Shifting Trends in In Vitro Antibiotic Susceptibilities for Common Ocular Isolates During a Period of 15 Years

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• PURPOSE: **To assess the in vitro susceptibility of the most common ocular bacterial isolates to several antibiotics and verify changing trends in the antibiotic susceptibility in a 15-year period.**

• DESIGN: **Experimental study.**

• METHODS: **All cultures positive for *Staphylococcus aureus*, coagulase-negative *Staphylococcus* (CNS), *Streptococcus* sp, and *Pseudomonas* sp in conjunctival (n = 4,585) and corneal (n = 3,779) samples from patients seen at the Federal University of São Paulo from 1985 to 2000 were evaluated. Cultures were performed in liquid and solid media, and susceptibility tests were done to amikacin, gentamicin, neomycin, tobramycin, ciprofloxacin, norfloxacin, ofloxacin, cephalothin, and chloramphenicol.**

• RESULTS: **Amikacin and neomycin showed an improvement of their sensitivity during the study period (88%–95% and 50%–85%, respectively) for corneal and conjunctival samples. Gentamicin and tobramycin revealed a decrease of sensitivity in time, from 95% to less than 80% in corneal and conjunctival samples. Ciprofloxacin, norfloxacin, and ofloxacin had good sensitivity to all evaluated bacteria, better in conjunctiva (95%) than in cornea (90%). Sensitivity of *S. aureus* to cephalothin decreased during the study but was still 98% for CNS. Chloramphenicol had good sensitivity to *S. aureus* (85% in corneal and 92%in conjunctival samples), CNS (87% and 88.5%), and *Streptococcus* sp (95% and 96%).**

• CONCLUSIONS: **Gentamicin, tobramycin, and cephalothin decreased their in vitro susceptibility to all tested pathogens. The fluoroquinolones remained a good choice in the treatment of ocular infections, with high susceptibility to all pathogens tested. Chloramphenicol also revealed an increase in its susceptibility to all bacteria evaluated. (Am J Ophthalmol 2004;137:43–51. © 2004 by Elsevier Inc. All rights reserved.)**

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BACTERIAL OCULAR INFECTIONS ARE POTENTIALLY sight threatening if appropriate antibiotic therapy is not instituted rapidly.[1,2] In the medical management of bacterial eye infections, the treatment must be initiated usually before pathogen identification and antibiotic susceptibility test results are available. Therefore, the antibiotic chosen must have demonstrated efficacy against a broad spectrum of possible ocular pathogens, providing good coverage against most gram-positive and gram-negative bacteria.[3]

The frequent and sometimes indiscriminate use of antibiotics has led to the development of bacterial strains resistant to many commonly used agents.[4–6] Hence, periodic susceptibility testing should be performed to ensure that the currently available antibiotics are providing good coverage against recent clinical isolates of pathogenic bacteria. As suggested by Jensen and coworkers,[3] this type of testing should be done every 2 to 3 years to detect resistance trends with currently used antibiotics. Such studies are of paramount value to health care providers who often have to select a first-line antibiotic treatment without the benefit of having the microbiological testing results.[7]

The purpose of this study is to assess the in vitro susceptibility of the most common ocular bacterial isolates to several antibiotics and verify changing trends in the antibiotic susceptibility of these bacteria during 15 consecutive years.

METHODS

ALL CONSECUTIVE CASES OF BACTERIAL KERATITIS AND bacterial conjunctivitis that underwent a diagnostic corneal

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TABLE 1. Total Number of Tested Bacteria for Each Antibiotic, Divided by Sample Site, From 1985 to 2000

	Pseudomonas sp		Coagulase-negative *Staphylococcus*		*Staphylococcus aureus*		*Streptococcus* sp	
	Conjunctiva	Cornea	Conjunctiva	Cornea	Conjunctiva	Cornea	Conjunctiva	Cornea
Total number of isolated bacteria	12	158	831	173	1,861	399	128	186
Amikacin	12	148	824	172	1,352	283	106	141
Gentamicin	11	158	818	167	1,792	383	112	148
Neomycin	8	115	814	169	955	195	105	136
Tobramycin	10	155	828	172	1,754	383	124	160
Ciprofloxacin	5	101	778	159	516	163	94	131
Norfloxacin	7	104	799	163	723	182	97	135
Ofloxacin	5	99	792	157	500	143	87	125
Cephalothin	10	117	831	173	1,861	399	128	186
Chloramphenicol	9	111	815	166	947	191	106	140

or conjunctival culture from January 1985 through May 2000 at the Federal University of Sao Paulo, Brazil were reviewed. Cultures were performed using both liquid (brain and heart infusion) and solid media (5% sheep blood agar, chocolate agar) and all isolates were identified. The four most frequent were analyzed and had their in vitro susceptibility test performed (coagulase negative *Staphylococcus* (CNS), *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa*). In vitro susceptibility testing was determined by the Kirby-Bauer disk diffusion method and interpreted using the National Committee for Clinical Laboratory Standards (NCCLS) serum standards.[8] Isolates of intermediate sensitivity were categorized with the resistant organisms, because the number of intermediates was insignificant compared with the whole sample. Susceptibility testing of the bacterial isolates was performed to the fluoroquinolones (ciprofloxacin, norfloxacin, and ofloxacin), aminoglycosides (amikacin, neomycin, gentamicin, and tobramycin), cephalosporins (cephalothin), and chloramphenicol. Samples from conjunctiva and from cornea were analyzed separately. All laboratory tests were performed using the same methodology over the analyzed period and by the same microbiologist.

For statistical analysis, logistic regression was used to fit odds of susceptibility vs month. Plots were shown as kernel-smoothed estimates of probability of susceptibility vs time. A gaussian (normal) kernel with a 4-year bandwidth was used for smoothing.

RESULTS

FROM 1985 TO 2000, 3,779 CORNEAL CULTURES AND 4,585 conjunctival cultures were obtained. The number of corneal and conjunctival cultures obtained and the percentage of positive cultures remained relatively constant with no statistically difference between years. The total number of each bacterial group isolates in this period was as follows: *Pseudomonas* sp, 170 samples (12 conjunctival; 158 corneal); *S epidermidis*, 1,004 samples (831 conjunctival; 173 corneal); *S. aureus*, 2,260 samples (1,861 conjunctival; 399 corneal); and *Streptococcus* sp, 314 samples (128 conjunctival; 186 corneal). The number of bacteria isolated from corneal and conjunctival cultures that were tested for each antibiotic susceptibility is shown in Table 1. The total number and statistical data for each type of bacteria analyzed from corneal and conjunctival samples, respectively, are shown in Tables 2 and 3.

The antibiotic-susceptibility profiles for the four different bacteria tested are represented in all of the Tables.

For the aminoglycosides, Table 4 shows a decrease of *Streptococcus* sp susceptibility to amikacin in corneal and conjunctival samples ($P = .0002$ and $P = .0004$, respectively) and an increase in CNS ($P \leq .0001$) and *Staphylococcus aureus* ($P = .0001$) in conjunctival samples over time. The analysis of gentamicin in vitro activity over time revealed a significant decrease of *Streptococcus* and *S. aureus* susceptibilities in corneal samples ($P = .004$ and $P \leq .0001$, respectively) and conjunctival samples ($P = .006$ and $P = .002$, respectively; Table 5). Table 6 demonstrated an increase of *S. aureus* and CNS susceptibilities to neomycin in corneal samples ($P \leq .0001$ and $P = .001$, respectively) and conjunctival samples ($P \leq .0001$ in both) and an increase of *Pseudomonas* susceptibility in corneal samples ($P \leq .0001$). The analysis of tobramycin in vitro activity showed a decrease of *Streptococcus* and *S. aureus* susceptibilities in corneal samples ($P \leq .0001$ and $P = .0003$, respectively) and conjunctival samples ($P \leq .0001$ in both) but an increase of CNS susceptibility in conjuctival samples ($P = .009$; Table 7).

TABLE 2. Total Number of Samples Analyzed From 1985 to 2000

Antibiotic	Bacteria	N	OR (95% CI)	*P* Value
Amikacin	*Pseudomonas aeruginosa*	148	0.896 (0.753–1.065)	.21
	Staphylococcus aureus	283	1.036 (0.995–1.078)	.09
	CNS	172	1.076 (0.98–1.181)	.13
	Streptococcus pneumoniae	141	0.869 (0.81–0.933)	.0002*
Gentamicin	*P. aeruginosa*	158	1.009 (0.954–1.066)	.75
	S. aureus	383	0.894 (0.858–0.931)	≤.0001*
	CNS	167	0.993 (0.932–1.058)	.83
	S. pneumoniae	148	0.934 (0.892–0.978)	.004*
Neomycin	*P. aeruginosa*	115	1.243 (1.129–1.368)	≤.0001*
	S. aureus	195	1.105 (1.061–1.151)	≤.0001*
	CNS	169	1.13 (1.105–1.216)	.001*
	S. pneumoniae	136	1.067 (0.996–1.144)	.07
Tobramycin	*P. aeruginosa*	155	0.982 (0.915–1.054)	.62
	S. aureus	383	0.918 (0.877–0.962)	.0003*
	CNS	172	1.001 (0.944–1.062)	.97
	S. pneumoniae	160	0.891 (0.846–0.937)	≤.0001*
Ciprofloxacin	*P. aeruginosa*	101	1.15 (1.021–1.296)	.02*
	S. aureus	163	1.098 (1.012–1.19)	.02*
	CNS	159	1.005 (0.89–1.135)	.94
	S. pneumoniae	131	1.33 (1.179–1.499)	≤.0001*
Norfloxacin	*P. aeruginosa*	104	1.14 (1.041–1.249)	.005*
	S. aureus	182	1.011 (0.965–1.06)	.64
	CNS	163	0.974 (0.867–1.093)	.65
	S. pneumoniae	135	1.151 (1.066–1.243)	.0004*
Ofloxacin	*P. aeruginosa*	99	1.182 (11.056–1.323)	.004*
	S. aureus	143	1.094 (1.004–1.192)	.04*
	CNS	157	1.003 (0.875–1.15)	.97
	S. pneumoniae	125	1.321 (1.169–1.492)	≤.0001*
Cephalothin	*P. aeruginosa*	117	0.838 (0.785–0.894)	≤.0001*
	S. aureus	399	0.891 (0.822–0.966)	.005*
	CNS	173	1.089 (0.989–1.2)	.08
	S. pneumoniae	186	0.931 (0.839–1.032)	.17
Chloramphenicol	*P. aeruginosa*	111	1.149 (1.008–1.31)	.04*
	S. aureus	191	1.162 (1.101–1.227)	≤.0001*
	CNS	166	1.094 (1.024–1.168)	.008*
	S. pneumoniae	140	1.154 (1.079–1.236)	≤.0001*

CI = confidence interval; CNS = coagulase-negative staphylococcus.
Odds ratio (OR) representing the change in odds every year, *P* values of all bacteria analyzed, divided by antibiotic, in corneal samples.
*Statistically significant.

For the fluoroquinolones, Table 8 shows an increase of *Streptococcus*, *S. aureus*, and *Pseudomonas* susceptibilities to ciprofloxacin in corneal samples ($P \leq .0001$, $P = .02$, $P = .02$, respectively) and an increase of *Streptococcus* and CNS susceptibilities to ciprofloxacin in conjunctival samples ($P = .04$ and $P \leq .0001$, respectively). The analysis of norfloxacin in vitro activity demonstrated an increase of *Streptococcus* and *Pseudomonas* susceptibilities in corneal samples ($P = .0004$ and $P = .005$, respectively) and an increase of CNS susceptibility in conjunctival samples ($P \leq .0001$; Table 9). Table 10 shows an increase in *Streptococcus*, *S. aureus*, and *Pseudomonas* susceptibilities to ofloxacin in corneal samples ($P \leq .0001$, $P = .04$, and $P = .004$, respectively) and also an increase in *S. aureus* and CNS susceptibilities to ofloxacin in conjunctival samples ($P = .04$ and $P = .0001$, respectively).

For the cephalosporins, Table 11 shows a decrease of *S. aureus* and *Pseudomonas* susceptibilities to cephalothin in corneal samples ($P = .005$ and $P \leq .0001$, respectively) and a decrease of *S. aureus* susceptibility in conjunctival samples ($P \leq .0001$).

TABLE 3. Total Number of Samples Analyzed From 1985 to 2000

Antibiotic	Bacteria	N	OR (95% CI)	*P* Value
Amikacin	*Pseudomonas aeruginosa*	12	All susceptible (12)	1
	Staphylococcus aureus	1352	1.038 (1.019–1.057)	.0001*
	CNS	824	1.123 (1.071–1.177)	≤.0001*
	Streptococcus pneumoniae	106	0.85 (0.779–0.928)	.0004*
Gentamicin	*P. aeruginosa*	11	All susceptible (11)	1
	S. aureus	1792	0.973 (0.955–0.99)	.002*
	CNS	818	1.014 (0.978–1.05)	.45
	S. pneumoniae	112	0.908 (0.849–0.972)	.006*
Neomycin	*P. aeruginosa*	8	1.262 (0.736–2.165)	.33
	S. aureus	955	1.141 (1.122–1.159)	≤.0001*
	CNS	814	1.242 (1.191–1.295)	≤.0001*
	S. pneumoniae	105	1.044 (0.977–1.115)	.2
Tobramycin	*P. aeruginosa*	10	All susceptible (10)	1
	S. aureus	1754	0.914 (0.893–0.936)	≤.0001*
	CNS	828	1.044 (1.011–1.078)	.009*
	S. pneumoniae	124	0.876 (0.822–0.934)	≤.0001*
Ciprofloxacin	*P. aeruginosa*	5	All resistant (5)	1
	S. aureus	516	1.06 (0.996–1.127)	.06
	CNS	778	1.255 (1.139–1.382)	≤.0001*
	S. pneumoniae	94	1.152 (1.009–1.314)	0.04*
Norfloxacin	*P. aeruginosa*	7	All susceptible (7)	1
	S. aureus	723	0.982 (0.95–1.0140)	.27
	CNS	799	1.234 (1.142–1.335)	≤.0001*
	S. pneumoniae	97	1.086 (0.974–1.211)	.14
Ofloxacin	*P. aeruginosa*	5	All susceptible (5)	1
	S. aureus	500	1.067 (1.002–1.136)	.04*
	CNS	792	1.241 (1.114–1.384)	.0001*
	S. pneumoniae	87	1.139 (0.94–1.38)	.18
Cephalothin	*P. aeruginosa*	10	0.74 (0.292–1.874)	.48
	S. aureus	1861	0.917 (0.886–0.948)	≤.0001*
	CNS	831	1.05 (0.939–1.174)	.39
	S. pneumoniae	128	1.038 (0.977–1.104)	.22
Chloramphenicol	*P. aeruginosa*	9	22.24 (0–123936)	.6
	S. aureus	947	1.145 (1.127–1.163)	≤.0001*
	CNS	815	1.186 (1.143–1.231)	≤.0001*
	S. pneumoniae	106	1.188 (1.093–1.291)	≤.0001*

CI = confidence interval; CNS = coagulase-negative *staphylococcus*.
Odds ratio (OR) representing the change in odds every year, *P* values of all bacteria analyzed, divided by antibiotic, in conjunctival samples.
*Statistically significant.

The analysis of chloramphenicol in vitro activity revealed an increase of all tested bacteria susceptibilities in corneal samples and also an increase in *Streptococcus*, *S. aureus* and CNS susceptibilities in conjunctival samples (Table 12).

DISCUSSION

BACTERIAL EXTERNAL OCULAR INFECTIONS, ESPECIALLY bacterial keratitis, can be sight-threatening, leading to a prompt treatment even before pathogen identification and antibiotic susceptibility testing can be completed.[9] Commonly, a broad-spectrum antibiotic is chosen as a first-line treatment. Although the results of standardized in vitro tests are based on drug concentrations that are safely achievable in plasma and do not reflect the concentrations achievable with topical application or the effect of local factors on drug activity, they provide a useful estimate of the comparative susceptibility of organisms to different antibiotics.[3]

The emergence of antibiotic-resistant ocular isolates has always been a concern.[10,11] The resistance pattern varies in different countries and different parts of the globe owing to

TABLE 4. Number of Bacteria Susceptible to Amikacin, Total Number of Bacteria Tested, and Susceptibility Percentage of the Different Bacterial Groups in Cornea and Conjunctiva Samples, Grouped in 4-Year Analysis

	1985–1988			1989–1992			1993–1996			1997–2000		
	S	N	%S	S	N	%S	S	N	%S	S	N	%S
Pseudomonas aeruginosa												
Cornea	23	23	100	33	33	100	31	31	100	67	71	94.37
Conjunctiva	5	5	100	2	2	100	2	2	100	3	3	100
Staphylococcus aureus												
Cornea	27	38	71.05	72	72	100	106	119	89.07	49	54	90.74
Conjunctiva	446	520	85.77	328	328	100	269	313	85.94	190	191	99.48
CNS												
Cornea	1	1	100	1	1	100	68	76	89.47	91	94	96.80
Conjunctiva	8	8	100	4	4	100	262	292	89.73	516	520	99.23
Streptococcus pneumoniae												
Cornea	3	4	75	4	4	100	36	55	65.45	38	78	48.72
Conjunctiva	7	7	100	2	2	100	26	47	55.32	14	50	28

CNS = coagulase-negative *Staphylococcus;* N = number of bacteria; %S = percentage of susceptible organisms; S = number of susceptible bacteria.

TABLE 5. Number of Bacteria Susceptible to Gentamicin, Total Number of Bacteria Tested, and Susceptibility Percentage of the Different Bacterial Groups in Cornea and Conjunctiva Samples, Grouped in 4-Year Analysis

	1985–1988			1989–1992			1993–1996			1997–2000		
	S	N	%S	S	N	%S	S	N	%S	S	N	%S
Pseudomonas aeruginosa												
Cornea	26	28	92.86	28	29	96.55	28	31	90.32	65	70	92.86
Conjunctiva	4	4	100	2	2	100	2	2	100	3	3	100
Staphylococcus aureus												
Cornea	80	81	98.76	134	135	99.26	82	112	73.21	43	55	78.18
Conjunctiva	694	744	93.28	544	546	99.63	246	312	78.85	180	190	94.74
CNS												
Cornea	2	2	100	2	2	100	51	69	73.91	75	94	79.79
Conjunctiva	9	9	100	8	8	100	244	281	86.83	453	520	87.11
Streptococcus pneumoniae												
Cornea	11	11	100	5	5	100	19	54	35.18	33	78	42.31
Conjunctiva	7	7	100	9	9	100	28	46	60.87	28	50	56

CNS = coagulase-negative *Staphylococcus*; N = number of bacteria; %S = percentage of susceptible organisms; S = number of susceptible bacteria.

seasonal and climatic differences as well as cultural differences.[12–15] The bacteria analyzed were the most frequent causes of bacterial conjunctivitis and keratitis described in the literature.[16–20]

The analysis of aminoglycosides susceptibilities to each pathogen tested varied from each antibiotic tested. Amikacin, an aminoglycoside that is not frequently and routinely used in our community, showed an improvement in its sensitivity during the study period. Neomycin was used more in the past, and its increase of susceptibility may be due to its lesser use nowadays. Conversely, gentamicin and tobramycin, two antibiotics widely used, showed a decrease of their sensitivity over time. This finding correlates with some published reports of emerging resistance to aminoglycosides.[21,22]

The fluoroquinolones started to be tested in our laboratory in 1990. All three different fluoroquinolones tested presented the same sensitivity pattern during the study period. There was a slight decrease in all pathogen susceptibilities to quinolones in 1993 and 1994, with a subsequent increase. Interestingly, this decrease in sensitivity happened at the same time that several cases of emerging

TABLE 6. Number of Bacteria Susceptible to Neomycin, Total Number of Bacteria Tested, and Susceptibility Percentage of the Different Bacterial Groups in Cornea and Conjunctiva Samples, Grouped in 4-Year Analysis

	1985–1988			1989–1992			1993–1996			1997–2000		
	S	N	%S	S	N	%S	S	N	%S	S	N	%S
Pseudomonas aeruginosa												
Cornea	0	12	0	3	4	75	13	29	44.83	60	70	85.71
Conjunctiva	0	3	0		0		2	2	100	2	3	66.67
Staphylococcus aureus												
Cornea	0	27	0	10	11	90.91	39	103	37.86	39	54	72.22
Conjunctiva	4	430	0.93	63	65	96.92	119	273	43.59	148	187	79.14
CNS												
Cornea	0	1	0		0		52	74	70.27	84	94	89.36
Conjunctiva	0	8	0	1	1	100	187	285	65.61	484	520	93.08
Streptococcus pneumoniae												
Cornea	0	4	0		0		15	54	27.78	29	78	37.18
Conjunctiva	0	7	0	1	1	100	11	47	23.40	15	50	30

CNS = coagulase-negative *Staphylococcus*; N = number of bacteria; %S = percentage of susceptible organisms; S = number of susceptible bacteria.

TABLE 7. Number of Bacteria Susceptible to Tobramycin, Total Number of Bacteria Tested, and Susceptibility Percentage of the Different Bacterial Groups in Cornea and Conjunctiva Samples, Grouped in 4-Year Analysis

	1985–1988			1989–1992			1993–1996			1997–2000		
	S	N	%S	S	N	%S	S	N	%S	S	N	%S
Pseudomonas aeruginosa												
Cornea	28	28	100	25	26	96.15	28	31	90.32	66	70	94.28
Conjunctiva	5	5	100		0		2	2	100	3	3	100
Staphylococcus aureus												
Cornea	76	78	97.43	128	129	99.22	105	122	86.06	45	54	83.33
Conjunctiva	720	731	98.49	527	527	100	262	307	85.34	170	189	89.95
CNS												
Cornea	2	2	100	2	2	100	54	74	72.97	74	94	78.72
Conjunctiva	9	9	100	6	6	100	238	293	81.23	467	520	89.81
Streptococcus pneumoniae												
Cornea	13	13	100	18	18	100	23	51	45.10	34	78	43.59
Conjunctiva	11	11	100	16	16	100	23	47	48.94	23	50	46

CNS = coagulase-negative *Staphylococcus*; N = number of bacteria; %S = percentage of susceptible organisms; S = number of susceptible bacteria.

fluoroquinolone resistance were published in the literature.[4–6,23–25] Another interesting finding was that the decrease in sensitivity was exacerbated in corneal samples compared with conjunctival samples. One of the possible explanations for this is that bacteria that cause corneal infections tend to be more pathogenic than the ones causing conjunctival infections. Besides that, all fluoroquinolones tested in this study showed high efficacy when analyzing the four different bacteria since 1998, proving that in our community quinolones are still a good choice for treating ocular infections.

The analysis of the first-generation cephalosporin, cephalothin, revealed a decrease of *S. aureus* and *Pseudomonas* susceptibility with time. The *Pseudomonas* sensitivity to cephalotin was expected to be low, as the first-generation cephalosporin has its main activity against gram-positive microorganisms. Cephalothin is frequently used in our community as a fortified antibiotic in treating bacterial keratitis. This frequent use could have contributed to the decrease of *S. aureus* sensitivity during the study period. It is important to point out that the first-generation cephalosporin used in ophthalmology in the United States is

TABLE 8. Number of Bacteria Susceptible to Ciprofloxacin, Total Number of Bacteria Tested, and Susceptibility Percentage of the Different Bacterial Groups in Cornea and Conjunctiva Samples, Grouped in 4-Year Analysis

	1985–1988			1989–1992			1993–1996			1997–2000		
	S	N	%S	S	N	%S	S	N	%S	S	N	%S
Pseudomonas aeruginosa												
Cornea		0		3	3	100	19	27	70.37	66	71	92.96
Conjunctiva		0			0		0	2	0	0	3	0
Staphylococcus aureus												
Cornea		0		5	5	100	73	103	70.87	51	55	92.73
Conjunctiva		0		65	65	100	233	262	88.93	187	189	98.94
CNS												
Cornea		0			0		60	66	90.91	88	93	94.62
Conjunctiva		0			0		242	258	93.80	515	520	99.04
Streptococcus pneumoniae												
Cornea		0			0		30	53	56.60	73	78	93.59
Conjunctiva		0			0		32	44	72.73	48	50	96

CNS = coagulase-negative *Staphylococcus*; N = number of bacteria; %S = percentage of susceptible organisms; S = number of susceptible bacteria.

TABLE 9. Number of Bacteria Susceptible to Norfloxacin, Total Number of Bacteria Tested, and Susceptibility Percentage of the Different Bacterial Groups in Cornea and Conjunctiva Samples, Grouped in 4-Year Analysis

	1985–1988			1989–1992			1993–1996			1997–2000		
	S	N	%S	S	N	%S	S	N	%S	S	N	%S
Pseudomonas aeruginosa												
Cornea		0		7	7	100	14	26	53.85	67	71	94.37
Conjunctiva		0		2	2	100	2	2	100	3	3	100
Staphylococcus aureus												
Cornea		0		29	29	100	58	99	58.58	50	54	92.59
Conjunctiva		0		258	258	100	212	276	76.81	187	189	98.94
CNS												
Cornea		0		1	1	100	63	69	91.30	87	93	93.55
Conjunctiva		0		2	2	100	256	279	91.76	513	518	99.03
Streptococcus pneumoniae												
Cornea		0		5	5	100	25	53	47.17	70	77	90.91
Conjunctiva		0		2	2	100	33	45	73.33	48	50	96

CNS = coagulase-negative *Staphylococcus*; N = number of bacteria; %S = percentage of susceptible organisms; S = number of susceptible bacteria.

cefazolin, and in Brazil it is cephalothin, but cefazolin is considered comparable to cephalothin.

Chloramphenicol, a broad-spectrum antibiotic, fell into discredit since the mid 1980s owing to its high resistance indexes against most ocular pathogens. In our community, chloramphenicol was the most commonly used antibiotic in the 1960s and 1970s, but most of the ophthalmologists stopped using chloramphenicol in the 1990s because bacteria susceptibility was very low. The analysis of chloramphenicol susceptibility to the four pathogens tested showed an important increase with time. One possible explanation for this is that after so many years of this drug being discredited and not being used, the bacterial susceptibility to it improved again, especially for gram-positive pathogens.

There are some points in this study that should be addressed. Resistance based on in vitro testing may not reflect true clinical resistance, because topical intensive use of antibiotics can produce corneal concentrations that exceed mean inhibitory concentration values.[5] Antibiotic sensitivities may not correspond to clinical response to an antibiotic because of host factors and penetration of the

TABLE 10. Number of Bacteria Susceptible to Ofloxacin, Total Number of Bacteria Tested, and Susceptibility Percentage of the Different Bacterial Groups in Cornea and Conjunctiva Samples, Grouped in 4-Year Analysis

	1985–1988			1989–1992			1993–1996			1997–2000		
	S	N	%S	S	N	%S	S	N	%S	S	N	%S
Pseudomonas aeruginosa												
Cornea		0		3	3	100	15	25	60	65	71	91.55
Conjunctiva		0			0		2	2	100	3	3	100
Staphylococcus aureus												
Cornea		0		3	3	100	63	86	73.25	50	54	92.59
Conjunctiva	1	1	100	68	68	100	217	241	90.04	187	189	98.94
CNS												
Cornea		0			0		60	64	93.75	89	93	95.70
Conjunctiva		0		1	1	100	263	273	96.33	513	518	99.03
Streptococcus pneumoniae												
Cornea		0			0		28	46	60.87	74	79	93.67
Conjunctiva		0			0		33	37	89.19	49	50	98

CNS = coagulase-negative *Staphylococcus*; N = number of bacteria; %S = percentage of susceptible organisms; S = number of susceptible bacteria.

TABLE 11. Number of Bacteria Susceptible to Cephalothin, Total Number of Bacteria Tested, and Susceptibility Percentage of the Different Bacterial Groups in Cornea and Conjunctiva Samples, Grouped in 4-Year Analysis

	1985–1988			1989–1992			1993–1996			1997–2000		
	S	N	%S	S	N	%S	S	N	%S	S	N	%S
Pseudomonas aeruginosa												
Cornea	15	17	88.23	2	2	100	1	28	3.57	4	70	5.71
Conjunctiva	4	5	80		0		0	2	0	0	3	0
Staphylococcus aureus												
Cornea	81	81	100	139	139	100	118	125	94.40	51	54	94.44
Conjunctiva	742	747	99.33	604	604	100	297	319	93.10	184	191	96.33
CNS												
Cornea	2	2	100	2	2	100	70	76	92.10	91	93	97.85
Conjunctiva	9	9	100	8	8	100	291	294	98.98	517	520	99.42
Streptococcus pneumoniae												
Cornea	13	13	100	37	37	100	55	58	94.83	73	78	93.59
Conjunctiva	11	13	84.61	18	18	100	39	47	82.98	49	50	98

CNS = coagulase-negative *Staphylococcus*; N = number of bacteria; %S = percentage of susceptible organisms; S = number of susceptible bacteria.

drug. However, in vitro susceptibility testing is the only established method of determining antibiotic resistance and was performed in a standardized method at our laboratory.[23] This study analyzed only the most common microorganisms isolated in our population, and the antibiotics analyzed were the most prescribed and used in our community. There are several different microorganisms and antibiotics that could be studied, and our plan is to analyze different bacteria and drugs in the near future.

In conclusion, this study showed that, in South America, the most used aminoglycosides, gentamicin and tobramycin, decreased their in vitro susceptibility to the pathogens tested over these 15 years, along with the cephalosporin tested, cephalothin. The gram-negative bacteria had a good response to the aminoglycosydes amikacin, gentamicin, and tobramycin, and we would suggest using aminoglycosides to treat these infections. All the fluoroquinolones tested in this study, conversely, represented a great choice for treating ocular infections, with high susceptibility to all pathogens tested. Chloramphenicol also revealed an increase in its susceptibility to all pathogens, showing that after a long period without using this drug, the sensitivity tends to get better.

TABLE 12. Number of Bacteria Susceptible to Chloramphenicol, Total Number of Bacteria Tested, and Susceptibility Percentage of the Different Bacterial Groups in Cornea and Conjunctiva Samples, Grouped in 4 Years Analysis

	1985–1988			1989–1992			1993–1996			1997–2000		
	S	N	%S	S	N	%S	S	N	%S	S	N	%S
Pseudomonas aeruginosa												
Cornea	0	12	0	1	1	100	2	28	7.14	13	70	18.57
Conjunctiva	0	4	0		0		0	2	0	2	3	66.67
Staphylococcus aureus												
Cornea	0	27	0	7	7	100	41	103	39.80	46	54	85.18
Conjunctiva	22	431	5.10	51	53	96.23	129	274	47.08	175	189	92.59
CNS												
Cornea	1	1	100		0		46	73	63.01	80	92	86.95
Conjunctiva	1	6	16.67		0		174	289	60.21	460	520	88.46
Streptococcus pneumoniae												
Cornea	4	7	57.14	2	2	100	26	52	50	75	79	94.94
Conjunctiva	0	7	0	2	2	100	35	47	74.47	48	50	96

CNS = coagulase-negative *Staphylococcus*; N = number of bacteria; %S = percentage of susceptible organisms; S = number of susceptible bacteria.

REFERENCES

1. Jones DB. Decision-making in the management of microbial keratitis. Ophthalmology 1981;88:814–820.
2. Tuft SJ, Matherson M. In vitro antibiotic resistance in bacterial keratitis in London. Br J Ophthalmol 2000;84:687–691.
3. Jensen HG, Felix C. In vitro antibiotic susceptibilities of ocular isolates in North and South America. Cornea 1998; 17:79–87.
4. Kunimoto DY, Sharma S, Garg P, Rao GN. In vitro susceptibility of bacterial keratitis pathogens to ciprofloxacin. Ophthalmology 1999;106:80–85.
5. Goldstein MH, Kowalski RP, Gordon YJ. Emerging fluoroquinolone resistance in bacterial keratitis. Ophthalmology 1999;106:1313–1318.
6. Garg P, Sharma S, Rao GN. Ciprofloxacin-resistant Pseudomonas keratitis. Ophthalmology 1999;106:1319–1323.
7. Molinari H, Belfort R Jr, Almada AT, Silva VLP. Laboratorial analysis of 247 patients with conjuctivitis, keratitis and endophthalmitis [translation]. Rev Bras Oftalmol 1978;37: 63–73.
8. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility test. The National Committee for Clinical Laboratory Standards. 1993;13(24):1–31.
9. Gritz DC, Hwang DG, Whitcher JP Jr. New horizons in antibacterial therapy. Int Ophthalmol Clin 1996;36:153–170.
10. Knauf HP, Silvany R, Southern PM, Risser RC, Wilson SE. Susceptibility of corneal and conjunctival pathogens to ciprofloxacin. Cornea 1996;15:66–71.
11. Serdarevic ON. Role of the fluoroquinolones in ophthalmology. Int Ophthalmol Clin 1993;33:163–178.
12. Carmichael TR, Wolpert M, Koornhof HJ. Corneal ulceration at an urban African hospital. Br J Ophthalmol 1985; 69:920–926.
13. Wahl JC, Katz HR, Abrams DA. Infectious keratitis in Baltimore. Ann Ophthalmol 1991;23:234–237.
14. Liesegang TJ, Forster RK. Spectrum of microbial keratitis in South Florida. Am J Ophthalmol 1980;90:38–47.
15. Upadhyay MP, Karmacharya PCD, Koirala S, Tuladhar NR, Bryan LE, Smolin G. Epidemiologic characteristics, predisposing factors and etiologic diagnosis of corneal ulceration in Nepal. Am J Ophthalmol 1991;111:92–99.
16. Everett SL, Kowalski RP, Karenchak LM, Landsittel D, Day R, Gordon YJ. An in vitro comparison of the susceptibilities of bacterial isolates from patients with conjunctivitis and blepharitis to newer and established topical antibiotics. Cornea 1995;14:382–387.
17. Osato MS, Jensen HG, Trousdale MD, et al. The comparative in vitro activity of ofloxacin and selected ophthalmic antimicrobial agents against ocular bacterial isolates. Am J Ophthalmol 1989;108:380–386.
18. Montan P. Endophthalmitis. Curr Opin Ophthalmol 2001; 12:75–81.
19. Ooishi M, Miyao M. Antibiotic sensitivity of recent clinical isolates from patients with ocular infections. Ophthalmologica 1997;211(Suppl 1):15–24.
20. Liesegang TJ. Use of antimicrobials to prevent postoperative infection in patients with cataract. Curr Opin Ophthalmol 2001;12:68–74.
21. Bovelacci A, Montini G, Ramacciotti PG. Changes in resistance to aminoglycoside antibiotics of different bacterial strains isolated during the period 1978 to 1983. Chemiotherapia 1985;4:439–444.
22. Gwon A. Topical ofloxacin compared with gentamicin in the treatment of external ocular infection. Br J Ophthalmol 1992;76:714–718.
23. Alexandrakis G, Alfonso EC, Miller D. Shifting trends in bacterial keratitis in South Florida and emerging resistance to fluoroquinolones. Ophthalmology 2000;107:1497–1502.
24. von Rosenstiel N, Adam D. Quinolone antibacterials: an update of their pharmacology and therapeutic use. Drugs 1994;47:872–901.
25. Chaudhry NA, Flynn HW, Murray TG, Tabandeh H, Mello MO, Miller D. Emerging ciprofloxacin-resistant *Pseudomonas aeruginosa*. Am J Ophthalmol 1999;128:509–510.