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ORIGINAL ARTICLE

Antimicrobial susceptibility of Gram-positive cocci isolated from patients with conjunctivitis and keratitis in Crete, Greece

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Purpose: To assess the *in vitro* susceptibility of *Streptococcus pneumoniae*, *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) ocular isolates to antibiotics, and identify changing trends in resistance over a 10-year period.
Methods: All isolates from ocular infections collected between 2000 and 2009 were prospectively tested against several antibiotics *in vitro*. *S. pneumoniae* isolates ($n = 93$) were tested against 20 and *S. aureus* ($n = 120$) and CoNS ($n = 214$) against 19 antibiotics. To identify changes in susceptibility patterns, we compared results from 2000—2004 with those from 2005—2009. We also compared the antibiotic susceptibilities against aminoglycosides and quinolones between methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) isolates.
Results: All *S. pneumoniae* isolates were susceptible to quinolones, and 99% were susceptible to chloramphenicol. Regarding *S. aureus*, we noted a significant increase in resistance against penicillin in recent years ($p = 0.016$). Over 90% of *S. aureus* isolates were susceptible to quinolones and aminoglycosides. MRSA isolates were more resistant to ciprofloxacin and ofloxacin than MSSA isolates were ($p = 0.016$). Concerning CoNS, a significant increase in susceptibility to amikacin was noted in the second study period ($p = 0.01$).

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Conclusion: Quinolones remain an excellent treatment option for bacterial conjunctivitis and keratitis due to Gram-positive cocci in our region.

Introduction

The eye can be affected by a wide spectrum of infections, some of which are vision-threatening. Adequate preventive measures, knowledge of the most common bacterial pathogens and their antibiotic susceptibility patterns in a given locale, and therapy with suitable antibiotics, constitute the cornerstones for the successful management of ocular infections.[1]

Streptococcus pneumoniae, *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) are important ophthalmic pathogens implicated frequently in ocular infections. In most series published to date, these Gram-positive cocci are the most prevalent bacterial pathogens implicated in cases of bacterial conjunctivitis and keratitis, along with *Pseudomonas aeruginosa* and *Moraxella* spp.[2]

Distinction of ocular isolates into those that cause bacterial conjunctivitis versus keratitis is important. Bacterial conjunctivitis is considered a mild and self-limited infection. In this infection, although topical antibiotics are associated with higher rates of early clinical remission, their benefit is minimal at later time points.[3] On the other hand, keratitis may be vision-threatening without prompt and adequate treatment. Moreover, bacterial isolates from cases of keratitis are frequently more resistant to antibiotics, suggesting that virulence may correlate with increased antibacterial resistance.[4]

The goal of the present study was to describe the *in vitro* antibiotic susceptibility patterns of *S. pneumoniae*, *S. aureus* and CoNS ophthalmic isolates derived from children (patients aged $\leq$ 14 years) and adults with bacterial conjunctivitis and keratitis that were cared for over a 10-year period in the University Hospital of Heraklion, the only tertiary hospital in the island of Crete, Greece.

Materials and methods

We prospectively tested against several antibiotics all ophthalmic isolates of *S. pneumoniae*, *S. aureus* and CoNS that were recovered from patients with bacterial conjunctivitis and keratitis and cared for in the University Hospital of Heraklion over the period January 2000—December 2009. One bacterial isolate was tested per patient. Our study met the ethical guidelines of the Declaration of Helsinki, and adhered to the legal requirements of the University Hospital of Heraklion regarding institutional review board approval for *in vitro* investigations of antibiotic susceptibilities of human isolates. For epidemiological reasons, we did not limit the susceptibility testing only to antibiotics administered topically for ocular infections. We also used antibiotics that are administered parenterally, since antibacterial resistance to one antibiotic frequently heralds the development of resistance to other antibacterials. For example, the emergence of resistance to β-lactams among *S. aureus* isolates is frequently associated with resistance to quinolones and even glycopeptides.[5]

The antibiotics that were tested against *S. pneumoniae*, *S. aureus* and CoNS ophthalmic isolates are shown in Table 1. The ophthalmic specimens were collected with sterile applicator swabs and transported to the laboratory within <2 hours after collection, along with written documentation about the type of underlying ocular infection necessitating specimen collection.

Identification of *S. pneumoniae* was based on colony and microscopic morphology, hemolytic activity on sheep blood agar medium, catalase test, optochin susceptibility, bile solubility, and biochemical profile using the API 20 Strep System (BioMérieux, Marcy l' Etoile, France). *S. aureus* and CoNS were identified on the basis of colony morphology, Gram stain, catalase and coagulase test, and the API 20 Staph System (BioMérieux). Antimicrobial susceptibility tests were performed by employing the disk diffusion method and the results were interpreted according to the 2010 Clinical and Laboratory Standards Institute criteria.[6] Intermediate isolates were grouped along with resistant isolates. For *S. pneumoniae* isolates, minimum inhibitory concentrations (MICs) were determined for penicillin G, cefuroxime, cefotaxime, ceftriaxone, erythromycin and clindamycin using the E-test method (AB Biodisk, Solna, Sweden).

To test for differences in the antibiotic susceptibility patterns between the earlier and later study years for a given antibiotic, we compared the resistance profiles of ophthalmic isolates from 2000—2004 with those from 2005—2009. Finally, we compared the quinolone susceptibility patterns between ophthalmic and non-ophthalmic isolates of *S. aureus*, that is, isolates obtained from all other body sites for the whole 10-year study period.

Statistical analysis was conducted by Mann—Whitney, Fisher's exact and χ^2 tests, as appropriate. Statistical significance was set at $p < 0.05$ (two-tailed). All analyses were performed with Graphpad Prism version 4 (GraphPad Software, San Diego, CA, USA).

Results

Overall, 427 ocular isolates of the Gram-positive cocci of interest were recovered during the 10-year study period from patients with bacterial conjunctivitis ($n = 340$) and keratitis ($n = 87$). These included 214 (50.1%) isolates of CoNS, 120 (28.1%) of *S. aureus* and 93 (21.8%) of *S. pneumoniae*. During the study period, we also isolated 278 other ophthalmic pathogens (130 during 2000—2004 and 148 during 2005—2009). These belonged to 37 different

Table 1 Resistance profiles of 93 *Streptococcus pneumoniae*, 120 *Staphylococcus aureus* and 214 CoNS isolates from patients with keratitis and conjuctivitis

		2000–2004		2005–2009		*p* value
		Resistant	Sensitive	Resistant	Sensitive	
S. pneumoniae						
01	Penicillin	10 (23.8%)	32 (76.2%)	13 (25.5%)	38 (74.5%)	1
02	Cefuroxime	7 (16.7%)	35 (83.3%)	6 (11.8%)	45 (88.2%)	0.56
03	Cefotaxime	2 (4.8%)	40 (95.2%)	1 (2%)	50 (98%)	0.59
04	Ceftriaxone	1 (2.4%)	41 (97.6%)	1 (2%)	50 (98%)	1
05	Cefepime	4 (9.5%)	38 (90.5%)	3 (5.9%)	48 (94.1%)	0.70
06	Imipenem	6 (14.3%)	36 (85.7%)	4 (7.8%)	47 (92.2%)	0.34
07	Meropenem	2 (4.8%)	40 (95.2%)	0 (0%)	51 (100%)	0.20
08	Erythromycin	13 (31%)	29 (69%)	16 (31.4%)	35 (68.6%)	1
09	Clarithromycin	13 (31%)	29 (69%)	16 (31.4%)	35 (68.6%)	1
10	Clindamycin	4 (9.5%)	38 (90.5%)	7 (13.7%)	44 (86.3%)	0.75
11	Roxithromycin	13 (31%)	29 (69%)	16 (31.4%)	35 (68.6%)	1
12	Ciprofloxacin	0 (0%)	42 (100%)	0 (0%)	51 (100%)	—
13	Ofloxacin	0 (0%)	42 (100%)	0 (0%)	51 (100%)	—
14	Levofloxacin	0 (0%)	42 (100%)	0 (0%)	51 (100%)	—
15	Moxifloxacin	0 (0%)	42 (100%)	0 (0%)	51 (100%)	—
16	Gatifloxacin	0 (0%)	42 (100%)	0 (0%)	51 (100%)	—
17	Chlorampenicol	1 (2.4%)	41 (97.6%)	0 (0%)	51 (100%)	0.45
18	Tetracycline	13 (31%)	29 (69%)	9 (17.6%)	42 (82.4%)	0.15
19	TMP–SMX	12 (28.6%)	30 (71.4%)	12 (23.5%)	39 (76.5%)	0.64
20	Vancomycin	0 (0%)	42 (100%)	0 (0%)	51 (100%)	—
S. aureus						
01	Penicillin	37 (72.5%)	14 (27.5%)	62 (89.9%)	7 (10.1%)	0.016
02	Oxacillin	16 (31.4%)	35 (68.6%)	28 (40.6%)	41 (59.4%)	0.34
03	Cephalothin	16 (31.4%)	35 (68.6%)	29 (42%)	40 (58%)	0.26
04	Gentamicin	5 (9.8%)	46 (90.2%)	1 (1.4%)	68 (98.6%)	0.08
05	Tobramycin	15 (29.4%)	36 (70.6%)	10 (14.5%)	59 (85.5%)	0.068
06	Amikacin	15 (29.4%)	36 (70.6%)	10 (14.5%)	59 (85.5%)	0.068
07	Netilmicin	5 (9.8%)	46 (90.2%)	1 (1.4%)	68 (98.6%)	0.08
08	Chloramphenicol	13 (25.5%)	38 (74.5%)	13 (18.8%)	56 (81.2%)	0.50
09	Tetracycline	9 (17.6%)	42 (82.4%)	9 (13%)	60 (87%)	0.60
10	Erythromycin	11 (21.6%)	40 (78.4%)	19 (27.5%)	50 (72.5%)	0.53
11	Clindamycin	3 (5.9%)	48 (94.1%)	8 (11.6%)	61 (88.4%)	0.35
12	Ciprofloxacin	2 (3.9%)	49 (96.1%)	2 (2.9%)	67 (97.1%)	1
13	Ofloxacin	2 (3.9%)	49 (96.1%)	2 (2.9%)	67 (97.1%)	1
14	Levofloxacin	—	—	1 (2.7%)	36 (97.3%)	—
15	Moxifloxacin	—	—	1 (2.7%)	36 (97.3%)	—
16	TMP–SMX	4 (7.8%)	47 (92.2%)	0 (0%)	69 (100%)	0.03
17	Rifampicin	1 (2%)	50 (98%)	0 (0%)	69 (100%)	0.43
18	Fucidin	9 (17.6%)	42 (82.4%)	5 (7.2%)	64 (92.8%)	0.09
19	Fosfomycin	1 (2%)	50 (98%)	0 (0%)	69 (100%)	0.43
20	Vancomycin	0 (0%)	51 (100%)	0 (0%)	69 (100%)	—
21	Teicoplanin	0 (0%)	51 (100%)	0 0%)	69 (100%)	
CoNS						
01	Penicillin	97 (89%)	12 (11%)	93 (88.6%)	12 (11.4%)	1
02	Oxacillin	38 (34.9%)	71 (65.1%)	50 (47.6%)	55 (52.4%)	0.07
03	Cephalothin	38 (34.9%)	71 (65.1%)	50 (47.6%)	55 (52.4%)	0.07
04	Gentamicin	26 (23.9%)	83 (76.1%)	20 (19%)	85 (81%)	0.41
05	Tobramycin	62 (56.9%)	47 (43.1)	46 (43.8%)	59 (56.2)	0.07
06	Amikacin	61 (56%)	48 (44%)	41 (39%)	64 (61%)	0.01
07	Netilmicin	26 (23.9%)	83 (76.1%)	18 (17.1%)	87 (82.9)	0.24
08	Chloramphenicol	20 (18.3%)	89 (81.7%)	18 (17.1%)	87 (82.9)	0.86
09	Tetracycline	18 (16.5%)	91 (83.5)	26 (24.8%)	79 (75.2%)	0.17
10	Erythromycin	46 (42.2%)	63 (57.8)	47 (44.8%)	58 (55.2%)	0.78

(*continued on next page*)

Table 1 (*continued*)

		2000–2004		2005–2009		*p* value
		Resistant	Sensitive	Resistant	Sensitive	
11	Clindamycin	30 (27.5%)	79 (72.5%)	34 (32.4%)	71 (67.6%)	0.45
12	Ciprofloxacin	7 (6.4%)	102 (93.6%)	10 (9.5%)	95 (90.5%)	0.45
13	Ofloxacin	6 (5.5%)	103 (94.5%)	9 (8.6%)	96 (91.4)	0.43
14	TMP–SMX	9 (8.3%)	100 (91.7)	12 (11.4%)	93 (88.6%)	0.49
15	Rifampicin	3 (2.8%)	106 (97.2%)	17 (16.2%)	88 (83.8%)	0.0007
16	Fucidic acid	63 (57.8%)	46 (42.2%)	60 (57.1%)	45 (42.9%)	1
17	Fosfomycin	13 (11.9)	96 (88.1%)	12 (11.4%)	93 (88.6%)	1
18	Vancomycin	0 (0%)	109 (100%)	0 (0%)	105 (100%)	—
19	Teicoplanin	0 (0%)	109 (100%)	0 (0%)	105 (100%)	—

CoNS = coagulase-negative staphylococci; TMP–SMX = trimethoprim–sulfamethoxazole.

species. Among them, approximately 21% (58/278) were Gram-positive and the remaining 220 (79%) were Gram-negative, with no significant difference between the two study periods. The same was true for the prevalence of the studied ocular pathogens of interest within all the ophthalmic bacterial pathogens of the 10-year surveillance period (data not shown). The most prevalent other pathogens were *Haemophilus influenzae* and *P. aeruginosa* that were responsible for 46% of the positive ophthalmic cultures for pathogens other than *S. aureus*, *S. pneumoniae* and CoNS.

Approximately 53% (226/427) of the study isolates came from the day clinic of the Department of Ophthalmology, whereas the remaining were collected in one of the several departments and/or clinics of the University Hospital of Heraklion.

Demographic data of patients with bacterial conjunctivitis and keratitis are shown in Table 2. The distribution of patients into children and adults differed significantly by ophthalmic pathogen ($p = 0.0004$), whereas no significant difference in the distribution of the three ophthalmic pathogens was seen by sex ($p = 0.61$).

Among the 93 isolates of *S. pneumoniae*, 42 were collected during the years 2000–2004 and 51 during 2005–2009. Sixty-eight of these isolates (73.1%) came from patients who suffered from conjunctivitis and the remaining 25 (26.9%) from patients with keratitis. Comparing the antibiotic susceptibility patterns of *S. pneumoniae* isolates from the two periods, no statistically significant change was noted for any of the 20 antimicrobials tested (Table 1). All 93 ophthalmic *S. pneumoniae* isolates were susceptible to all five quinolones tested, that is, ciprofloxacin, ofloxacin, levofloxacin, moxifloxacin and gatifloxacin. Moreover, 99% of *S. pneumoniae* isolates were susceptible to chloramphenicol. Comparing the antibiotic susceptibility patterns between *S. pneumoniae* conjunctivitis versus keratitis isolates, there was no significant difference for any of the 20 antibiotics tested (data not shown).

Regarding *S. aureus*, 51 and 69 ophthalmic isolates of this pathogen were recovered during 2000–2004 and 2005–2009, respectively. Eighty-three of these isolates (69.2%) came from patients with conjunctivitis and the remaining 37 (30.8%) from patients with keratitis. Comparing the antibiotic susceptibility patterns between the two study periods, we noted a significant increase in resistance against penicillin in recent years, along with a significant decrease in resistance against trimethoprim–sulfamethoxazole (Table 1). Interestingly, methicillin-resistant *S. aureus* (MRSA) was not significantly more common during the more recent study years, as we had hypothesized. As seen from the data in Table 1, there was a trend for decreased resistance of *S. aureus* against aminoglycosides (gentamicin, tobramycin, amikacin and netilmicin) in recent years, which did not, however, reach statistical significance. Comparing the antibiotic susceptibility patterns between conjunctivitis and keratitis isolates, there was no significant difference for any of the 19 antibiotics tested (data not shown).

We also compared the susceptibilities to aminoglycosides, quinolones and chloramphenicol of MRSA ($n = 44$) and methicillin-sensitive *S. aureus* (MSSA; $n = 76$) isolates. MRSA isolates were more resistant to ciprofloxacin and ofloxacin, whereas no differences in susceptibility were seen for any of the four aminoglycosides or for chloramphenicol (Table 3).

Table 4 shows the comparison of quinolone susceptibility patterns between ophthalmic and nonophthalmic isolates of *S. pneumoniae* and *S. aureus* (2000–2009). As seen from these data and Table 1, ofloxacin susceptibility was not tested for nonophthalmic isolates of *S. pneumoniae*, whereas levofloxacin and moxifloxacin susceptibility data

Table 2 Demographic data of patients with bacterial conjunctivitis and keratitis ($n = 427$)

Pathogen	Sex, *n* (%)	Age (median, range)	Children ≤ 14 yr
Streptococcus pneumoniae	M: 46 (49.4%) F: 47 (50.6%)	7 yr (1 mo–86 yr)	52/93 (55.9%)
Staphylococcus aureus	M: 59 (49.2%) F: 61 (50.8%)	25.5 yr (1 mo–88 yr)	49/120 (40.8%)
CoNS	M: 123 (57.5%) F: 91 (42.5%)	32 yr (1 mo–89 yr)	68/214 (31.8%)
Total	M: 228 (53.4%) F: 199 (46.6%)	29.8 yr (1 mo–89 yr)	169/427 (39.6%)

CoNS = coagulase-negative staphylococci; F = female; M = male.

Table 3 Comparison of antibiotic susceptibilities to aminoglycosides and quinolones of MRSA and MSSA isolates

2000–2009	MRSA ($n = 44$)		MSSA ($n = 76$)		p value
	Resistant	Sensitive	Resistant	Sensitive	
Gentamicin	1	43	5	71	0.41
Tobramycin	10	34	14	62	0.64
Amikacin	10	34	14	62	0.64
Netilmicin	1	43	5	71	0.41
Ciprofloxacin	4	40	0	76	0.016
Ofloxacin	4	40	0	76	0.016
Chloramphenicol	13	31	13	63	0.167

MRSA = methicillin-resistant *Staphylococcus aureus*; MSSA = methicillin-sensitive *Staphylococcus aureus*.

were available only for *S. aureus* ocular isolates from 2005–2009.

Regarding CoNS, 109 isolates were recovered during 2000–2004, and 105 during 2005–2009 from 155 (72.4%) patients with conjunctivitis and 59 patients (27.6%) with keratitis. These isolates belonged to the following nine species: 176 *Staphylococcus epidermidis*, 17 *Staphyloccocus hominis*, seven *Staphylococcus haemolyticus*, five *Staphylocccus capitis*, three *Staphylococcus warneri*, two *Staphyloccocus xylosus*, two *Staphylococcus lungdunensis*, one *Staphylococcus simulans*, and one *Staphylococcus cohnii*. Comparing the antibiotic susceptibility patterns between the two study periods, we noted a significant decrease in resistance against amikacin in 2005–2009, along with a significant increase in resistance against rifampicin (Table 1). Moreover, there was a trend for decreased resistance against tobramycin and a trend for increased resistance to oxacillin and cephalothin in the second study period. No CoNS isolates were resistant to glycopeptides (vancomycin and teicoplanin). Comparing the antibiotic susceptibility patterns between CoNS isolates from conjunctivitis versus keratitis cases, there was no significant difference for any of the tested antibiotics (data not shown).

Discussion

The main finding of this study is that second-generation quinolones (ciprofloxacin and ofloxacin), and newer third- (levofloxacin) and fourth-generation (moxifloxacin and gatifloxacin) fluoroquinolones appear to be very effective *in vitro* against ophthalmic isolates of *S. pneumoniae*, because all isolates were susceptible to these antibiotics. Moreover, quinolone susceptibility was similar for ophthalmic and nonophthalmic isolates of *S. pneumoniae*. Chloramphenicol, an old, cheap, and widely used antibiotic for ocular infections was also highly effective, and 99% of the *S. pneumoniae* ophthalmic isolates were susceptible to it.

A second finding of our study was that both quinolones and aminoglycosides appeared to be effective (> 90% susceptibility rates) against ophthalmic isolates of *S. aureus*. This is important because corneal perforations can occur within <24 hours in the presence of particularly invasive bacterial pathogens, such as *S. aureus*, and topical ocular antibiotic solutions of fluoroquinolones and aminoglycosides appear to be the drugs of choice in this case.

We did not see a significant increase in recent years of ocular MRSA isolates, like the increase we have seen with this pathogen in other body sites at our institution. Although we did not see high rates of resistance of *S. aureus* isolates to quinolones, MRSA isolates were significantly more resistant to these antibiotics compared to MSSA isolates. In the Ocular Tracking Resistance in U.S. Today study, MSSA susceptibility for the fluoroquinolones was 79.9–81.1% and MRSA susceptibility was only 15.2%.[7] Elsahn et al have determined the *in vitro* susceptibility of 40 ophthalmic isolates of *S. aureus* including 21 MRSA isolates, and found that 90% of MRSA isolates were resistant to fourth-generation fluoroquinolones.[8] Therefore, in areas

Table 4 Comparison of quinolone susceptibility patterns between ophthalmic and nonophthalmic isolates of *Streptococcus pneumoniae* and *Staphylococcus aureus* (2000–2009)

		Ophthalmic		Nonophthalmic		p value
		Resistant	Sensitive	Resistant	Sensitive	
S. pneumoniae						
01	Ciprofloxacin	0 (0%)	93 (100%)	4 (1.1%)	387 (98.9%)	1
02	Ofloxacin	0 (0%)	93 (100%)	—	—	—
03	Levofloxacin	0 (0%)	93 (100%)	1 (0.3%)	390 (99.7%)	1
04	Moxifloxacin	0 (0%)	93 (100%)	1 (0.3%)	390 (99.7%)	1
S. aureus						
01	Ciprofloxacin	4 (3.4%)	116 (96.6%)	407 (15.9%)	2160 (84.1%)	0.0001
02	Ofloxacin	4 (3.4%)	116 (96.6%)	408 (15.9%)	2160 (84.1%)	0.0001
03	Levofloxacin	1 (2.7%)	36 (97.3%)	206 (13.1%)	1367 (86.9%)	0.08
04	Moxifloxacin	1 (2.7%)	36 (97.3%)	165 (10.2%)	1467 (89.8%)	0.17

with high-level *in vitro* MRSA resistance, one should consider alternative therapies to fluoroquinolones, if MRSA is a likely ophthalmic pathogen. The In Vitro Antibiotic Testing Group has studied the antibiotic susceptibilities of 1291 ocular isolates in North and South America by both disk- and broth-dilution methods. The fluoroquinolones tested (ciprofloxacin, ofloxacin and norfloxacin) had higher overall *in vitro* efficacy than the other antibiotics tested. Among them, ofloxacin had the highest *in vitro* efficacy against Gram-positive organisms.[9]

In our study, we saw an increase in resistance of *S. aureus* to aminoglycosides (gentamicin, tobramycin, amikacin and netilmicin) in recent years, which did not, however, reach statistical significance. However, *S. aureus* isolates showed >96% susceptibility to quinolones. Moreover, *S. aureus* ocular isolates were more susceptible to quinolones compared to nonophthalmic isolates (Table 4). Emerging fluoroquinolone resistance is an issue in several parts of the world.[10] Goldstein et al have reviewed all cases of bacterial keratitis presenting to the Eye and Ear Institute in Pittsburgh from 1993 to 1997, and showed significantly increasing resistance rates of *S. aureus* keratitis isolates to ciprofloxacin, from 5.8% in 1993 to 35% in 1997.[11] In the same study, *Streptococcus* spp. and CoNS also showed significant resistance to fluoroquinolones, but no change in resistance over the years.

Marangon et al from the Bascom Palmer Eye Institute in Miami, USA, have shown that ciprofloxacin and levofloxacin resistance among MSSA corneal and conjunctival isolates is increasing.[12] More specifically, ciprofloxacin resistance among corneal and conjunctival *S. aureus* isolates during 1990–1995 was 8% (range: 3–11%), whereas the rate of resistance during 1996–2001 was 20.7%. Baseline ciprofloxacin resistance for MSSA isolates was 2% versus 55.8% for MRSA isolates. The levofloxacin-resistance rate for MSSA was 4.7% versus 11.9% for ciprofloxacin. In MRSA isolates, a significantly higher resistance rate was found for ciprofloxacin (95.7%) versus levofloxacin (82.1%).[12] The claim that fourth-generation fluoroquinolones are statistically more potent than second-generation fluoroquinolones against Gram-positive bacteria has been shown in other studies as well.[13] However, due to pharmacokinetic differences, not all fourth-generation fluoroquinolones are similar in the way that they eradicate bacterial ophthalmic pathogens. For example, the ophthalmic solution of 0.3% gatifloxacin eradicates bacteria implicated in postoperative ocular infections substantially faster than the ophthalmic solution of 0.5% moxifloxacin.[14] Regarding moxifloxacin, it has been shown to kill bacteria more rapidly than nonfluoroquinolone topical ocular antibiotics.[15] Scoper has reviewed studies that investigated the therapeutic potential of levofloxacin and has compared it to that of existing fourth-generation fluoroquinolones.[16] Eight eligible studies published between 2002 and 2008 have been reviewed. The five *in vitro* studies have demonstrated that moxifloxacin and gatifloxacin are statistically more potent than levofloxacin against Gram-positive organisms. *In vivo* animal models testing moxifloxacin or gatifloxacin against 0.5% levofloxacin have demonstrated that fourth generation agents are superior to levofloxacin for prophylaxis of infections due to Gram-positive bacteria.

We saw an increase in resistance of *S. aureus* against penicillin and a decrease in resistance of this pathogen against trimethoprim–sulfamethoxazole in recent years. Spread of penicillin resistance among *S. pneumoniae* ocular strains has been well known for several years. In a study from India, among the 25 isolates of *S. pneumoniae* derived from 617 ophthalmic specimens, only four (16%) exhibited intermediate resistance to penicillin.[17] By contrast, among the 25 non-typable ocular isolates of *S. pneumoniae* tested by Kojima et al in 2006, 40% were intermediately resistant to penicillin (MIC: 0.1–1 μg/mL).[18]

With regard to chloramphenicol, this agent has been shown to be very effective against ocular infections due to Gram-positive bacteria. In the study by Schaefer et al, the sensitivity rate of Gram-positive strains to chloramphenicol was 98%.[1] Fukuda et al have found that chloramphenicol sensitivity of MRSA isolates from bacterial conjunctivitis cases was 98%.[19] Moreover, in the same study, the *in vivo* efficacy of chloramphenicol eye drops in the treatment of MRSA conjunctivitis was 81%. In our study, 99% of the *S. pneumoniae* isolates, and 79% of the *S. aureus* isolates were susceptible to this agent *in vitro*.

Regarding CoNS, 92% of the isolates were susceptible to ciprofloxacin and 93% to ofloxacin. In the study by Elsahn et al, 65% of the 29 methicillin-resistant CoNS were susceptible to the fourth generation fluoroquinolones and gentamicin.[8] We saw a significant increase in susceptibility of CoNS to aminoglycosides, predominantly amikacin and to a lesser degree tobramycin, over the period 2005–2009; probably due to the decreased use of topical aminoglycosides in favor of quinolones for ophthalmic infections in recent years. Chlorampenicol was effective *in vitro* against 82% (176/214) of CoNS isolates; a finding similar to previous studies. In a study by Chalita et al, chloramphenicol was effective against 87% of the corneal and 88.5% of the conjunctival isolates of CoNS.[20] Regarding cephalothin, 41.1% (88/214) of our CoNS isolates were resistant to this first-generation cephalosporin; a similar percentage to *S. aureus* (45/120 or 37.5%). In a study from Australia, 80% of Gram-positive isolates from patients with keratitis over a 5-year period were susceptible to cephalothin.[21] Hence, the antibiotic susceptibility patterns of CoNS show substantial geographic variability that should be taken into account when choosing antibiotic therapy for ophthalmic infections.

The overall *in vitro* susceptibility rate of the three Gram-positive organisms of interest to ciprofloxacin was high at 95.1% (406/427) with the low resistance being limited to *S. aureus* (3.3% or 4/120) and CoNS (7.9% or 17/214) isolates alone. Among 485 cases of bacterial keratitis referred to the Massachusetts Eye and Ear Infirmary over two consecutive annual 10-month periods in 1999 and 2000, the cumulative resistance of Gram-positive isolates to ciprofloxacin was 12% and 22%, respectively; figures substantially higher than ours.[22]

Our study was limited by the lack of clinical follow-up. Thus, we did not capture data for the type of antibiotic chosen by the treating physicians, the possible modifications made in the initial antibiotic regimens based on the results of the *in vitro* antibiotic susceptibility testing, and most importantly, the clinical outcomes of the patients treated.

In conclusion, the *in vitro* antibiotic susceptibilities of *S. pneumoniae* and to a lesser extend of *S. aureus* and of

CoNS have not changed substantially in our region over the past decade. Quinolones appear to be an excellent treatment option for cases of bacterial conjunctivitis and keratitis in Crete, because they cover almost all ocular *S. pneumoniae* and *S. aureus* ophthalmic isolates, and >90% of CoNS isolates. Although MRSA ocular isolates are more resistant to quinolones compared to MSSA isolates, the overall low rates of resistance to quinolones make these agents the antibiotics of choice in cases of bacterial conjunctivitis and keratitis. Additionally, chloramphenicol is an excellent treatment option for ocular infections due to *S. pneumoniae*. Continued surveillance of the antibiotic susceptibility patterns of bacterial pathogens implicated in ocular infections in our region will provide valuable data to ophthalmologists and other clinicians for the selection and use of the most appropriate empirical antimicrobial agents.

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