Fluoroquinolone susceptibilities to methicillin-resistant and susceptible coagulase-negative *Staphylococcus* isolated from eye infection

Suscetibilidade dos Staphylococcus *coagulase negativo meticilina-resistentes e suscetíveis isolados em infecções oculares*

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ABSTRACT

Purpose: To evaluate the fluoroquinolone susceptibilities of ocular isolate coagulase-negative staphylococci (CoNS), identified at the Microbiology Laboratory - UNIFESP. **Design**: Experimental laboratory investigation. **Methods:** The minimum inhibitory concentrations (MICs) of 21 strains of methicillin-resistant coagulase-negative staphylococci (MRCoNS) and 22 methicillin-sensitive coagulase-negative staphylococci (MSCoNS) to ciprofloxacin, ofloxacin, gatifloxacin and moxifloxacin were determined, using the E-test method standardized by the Clinical and Laboratory Standards Institute (CLSI/NCCLS). **Results**: The MIC^{90s} (μg/ml) for the second generation of tested fluoroquinolones were higher than the fourth generation, especially for the methicillin-resistant coagulase-negative staphylococci group. **Conclusion:** Our results indicate that methicillin-sensitive coagulase-negative staphylococci are more susceptible to quinolones than are methicillin-resistant coagulase-negative staphylococci and that fourth generation fluoroquinolones appear to be more potent, affecting even coagulase-negative staphylococcal strains resistant to second generation fluoroquinolones.

Keywords: Drug resistance, microbial; Eye infections, bacterial/microbiology; Staphylococcus/isolation & purification; Coagulase; Fluoroquinolones; Methicillin-resistance

INTRODUCTION

Coagulase-negative staphylococci are commonly isolated bacteria mixed with the more typical ocular flora, and can lead to major infections, including keratitis, conjunctivitis and endophthalmitis. These infections represent serious ocular conditions with significant sight-threatening consequences, particularly if aggressive, appropriate therapy is not instituted in a timely manner[1-4].

In recent years, many new antibiotics and synthetic antimicrobial eye drops have been developed and used in the treatment of ocular infections. Any one of these, however, may alter drug sensitivity of bacteria on the ocular surface. There are many reports in the ophthalmic literature concerning infections due to methicillin-resistant coagulase-negative staphylococci[5-7].

The quinolones are one class of antibiotics that has been used to treat these infections, since their introduction into the ophthalmic community in 1991[8-9].

Ciprofloxacin and ofloxacin have soon achieved great acceptance in the treatment of ocular infections. These antibiotics (ciprofloxacin, ofloxacin,

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moxifloxacin and gatifloxacin) provide: broad-spectrum coverage against most aerobic gram-negative and gram-positive bacteria, low toxicity, safety, good ocular surface penetration, prolonged tear film concentration, stability at room temperature, and good availability[5,10]. Unfortunately, recent reports of bacterial resistance to these second generation fluoroquinolones have had an impact on their acceptance, with a decrease in their use and the introduction of the fourth generation fluoroquinolones as an alternative to increase the spectrum of action, mostly against gram-positive cocci[5,10-14].

Microbial resistance to fluoroquinolones is the result of genetic changes in one or more of four major bacterial mechanisms: enzymes for DNA synthesis; gyrase protecting proteins; cell permeability or drug efflux[15].

The 8-methoxyfluoroquinolones, gatifloxacin and moxifloxacin, have enhanced activity against gram-positive pathogens, thereby increasing the probability that strains resistant to the older fluoroquinolones will be susceptible. These newer fluoroquinolones are less prone to encourage the development of resistance on a number of fronts, primarily because of their higher activity against gram-positive pathogens, but for other reasons as well, such as the necessity of two mutations: one in the topoisomerase IV and a second in the DNA gyrase gene[12].

The purpose of this study was to evaluate and compare the antibiotic susceptibility results of CoNS isolated from eye infections at the Microbiology Laboratory of the Vision of Institute, Federal University of São Paulo, Brazil.

METHODS

Forty-three CoNS ocular isolates were studied and divided into two groups according to resistance to methicillin; these consisted of 21 methicillin-resistant organisms (3 endophthalmitis, 10 keratitis and 8 conjunctivitis) and 22 methicillin-sensitive organisms (11 endophthalmitis, 9 keratitis and 2 conjunctivitis).

Methicillin-resistance of the strains was determined by the Kirby-Bauer disk diffusion method using 1 µg oxacillin disk (Cecon, São Paulo, Brazil) according to the criteria of the Clinical and Laboratory Standards Institute (CLSI/NCCLS)[16].

Antibiotic susceptibility of each bacterial isolate to ciprofloxacin, ofloxacin, gatifloxacin and moxifloxacin was determined by two methods: Kirby-Bauer disk diffusion (Cecon, São Paulo, Brazil) and E-test (AB Biodisk, Solna, Sweden)[14]. Interpretation for susceptible and non-susceptible (intermediate and resistant) responses were in accordance with the criteria of the Clinical and Laboratory Standards Institute (CLSI/NCCLS) document M100-S15, Vol. 25 Nº1NCCLS: ciprofloxacin S= <1 µg/ml, R= >4 µg/ml; ofloxacin S= <1 µg/ml, R= >4 µg/ml; gatifloxacin S= <0,5 µg/ml, R= >2 µg/ml; moxifloxacin S= <0,5 µg/ml, R= >2 µg/ml[16].

Minimal inhibitory concentration 90s (MIC^{90}) were determinated for each group, methicillin-resistant and methicillin-sensitive coagulase-negative staphylococci.

The results were statistically evaluated by the Mann-Whitney and the Friedman tests.

RESULTS

Table 1 summarizes the MIC^{90} of the 21 methicillin-resistant isolates and table 2 summarizes the MIC^{90}s of the 22 methicillin-sensitive isolates to ciprofloxacin, ofloxacin, gatifloxacin, and moxifloxacin.

For all tested antibiotics, MIC values were statistically higher for the methicillin-resistant group than for the methicillin-sensitive group ($p<0.05$) (Table 3).

Comparing the MIC values for the four antibiotics by the Friedman test, those for ofloxacin were statistically significantly higher in both groups: ofloxacin > ciprofloxacin > (gatifloxacin=moxifloxacin) ($p<0.001$) (Table 3).

There was no statistically significantly difference between both groups comparing the resistance of the four antibiotics by the two methods (Tables 4 and 5).

Table 1. Susceptibilities of methicillin-resistant coagulase-negative *Staphylococcus* isolates to fluoroquinolones

	Range (µg/ml)	MIC^{90} (µg/ml)	% Sensitivity disc	% Sensitivity E-test
Cipro	0.047 – 32	32	52.4%	28.60%
Oflox	0.094 – 32	32	57.1%	23.80%
Gati	0.032 – 2.0	2.0	71.4%	38.10%
Moxi	0.023 – 3.0	3.0	81.0%	33.33%

MIC= minimum inhibitory concentrations; Cipro= ciprofloxacin; Oflox= ofloxacin; Gati= gatifloxacin; Moxi= moxifloxacin

Table 2. Susceptibilities of methicillin-sensitive coagulase-negative *Staphylococcus* isolates to fluoroquinolones

	Range (µg/ml)	MIC^{90} (µg/ml)	% Sensitivity disc	% Sensitivity E-test
Cipro	0.064 – 0.25	0.25	95.5%	95.5%
Oflox	0.190 – 1.0	1.0	95.5%	90.9%
Gati	0.047 – 0.125	0.125	100.0%	95.5%
Moxi	0.032 – 0.125	0.125	95.5%	95.5%

MIC= minimum inhibitory concentrations; Cipro= ciprofloxacin; Oflox= ofloxacin; Gati= gatifloxacin; Moxi= moxifloxacin

Table 3. Descriptives measurements: 1st and 3rd quartiles and medians of MIC values of both groups

Antibiotics	MR CoNS (n=21)	MS CoNS (n=22)	Mann-Whitney test
Ciprofloxacin	0.820 – 7.000 3.00	0.125 – 0.190 0.158	p<0.001
Ofloxacin	1.190 – 32.000 8.00	0.250 – 0.500 0.380	p=0.001
Gatifloxacin	0.188 – 2.000 1.50	0.064 – 0.102 0.094	p<0.001
Moxifloxacin	0.380 – 1.750 1.00	0.064 – 0.094 0.094	p<0.001

MIC= minimum inhibitory concentrations; MR CoNS= Methicillin-resistant coagulase-negative *Staphylococcus*; MS CoNS= Methicillin-susceptible coagulase-negative *Staphylococcus*

Table 4. Antibiotic % susceptibilities of both groups by the Kirby-Bauer disk method

Groups	Result	Cipro	Oflox	Gati	Moxi	Friedman test
MR CoNS	Not susceptible	10 (47.6%)	9 (42.9%)	6 (28.6%)	4 (19.0%)	p=0.096
(n=21)	Susceptible	11 (52.4%)	12 (57.1%)	15 (71.4%)	17 (81.0%)	
MS CoNS	Not susceptible	1 (4.5%)	1 (4.5%)	0 (0.0%)	1 (4.5%)	p=0.733
(n=22)	Susceptible	21 (95.5%)	21 (95.5%)	22 (100%)	21 (95.5%)	

Cipro= ciprofloxacin; Oflox= ofloxacin; Gati= gatifloxacin; Moxi= moxifloxacin; MR CoNS= Methicillin-resistant coagulase-negative *Staphylococcus*; MS CoNS= Methicillin-susceptible coagulase-negative *Staphylococcus*

Table 5. Antibiotic % susceptibilities of both groups using the E-test method

Groups	Result	Cipro	Oflox	Gati	Moxi	Friedman test
MR CoNS	Not susceptible	15 (71.4%)	6 (76.2%)	13 (61.9%)	14 (66.7%)	p=0.232
(n=21)	Susceptible	6 (28.6%)	5 (23.8%)	8 (38.1%)	7 (33.3%)	
MS CoNS	Not susceptible	1 (4.5%)	2 (9.1%)	1 (4.5%)	1 (4.5%)	p=0.392
(n=22)	Susceptible	21 (95.5%)	20 (90.9%)	21 (95.5%)	21 (95.5%)	

Cipro= ciprofloxacin; Oflox= ofloxacin; Gati= gatifloxacin; Moxi= moxifloxacin; MR CoNS= Methicillin-resistant coagulase-negative *Staphylococcus*; MS CoNS= Methicillin-susceptible coagulase-negative *Staphylococcus*

DISCUSSION

As has been well demonstrated in the infectious disease literature, resistant strains are developing with increasing use of an antibiotic[5]. As a result, periodic susceptibility surveys are important to detect emerging resistance patterns, which may become clinically significant.

In the here described study, we examined the in vitro susceptibility of coagulase-negative staphylococci to fluoroquinolone antibiotics. The MIC^{90} values for the methicillin-resistant group were higher then those for the methicillin-susceptible group. Both groups of staphylococci were more susceptible to gatifloxacin and moxifloxacin than they were to ciprofloxacin and ofloxacin.

Table 6. Comparison between MIC^{90} values in our studies and Kolwaski's 2003/USA[13]

		MIC^{90}			
		Moxi	Gati	Cipro	Oflox
MR CoNS	Brazil	3	2	32	32
	Kolwaski 2003	3	3	64	64
MS CoNS	Brazil	0.75	1	2	6
	Kolwaski 2003	0.125	0.19	0.38	0.75

Moxi= moxifloxacin; Gati= gatifloxacin; Cipro= ciprofloxacin; Oflox= ofloxacin; MR CoNS= Methicillin-resistant coagulase-negative *Staphylococcus*; MS CoNS= Methicillin-susceptible coagulase-negative *Staphylococcus*

The differences observed for the diffusion susceptibilities compared with the E-test results for CoNS could be explained by the fact that CLSI/NCCLS considers the standards for all drugs with no deterrents regarding the species. Probably in the future new studies would differentiate the level of antibiotic susceptibility based not on the group of CoNS but based on the species.

In 2002, Mather and colleagues determined the median MICs for 93 bacterial endophthalmitis isolates using the E-test approach. The results of the study demonstrated that comparing the median MICs for the fourth-generation agents, moxifloxacin was more potent than gatifloxacin against fluoroquinolone susceptible CoNS; and equally potent against fluoroquinolone-resistant CoNS[17].

Our results are supported by those of Stroman and associates, who similarly presented MIC data demonstrating the high potency of moxifloxacin[18].

Some authors studying bacterial keratitis isolates, found that fluoroquinolone-susceptible and resistant CoNS were equally susceptible to the second and fourth generation. The MIC of moxifloxacin was equivalent to that of gatifloxacin for fluoroquinolone-resistant CoNS. In our study, the MIC^{90} was lower for gatifloxacin than that of moxifloxacin for MR CoNS, but equal for CoNS[13] (Tables 6 and 7).

Other authors published that ocular *S. epidermidis* retrieved from conjunctivitis are clearly more susceptible to moxi-

Table 7. MIC^{90} values and potency for coagulase-negative *Staphylococcus* isolates to fluoroquinolones

			MIC^{90}			
	n	Potency	Cipro	Oflox	Gati	Moxi
MRCoNS	21	gati > moxi > cipro = oflox	32	32	2	3
MSCoNS	22	moxi > gati > cipro > oflox	0.25	1.0	0.125	0.125

MIC= minimum inhibitory concentrations; Cipro= ciprofloxacin; Oflox= ofloxacin; Gati= gatifloxacin; Moxi= moxifloxacin; MR CoNS= Methicillin-resistant coagulase-negative *Staphylococcus*; MS CoNS= Methicillin-susceptible coagulase-negative *Staphylococcus*

floxacin than ciprofloxacin throughout three different parts of world (USA, Europe and India), based on the MIC^{90}s[19].

Fluoroquinolone antibiotics are concentration-dependent killers in that the MIC must be reached for the antibiotic to be effective. These values are based on CLSI/NCCLS criteria related to the drug concentration that is safely achievable in plasma, and do not reflect the concentration achievable with topical application. Therefore, some isolates classified as intermediate or resistant might be sensitive if the ocular tissue levels are much greater than those of serum. The CLSI/NCCLS standards indicate also that systemic penetration for effective therapy is similar among the fluoroquinolones tested in our study. It would thus be reasonable to assume that similar, perhaps even higher levels can be reached in the ocular tissues. If we assume that antibiotic concentrations in ocular tissues following topical administration are at least equivalent to (if not higher than) systemic penetration, the key parameter to consider in comparing antibiotic effectiveness is the MIC value: the antibiotic with the lowest MIC for a bacterial group would be the most potent. Furthermore, the most potent antibiotic would have the least chance of causing resistance because the concentration in the ocular tissues would less likely be sublethal.

Primary use of the newer fluoroquinolones in preference to use of the older fluoroquinolones provides the potential for helping to forestall the development of resistance, but this approach must be coupled with the overall strategy of avoiding indiscriminate use of and ensuring proper dosing with these antimicrobials[3].

CONCLUSION

Our in vitro study suggests that the fourth generation fluoroquinolones are more potent than are the second and third generation fluoroquinolones against coagulase-negative staphylococci, particularly those resistant to methicillin.

RESUMO

Objetivos: Avaliar a suscetibilidade a fluorquinolonas dos *Staphylococcus* coagulase-negativo (SCoN) identificados no Laboratório de Microbiologia Ocular da UNIFESP. **Métodos**: Foi determinada a concentração inibitória mínima de 21 cepas de SCoN meticilina-resistentes e 22 meticilina-sensíveis para ciprofloxacina, ofloxacina, gatifloxacina e moxifloxacina, utilizando o E-test estandartizado pelo CLSI/NCCLS. **Resultados**: Os MIC^{90} (µg/ml) de 43 SCoN isolados para fluorquinolonas de segunda geração foram maiores do que os de quarta geração, principalmente para o grupo dos meticilina-resistentes. **Conclusão:** Nossos resultados indicam que *Staphylococcus* coagulase-negativo meticilina-sensíveis são mais suscetíveis às quinolonas do que os *Staphylococcus* coagulase-negativo meticilina-resistentes, fluorquinolonas de quarta geração parecem ser mais potentes, cobrindo inclusive cepas de *Staphylococcus* coagulase-negativo resistentes à segunda geração de fluorquinolonas.

Descritores: Resistência microbiana a drogas; Infecções oculares bacterianas/microbiologia; Staphylococcus/isolamento & purificação; Coagulase; Fluoroquinolonas; Resistência a meticilina

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