* 1996:122(1);1-17.
7. Liesegang TJ. Perioperative antibiotic prophylaxis in cataract surgery. *Cornea* 1999;18(4):383-402.
8. Isenberg SI, Apt L, Yoshimori R, et al. Chemical preparation of the eye in ophthalmic surgery. IV. Comparison of povidone-iodine on the conjunctiva with a prophylactic antibiotic. *Arch Ophthalmol* 1985:103(9);1340-1342.
9. Cosar CB, Cohen EJ, Rapuano CJ, et al. Clear corneal wound infection after phacoemulsification. *Arch Ophthalmol* 2001:119(12);1755-1759.
10. Hardman JG, Limbird LE, Gilman AG. Antimicrobial agents buy Chambers HV. In: *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. 10th ed. 2001:1143-1170.
11. Hoiby N, Jarlov JO, Kemp M, et al. Excretion of ciprofloxacin in sweat and multiresistant *Staphylococcus epidermidis*. *Lancet* 1997;349(9046):167-169.
12. Apt L, Isenberg SJ, Yoshimori R, et al. Outpatient topical use of povidone-iodine in preparing the eye for surgery. *Ophthalmology* 1989;96(3):289-292.
13. Apt L, Isenberg SJ, Yoshimori R, et al. Chemical preparation of the eye in ophthalmic surgery. III. Effect of povidone-iodine on the conjunctiva. *Arch Ophthalmol* 1984:102(5);728-729.
14. Speaker MG, Menikoff JA. Prophylaxis of endophthalmitis with topical povidone-iodine. *Ophthalmology* 1991;98(12):1769-1775.

DISCUSSION

DR RICHARD K. FORSTER. Dr Wood and his colleagues have undertaken a study to determine if there is a change in resistance patterns of coagulase-negative staphylococci after exposure to the antibiotics gentamicin and streptomycin in Optisol corneal preservation media. They compared the sensitivity pattern of bacteria on donor rims to that of bacteria isolated from host corneas undergoing corneal transplantation. They conclude that there was a significant increase in the resistance to ciprofloxacin and gentamicin after short-term exposure to antibiotics in the storage media.

After careful analysis of the data presented, I must conclude that Dr Wood and his coauthors may have demonstrated a difference in resistance patterns, but they have not demonstrated an increase in resistance to ciprofloxacin and gentamicin. How do I come to this conclusion? Basically, the study compares two different populations of bacteria. Those derived from the donor rims in storage media have been exposed to gentamicin and streptomycin for an average of 2.4 days. Short-term exposure to these antibiotics suppressed or killed all susceptible bacterial populations. Therefore, the resistant population of bacterial isolates was selected before being exposed to the various antibiotic sensitivity studies. By contrast, the bacteria isolated from the host corneas were not exposed to antibiotics before culturing, and thus would have higher susceptibilities to the tested antibiotics.

It is somewhat surprising that there was no demonstrable resistance to ciprofloxacin and gentamicin in 28 and 20 host cornea isolates, respectively. In South Florida, records from the ocular microbiology files of the Bascom Palmer Eye Institute in 2001 demonstrated approximately 33% resistance of *S epidermidis* to gentamicin and >50% resistance to ciprofloxacin. On the other hand, it is not surprising that 11 of 40 donor rim cultures were resistant to ciprofloxacin, since multidrug resistance probably develops from the gentamicin and streptomycin in the storage media.

Finally, in order to have two comparable populations of bacteria, cultures of the cornea and ocular surface would be required of both the donor eyes before removal and placement into the preservation media, and the host cornea and ocular surface. Standardization also assumes that no betadine or topical antibiotics are applied to either the donor or host eyes before obtaining cultures. In the present study the method of quantitation is unclear. In order for resistance to develop, there is need for a sufficient pool of bacteria that are growing, and therefore both groups need to be matched for temperature, time, and standard quantitation of bacterial growth. Detecting "rare" numbers of organisms in the first quadrant of the culture plate is only qualitative or at best semiquantitative.

Further to my communication with the author, I understand that this is a preliminary study, but these comments need to be considered and addressed in any prospective protocol.

DR VERINDER S. NIRANKARI. About 15 years ago, we were having significant problems with endophthalmitis from contaminated corneas coming from the eye bank. At that time the organisms were streptococcal species because we

were putting gentamicin in the solution. In our studies we found that although vancomycin was a pretty good antibiotic, there were cost issues and stability issues. Streptomycin was selected, and the incidence of clinical endophthalmitis after corneal transplantation using corneal storage media has rapidly declined

DR RICHARD L. LINDSTROM. The purpose of prophylactic antibiotics is to reduce the incidence of infection. Also, they should not be toxic to the cornea, especially the endothelium. The antibiotics selected should be uncommonly used, like streptomycin, so that if you did get an infection you'd have alternative antibiotics for treatment. We never sterilized the tissue, but you can reduce the load of bacteria that are presented. Then the body's immune system has a chance to handle the remaining organisms. I think if we took the antibiotics completely out of the media, we'd see a very high incidence of postoperative infections.

DR IVAN R. SCHWAB. *S epidermidis* is not a single organism, but rather a group of organisms, with perhaps 16 to 20 in the group, and some are more virulent than others. Did the authors look at the different variants of *S epidermidis*?

DR THOMAS O. WOOD. What led me to do this study was postoperative endophthalmitis, which occurs in about 1 in 100 cases, usually in patients who have bullous keratopathy and glaucoma. All the organisms were coagulase-negative *Staphylococcus*. Dr Forster believes that exposure to preoperative antibiotics in the optisol solution selected out resistant bacteria accounting for our results. Basically 4% CNS grew from both donor and host. Dr Dickson evaluated these numbers; the chances of selecting out the bacteria that were resistant are between 4 in 10,000 and 1 in 1,000,000. Dr Forster also mentioned a 33% resistance in bacteria to gentamicin and ciprofloxacin in South Florida, but we don't know if those cultures came from the inferior cul de sac or the cornea, or, if there were ulcers, and the corneas had been treated with antibiotics. I suspect if you have enough to get 33% resistance, a lot of those corneas, by the time they got to the Bascom Palmer, had been pretreated. Dr Schwab, we did not divide the Staphylococci into groups.