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LABORATORY STUDY

Comparison of *in vitro* susceptibilities of Gram-positive cocci isolated from ocular infections against the second and fourth generation quinolones at a tertiary eye care centre in South India

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Abstract

Purpose **To compare the *in vitro* antimicrobial susceptibilities of Gram-positive cocci isolated from the ocular infections to the second and fourth generation fluoroquinolones at a tertiary eye care centre in south India.**

Methods **A retrospective review of microbiology records at LV Prasad eye institute, Hyderabad, India, identified 787 Gram-positive cocci isolated from different ocular infections between January 2005 to May 2008.The isolates were identified using culture characteristics and biochemical tests. *In vitro* antibiotic susceptibility of the isolates was determined by using Kirby–Bauer disc diffusion method. We analysed the susceptibility data of ciprofloxacin, ofloxacin, gatifloxacin, and moxifloxacin.**

Results **Out of 787 isolates, 147 (18.7%) were *Staphylococcus aureus*, 279 (35.2%) were coagulase-negative Staphylococci, 357 (45.4%) were *Streptococcus pneumoniae*, and 4 (0.4%) were other Streptococcus species. Of the four quinolones, susceptibility to gatifloxacin was highest (85.6%) followed by ofloxacin (65.6%), moxifloxacin (63.9%), and ciprofloxacin (60.5%). In all, 33 (4.2%) of 787 isolates were resistant to all the four fluoroquinoles. *S. aureus* and coagulase-negative Staphylococcus isolates that were resistant to ciprofloxacin and ofloxacin were most susceptible to gatifloxacin. *S. pneumoniae* were more susceptible to gatifloxacin, ofloxacin, and ciprofloxacin than moxifloxacin.**

Conclusions **In our institute, we observed that gatifloxacin is more potent than moxifloxacin against Gram-positive cocci isolated from ocular infections.**

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Keywords: Staphylococcus aureus; coagulase-negative Staphylococcus; ciprofloxacin; gatifloxacin; moxifloxacin

Introduction

In 1962, the first quinolone, nalidixic acid was introduced. Nalidixic acid has moderate Gram-positive coverage and minimal systemic distribution.[1] The structural modifications of quinolones have resulted in the development of the second, third, and fourth generation quinoles.[2] The second generation quinolones (ciprofloxacin and ofloxacin) have expanded Gram-negative coverage, but limited Gram-positive coverage.[1] Ciprofloxacin was approved for use as topical therapy for bacterial corneal ulcers in 1990.[1] Ofloxacin, another second generation quinolone, was approved for treatment of corneal ulcers in 1996.[1] In the late

1990s, several studies showed excellent efficacy of the second generation fluoroquinolones in treating bacterial keratitis and conjunctivitis.[3–6] This resulted in widespread use of these molecules as monotherapy in treatment of various ocular infections. Development of the third generation quinolones (levofloxacin) improved Gram-positive coverage while retaining Gram-negative activity. Levofloxacin solution was approved for ophthalmic use in the year 2000.[1]

Around the beginning of the 20th century, a number of studies reported emerging resistance to the second and third generation quinolones, primarily among Gram-positive ocular isolates.[7–9] The fourth generation quinolones have been introduced to ophthalmology to counteract this resistance. The fourth generation quinolones (gatifloxacin and moxifloxacin) have improved Gram-positive coverage and retained Gram-negative activity.[1] Minimum inhibitory concentrations of the fourth generation fluoroquinolones suggests that the fourth generation fluoroquinolones are more effective than the second and third generation quinolones against Gram-positive bacteria, including the second and third generation fluoroquinolone resistant Staphylococcus species isolated from endophthalmitis and keratitis cases.[10,11] Mather *et al*[11] suggested that among the fourth generation quinolones, moxifloxacin is more potent than gatifloxacin for Gram-positive bacteria, and both are equally potent towards Gram-negative bacteria.

In this *in vitro* study, the susceptibility of Gram-positive cocci isolated from various ocular infections were tested against the fourth generation fluoroquinolones, and the results were compared with the second generation fluoroquinolones.

Materials and methods

A retrospective review of microbiology records at LV Prasad Eye Institute, Hyderabad, India, identified 787 Gram-positive cocci isolated from different ocular infections between January 2005 and May 2008. The organisms were identified by conventional biochemical tests and by using Mini API (bioMérieux, France).

Antibiotic susceptibility of the isolates was determined by using Kirby–Bauer disc diffusion method. Antibiotic discs were obtained from Hi media, Mumbai, India, and always tested for their potency once in 15 days and also as and when a new batch of disc is introduced in to the laboratory using standard ATCC (American Type Culture Collection) bacteria (*S. aureus*-25923, *P. aeruginosa*-27853, and *Escherichia coli*-25922) as a general quality control laboratory procedure.

Antibiotic susceptibility of Staphylococcus species was performed on Cation-adjusted Mueller Hinton agar, and that of the Streptococcus species was performed on Mueller Hinton agar with 5% sheep blood. Plates were incubated at 35°C for 16–18 h in non-CO_2 incubator. The results were interpreted as per the guidelines of the Clinical Laboratory Standards Institute.

The bacterial groups when applicable were designated as fluoroquinolone resistant or susceptible based on disc diffusion susceptibility testing to ciprofloxacin and ofloxacin. Selection of resistance to ciprofloxacin and ofloxacin was desired to test any possible advantage for moxifloxacin and gatifloxacin.

Statistical analysis

Statistical analysis was performed by using Fishers' exact test or χ^2-test as appropriate.

Results

Out of 787 isolates, 147 (18.7%) were *Staphylococcus aureus*, 279 (35.2%) were coagulase-negative Staphylocci, 357 (45.4%) were *Streptococcus pneumoniae*, and 4 (0.4%) were other Streptococcus species. Antibiotic susceptibility of 787 gram-positive cocci is shown in Table 1. Of all the four quinolones tested, Organisms showed the highest susceptibility to gatifloxacin (85.6%) followed by ofloxacin (65.6%), moxifloxacin (63.9%), and ciprofloxacin (60.5%). Out of 787 isolates tested, 33 (4.2%) were resistant to all the four fluoroquinoles. The number of isolates that were intermediately sensitive to ciprofloxacin (11.1%) was more when compared with other quinolones.

Comparison of susceptibilities of Gram-positive cocci isolated from keratitis, endophthalmitis, and other specimens (conjunctival swabs, swabs from orbital abscess, and lachrymal abscess) to quinolones is shown in Table 2. Susceptibilities of quinolones against Gram-positive cocci isolated from keratitis, endophthalmitis, and other specimens (conjunctival swabs, swabs from orbital abscess, and lachrymal abscess) were similar to over all susceptibility data; that is, gatifloxacin has increased susceptibility to all

Table 1 Antibiotic susceptibility of Gram-positive isolates to different quinolones

Antibiotic	*Susceptibility*		
	Sensitive (%)	*Intermediate (%)*	*Resistant (%)*
Ciprofloxacin	476 (60.5)	87 (11.1)	223 (28.3)
Ofloxacin	516 (65.6)	4 (0.5)	265 (33.7)
Gatifloxacin	674 (85.6)	66 (8.4)	41 (5.2)
Moxifloxacin	503(63.9)	29 (3.7)	185(23.5)

Table 2 Susceptibilities of Gram-positive cocci isolated from keratitis, endophthalmitis, and other specimens to quinolones

Antibiotis	*Keratitis isolates* (n = *409*)			*Endophthalmitis isolates* (n = *80*)			*Other specimens* (n = *298*)		
	Sensitive (%)	*Intermediate (%)*	*Resistant (%)*	*Sensitive (%)*	*Intermediate (%)*	*Resistant (%)*	*Sensitive (%)*	*Intermediate (%)*	*Resistant (%)*
Ciprofloxacin	261 (63.8)	48 (11.7)	100 (24.4)	66 (82.5)	9 (11.2)	5 (6.2)	146 (48.9)	30 (10.0)	122 (40.9)
Ofloxacin	284 (69.4)	2 (0.5)	123 (30.0)	69 (86.2)	0 (0)	11 (13.8)	160 (53.6)	2 (0.6)	136 (45.6)
Moxifloxacin	279 (68.2)	13 (3.1)	117 (28.6)	68 (85)	2 (2.5)	10 (12.5)	155 (52)	14 (4.6)	125 (41.9)
Gatifloxacin	361 (88.2)	27 (6.6)	21 (5.1)	74 (92.5)	3 (3.7)	3 (3.7)	236 (79.1)	35 (11.7)	23 (7.7)

Gram-positive cocci isolated from keratitis, endophthalmitis, and other specimens.

Table 3 details the comparisons of antibacterial susceptibilities to the second and fourth generation fluoroquinolones. Out of 39 ciprofloxacin- and ofloxacin-sensitive *S. aureus* isolates, all the 39 isolates were sensitive to gatifloxacin and only 37 (94.9%) were sensitive to moxifloxacin ($P = 0.494$). Out of 97 ciprofloxacin and ofloxacin resistant *S. aureus* isolates, 40 (41%) were sensitive to gatifloxacin and only 7 (7.2%) were sensitive to moxifloxacin ($P < 0.0001$). Of 120 ciprofloxacin- and ofloxacin-sensitive coagulase-negative staphylococci, 119 (99%) were susceptible to gatifloxacin and 103 (85%) were sensitive to moxifloxacin ($P < 0.0001$). Of 111 ciprofloxacin- and ofloxacin-resistant coagulase-negative staphylococci, 84 (75.7%) were susceptible to gatifloxacin and 28 (25.2%) were sensitive to moxifloxacin ($P < 0.0001$). Of 301 ciprofloxacin and ofloxacin sensitive *S. pneumoniae*, 294 (97%) were susceptible to gatifloxacin and 251 (83%) were sensitive to moxifloxacin ($P < 0.0001$). Of five ciprofloxacin- and Ofloxacin-resistant *S. pneumoniae*, two (40%) were susceptible to gatifloxacin and one (20%) was sensitive to moxifloxacin. Streptococcus species other than *S. pneumoniae* were in small numbers and were not included in the table for comparison.

Comparison of fourth generation fluoroquinolones showed that the organisms were highly susceptible to gatifloxacin when compared to moxifloxacin.

Discussion

The fluoroquinolone antibiotics are unique class of antibacterial agents with a broad spectrum of antimicrobial activity against most aerobic Gram-negative and Gram-positive bacteria. They have low toxicity, safety, good ocular surface penetration, prolonged tear film concentration, stability at room temperature, and easy availability.[12–14] The emergence of resistance among Gram-positive organisms to the second generation fluoroquinolones was first noticed in 1990 in patients with community-acquired pneumonia caused by *S. pneumoniae*.[15] A number of recent studies have reported resistance to the second and third generation fluoroquinolones among Gram-positive ocular isolates.[7–9] Goldstein *et al*[7] found increased resistance of *S. aureus*, coagulase-negative staphylococci, and Streptococcus species to the second and third generation fluoroquinolones. Alexandrakis *et al*[16] found that there was increasing laboratory resistance of *S. aureus* keratitis isolates to quinolones from 11% in 1990 to 28% in 1998.

The fourth generation fluoroquinolones moxifloxacin and gatifloxacin were introduced in 2003, and offer improved spectrum of activity, increased penetration into ocular tissues, and delayed propensity to the development of bacterial antibiotic resistance.[17] A retrospective *in vitro* study by Mather *et al*[11] compared the potencies and antibiotic susceptibilities of ciprofloxacin, ofloxacin, levofloxacin, gatifloxacin, and moxifloxacin against 93 bacterial isolates of endophthalmitis. The second generation fluoroquinolone-resistant *S. aureus* was statistically more susceptible to moxifloxacin than the other fluoroquinolones. Coagulase-negative Staphylococcus were statistically more susceptible to gatifloxacin and moxifloxacin than levofloxacin, ciprofloxacin, and ofloxacin. They concluded that the fourth generation fluoroquinolones are more potent than the second and third generation quinolones for Gram-positive bacteria, and the fourth generation fluoroquinolones appear to cover the second and third generation fluoroquinolones resistance among Staphylococcus species. Among the gatifloxacin and moxifloxacin, they found moxifloxacin to be more potent than gatifloxacin for Gram-positive bacteria. Stroman *et al*[18] presented similar data showing a high potency of moxifloxacin against fluoroquinolones-resistant isolates of *S. aureus* and *Staphylococcus epidermidis* collected from patients with bacterial conjunctivitis and blepharitis. Kowalski *et al*[10] compared the *in vitro* activity of gatifloxacin, moxifloxacin, levofloxacin, ofloxacin, and ciprofloxacin against 177 bacterial keratitis isolates. They found that the fourth generation fluoroquinolones provide greater antibacterial activity against the Gram-positive keratitis isolates and appear to cover many second generation fluoroquinolone-resistant Staphylococcus isolates. Moxifloxacin showed greater susceptibility for the second generation fluoroquinolone-resistant *S. aureus*.

Table 3 Comparison of susceptibilities of Gram-positive cocci isolated from different ocular infections to the second and fourth generation fluoroquinolones

Organism	*Isolates from different infections (keratitis, endophthalmitis, and other specimens)*			*Keratitis isolates*			*Endophthalmitis*		
	N	*GF sensitivity (%)*	*MF sensitivity (%)*	N	*GF sensitivity (%)*	*MF sensitivity (%)*	N	*GF sensitivity (%)*	*MF sensitivity (%)*
Ciprofloxacin- and ofloxacin-sensitive *S. aureus*	39	39 (100)	37 (94.9)	—	—	—	—	—	—
Ciprofloxacin- and ofloxacin-resistant *S. aureus*	97	40 (41.2)	7 (7.2)	29	13 (44.8)	4 (13.8)	1	1 (100)	0 (0)
Ciprofloxacin- and ofloxacin-sensitive CONS	120	120 (100)	103 (85.3)	—	—	—	—	—	—
Ciprofloxacin- and floxacin-resistant CONS	111	84 (75.7)	28 (25.2)	60	47 (78.3)	17 (28.3)	4	3 (75)	1 (25)
Ciprofloxacin- and floxacin-sensitive *S. pneumoniae*	301	294 (97.7)	251 (83.5)	—	—	—	—	—	—
Ciprofloxacin- and ofloxacin-resistant *S. pneumoniae*	5	2 (40)	1 (20)	—	—	—	—	—	—

CONS, coagulase-negative staphylococci; MF, moxifloxacin; GF, gatifloxacin.

Our results are contrary to these earlier reports. We found that, Gram-positive cocci were more susceptible to gatifloxacin (85.6%) when compared with other quinolones. Though moxifloxacin is a fourth generation fluoroquinolone, it showed activity comparable to that of (63.9%) ofloxacin (65.6%) and ciprofloxacin (60.1%). *S. aureus* isolates susceptible to ciprofloxacin and oflaxacin were also susceptible to gatifloxacin, but only 94% of these isolates were susceptible to moxifloxacin. Among *S. aureus* isolates that were resistant to ciprofloxacin and oflaxacin, only 7% of were sensitive to moxifloxacin, whereas 40% were sensitive to gatifloxacin.

We noticed similar trends for coagulase-negative Staphylococcus and *Streptococcus pneumoniae*. One must be careful in interpretation of these results and its applications in clinical practice because (1) the *in vitro* test is based on the blood concentrations achieved on systemic administration; (2) concentration achieved in ocular tissues after topical administration are much higher; (3) determination of minimum inhibitory concentration gives better approximation of the degree of resistance; (4) the concentration achieved in the ocular tissue depends on the concentration of the drug, and moxifloxacin is marketed as 0.5% solution, whereas gatifloxacin is marketed as 0.3%.The study results are from a single tertiary eye care centre, not representative of the whole region, and susceptibility of bacteria varies from region to region.

Conclusion

On the basis of *in vitro* antibiotic susceptibility test, using Kirby–Bauer disc diffusion test, gatifloxacin was found to have better acitivity against all Gram-positive cocci, including those resistant to the second generation quinolones at our institute.

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