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Age factor in the fluoroquinolone susceptibility of gram-positive cocci isolates from bacterial keratitis cases between 2008 and 2016

Koji Ueda[1,2] · Takuya Iwasaki[1] · Takashi Ono[1,2] · Jinhee Lee[1] · Ryohei Nejima[1] · Yosai Mori[1] · Yukari Noguchi[1] · Akiko Yagi[1] · Kazunori Miyata[1]

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Abstract

Purpose To determine the relationship between fluoroquinolone susceptibility of gram-positive cocci (GPC) isolated from patients with bacterial keratitis and the age of the patients or the date of onset.

Methods Bacterial isolates were obtained from corneal lesions of patients with infectious keratitis treated between January 2008 and December 2016. The fluoroquinolone susceptibility of GPC was assessed, and a retrospective review of microbiological records was performed. Fluoroquinolone susceptibility was measured through broth microdilution in accordance with protocols of the Clinical and Laboratory Standards Institute. Statistical analysis was performed using a generalized estimating equation and cubic spline to determine the association between fluoroquinolone susceptibility of GPC isolated from corneal lesions and patient age.

Results Of the 1200 bacterial isolates, 471 GPC were identified. They included *Staphylococcus epidermidis* (45.6%), other coagulase-negative *Staphylococcus* sp. (17.8%), and *Staphylococcus aureus* (18.3%). Levofloxacin susceptibility of GPC exhibited a negative relationship with age and had an odds ratio of 0.893 (95% confidence interval, 0.825–0.967) for every 10 years of age. A non-adjusted cubic spline curve was well correlated with year-adjusted data in a generalized additive model, and the levofloxacin susceptibility of GPC was initially stable but gradually declined after 40 years of age, before re-stabilizing again after 70 years of age.

Conclusion The fluoroquinolone susceptibility of GPC isolated from corneal lesions of infectious keratitis is high in children under 15 years of age and declines with an increase in age of patients using a generalized estimating equation and cubic spline.

Keywords Fluoroquinolone · Bacterial keratitis · Age · Gram-positive · *Staphylococcus*

Key messages

- Bacterial keratitis is a potentially vision-threatening disease that requires urgent treatment with antibiotics.
- We found a negative relationship between fluoroquinolone susceptibility of gram-positive cocci and age.
- Furthermore, our study revealed that fluoroquinolone resistance increased at around 40 years of age and plateaued around 70 years of age.

Introduction

Bacterial keratitis is a community-acquired infection and a potentially vision-threatening disease that requires urgent treatment. Broad-spectrum antibiotics are currently the first-line treatment for patients with bacterial keratitis. However, the treatment should be updated to include appropriate antibiotics based on the sensitivity of specimens isolated from the cornea [1]. Several studies have shown that fluoroquinolones

✉ Takashi Ono
taono-tky@umin.ac.jp

[1] Miyata Eye Hospital, Kurahara 6-3, Miyakonojo, Miyazaki 885-0051, Japan

[2] Department of Ophthalmology, Graduate School of Medicine and Faculty of Medicine, The University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-0033, Japan

have excellent efficacy as a primary monotherapy in the treatment of bacterial keratitis [2, 3]. However, fluoroquinolone-resistant species have been isolated from bacterial keratitis corneal lesions since the 1990s [4, 5].

The primary causative agents of bacterial keratitis include gram-positive cocci (GPC) such as *Staphylococcus epidermidis* and *Staphylococcus aureus*, and gram-negative bacteria, *Pseudomonas aeruginosa*. Variation in the prevalence of keratitis caused by *P. aeruginosa* is related to geographical and historical factors [6]. In contrast, the prevalence of GPC-related keratitis has remained relatively constant, possibly because GPCs are part of the normal microbiota of human skin and the ocular surface [7].

Fluoroquinolones interact with bacterial topoisomerase IV and gyrase [8], and have been marketed and widely prescribed for gram-negative and gram-positive bacterial infections since the 1980s. No limitations have been placed on prescribing topical fluoroquinolone for children; however, to mitigate the risk of side effects such as cartilage and tendon damage that affect physical growth, systemic prescription of fluoroquinolones for patients under 16 years of age has been restricted to a few diseases [9].

To reveal the influence of age or the date of the onset of infection on the fluoroquinolone susceptibility of bacteria causing keratitis, we analyzed the fluoroquinolone susceptibility of GPC isolated from bacterial keratitis in a single hospital.

Patients and methods

This study was conducted in accordance with the principles outlined in the Declaration of Helsinki and was approved by the Institutional Research Ethics Committee of the Miyata Eye Hospital. Written informed consent was obtained from all patients after explanation of the nature and possible consequences of the study.

We included patients who underwent bacteriologic analysis for keratitis at Miyata Eye Hospital between January 2008 and December 2016. Microbiological records of the patients were retrospectively reviewed for age, clinical diagnosis, microbiological samples, bacterial species, and antibiotic susceptibility. We excluded patients whose medical information was not consistently available.

Microbiological methods and isolates

All isolates were obtained after administration of topical anesthesia on the ocular surface. Preservative-free 0.4% oxybuprocaine hydrochloride was used and corneal lesions were scraped with a surgical blade under a microscope. Bacterial isolation, identification, and susceptibility tests were performed at the Research Foundation for Microbial Diseases of Osaka University, Suita, Japan.

Bacterial isolation was performed for 24–48 h at 36.5 °C on TSA II 5% sheep blood agar (Becton Dickinson Japan Co. Ltd.) and Drygalski improved medium (Kyokuto Pharmaceutical Industrial Co., Ltd, Tokyo, Japan) or Chocolate II agar medium (Becton Dickinson Japan Co. Ltd.), in an atmosphere containing 5% CO_2. In addition, cultivation under anaerobic conditions was conducted for 24–120 h at 36.5 °C in Chocolate II agar medium. Samples that could not be isolated through direct culture were cultured for 1–2 weeks at 36.5 °C in TGC medium (Nissui, Tokyo, Japan) and were separated.

Antibiotic susceptibility for levofloxacin (LVFX), moxifloxacin, and gatifloxacin was measured using the broth dilution method and was determined in accordance with protocols from the Clinical and Laboratory Standards Institute [10]. Isolates were graded as sensitive (S), intermediate (I), or resistant (R) to the tested antibiotics, with minimal inhibitory concentration (MIC) interpreted against breakpoints from the CLSI. The percentage of antibiotic susceptibility was calculated as 100 (S/S + I + R).

Statistical analysis

To estimate the susceptibility of bacterial isolates, a generalized linear mixed-effects model was used with patients designated as a random effect. The simple and adjusted smoothed curves of bacterial susceptibility with respect to age or year isolated were plotted using a generalized additive model (GAM) with 3 degrees of freedom. All analyses were performed using SAS version 9.4. A p value of less than 0.05 was considered to indicate a statistically significant difference. All values are presented as the mean and 95% confidence interval (CI) unless otherwise mentioned.

Results

Bacterial isolation

In total, 1200 bacterial isolates were obtained from corneal scrapings of 1167 eyes. The mean age of the patients was 50.4 ± 23.1 (mean ± standard deviation) years. Among them, 471 isolates (39.3%) were GPC, 500 were *Cutibacterium* (formerly *Propionibacterium*) species, and 112 were other gram-negative bacteria (GNB) (Table 1). The mean age of the group infected with the GNB was 50.5 years. Among

Table 1 Number of bacterial isolates obtained from the corneal lesions of patients with infectious keratitis

	Total	2008	2009	2010	2011	2012	2013	2014	2015	2016
Gram-positive cocci										
Staphylococcus aureus	86	6	9	6	8	11	18	8	7	13
(Methicillin-resistant *S. aureus*)	(21)	(2)	(4)	(0)	(2)	(3)	(6)	(0)	(1)	(3)
Staphylococcus epidermidis	215	20	21	37	29	26	27	20	21	14
Other coagulase-negative staphylococci	84	2	4	12	14	10	22	11	8	1
Streptococcus pneumoniae	17	1	1	2	1	3	1	2	3	3
Other streptococci	35	5	3	3	7	2	2	4	4	5
Enterococcus faecalis	15	2	3	2	0	2	1	2	1	2
Other gram-positive cocci	19	0	2	4	3	2	2	1	2	3
Gram-positive bacilli										
Corynebacterium sp.	95	9	2	7	9	14	10	13	15	16
Other gram-positive species	22	1	2	3	2	3	4	2	3	2
Gram-negative bacteria										
Haemophilus influenzae	3	0	1	0	0	1	0	1	0	0
Moraxella sp.	8	2	1	1	0	1	0	1	1	1
Pseudomonas aeruginosa	20	1	5	2	1	2	2	0	3	4
Other *Pseudomonas* sp.	8	5	3	0	0	0	0	0	0	0
Serratia marcescens	24	6	4	3	6	0	2	0	2	1
Other *Serratia* sp.	2	1	1	0	0	0	0	0	0	0
Acinetobacter sp.	13	10	3	0	0	0	0	0	0	0
Other gram-negative species	34	5	8	2	4	1	1	3	4	6
Anaerobe										
Cutibacterium sp.	500	53	67	62	66	64	59	46	41	42
Total	1200	129	140	146	150	142	151	114	115	113

the GPC, the majority were *S. epidermidis* (45.6%), other coagulase-negative *Staphylococci* (17.8%), and *S. aureus* (18.3%). Approximately 24.4% of the *S. aureus* isolates were methicillin-resistant. The annual number of infections caused by GPC was consistent enough to conduct statistical analysis. Among the GNB isolates, we identified *Pseudomonas* ($n=20$), *Serratia* ($n=24$), and *Acinetobacter* ($n=13$) species during the observation periods (Table 1). The isolation rate was about 70% during the observation periods (Online Resource Table 1S).

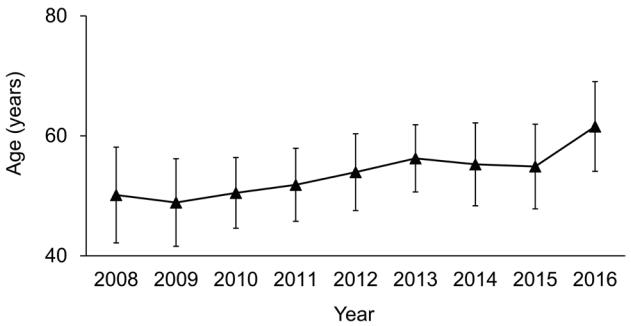

Fig. 1 Annual changes in the mean age (at 95% confidence interval) of patients with corneal lesions positive for gram-positive cocci (GPC), between 2008 and 2016. The average age of patients increased from 50.4 years in 2009 to 61.6 years in 2016 ($p=0.0407$)

Annual changes of isolates and patient age on fluoroquinolone susceptibility

The mean age of patients from whose corneal lesions GPC isolates were obtained gradually increased from 50.4 years in 2008 to 61.6 years in 2016 ($p=0.0407$, Fig. 1), and the proportion of older patients gradually increased (Fig. 2). The mean age of patients from whose corneal lesions *S. epidermidis* isolates were obtained also gradually increased

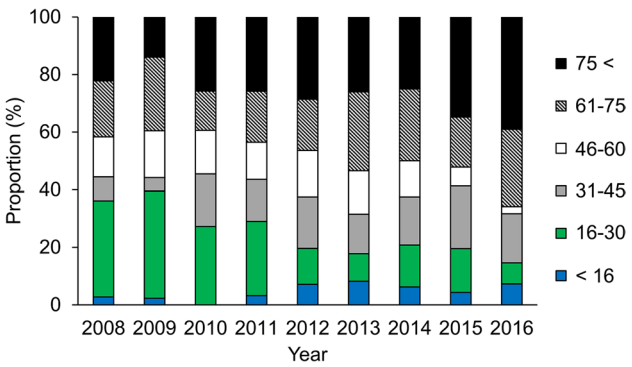

Fig. 2 Annual trend in the percentage distribution categorized per 15 years of life of the patients with corneal lesions positive for GPC from 2008 to 2016. The number of patients under 60 apparently decreased

Table 2 Influence of annual changes and aging on susceptibility of isolates to fluoroquinolone determined using the generalized linear mixed model

	Factor	Odds ratio		*p*
			95% confidence interval	
Gram-positive cocci	Age (10 years)	0.893	0.825–0.967	0.0055
	Annual change	1.009	0.931–1.094	0.8208
Staphylococcus epidermidis	Age (10 years)	0.787	0.682–0.907	0.0042
	Annual change	0.906	0.786–1.045	0.1530

(Online Resource Table 2S). There were only a few patients under the age of 15 in the study. Thus, the number of isolates obtained from these patients was small (Online Resources Table 3S and 4S).

Aging effect on fluoroquinolone susceptibility of GPC

In models that designated both the variables of annual change and patient age as fixed effects, the odds ratio for LVFX susceptibility gradually decreased: 0.893 (0.825–0.967) for GPC and 0.787 (0.682–0.907) for *S. epidermidis* ($p = 0.0055$ and 0.0042, respectively; Table 2). In contrast, no significant association was found between LVFX susceptibility of GPC and *S. epidermidis* with one year of their isolation (Table 2).

The gradual decrease in the LVFX susceptibility of GPC with age in patients was also confirmed by cubic spline analysis. Non-adjusted smooth curves of the mean LVFX susceptibility of GPC and *S. epidermidis* against patient age (blue plots, Figs. 3 and 4) suggested that the LVFX resistance of GPC increased at around 40 years of age and plateaued around 70 years of age. The same tendency was observed by the plots of onset-date-adjusted LVFX susceptibility of GPC and *S. epidermidis* (red circles, Figs. 3 and 4).

Comparison of different fluoroquinolone antibiotic susceptibilities of GPC

Finally, we compared the susceptibility of *S. epidermidis* isolates to three different fluoroquinolones: LVFX, gatifloxacin, and moxifloxacin. No significant differences were observed between the fluoroquinolones (Table 3).

Discussion

In this study, we retrospectively analyzed the microbiological data of isolates from corneal lesions of bacterial keratitis obtained between 2008 and 2016. We hypothesized that fluoroquinolone resistance of GPC in the general population would increase over the years. However, statistical analysis revealed that fluoroquinolone resistance increases around age 40 and plateaus around age 70. Systemic administration

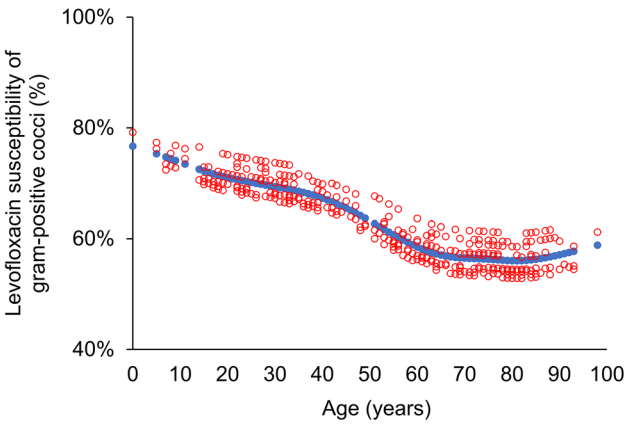

Fig. 3 Age-dependent changes in levofloxacin (LVFX) susceptibility of GPC isolated from corneal lesions. The non-adjusted smoothed curve (closed blue circle) indicates LVFX susceptibility of GPC with respect to age, using a generalized additive model with three degrees of freedom. Open red circle indicates onset-date-adjusted average LVFX susceptibility of GPC, adjusted to the year of isolation. Both plots show corresponding decrease with increase in age

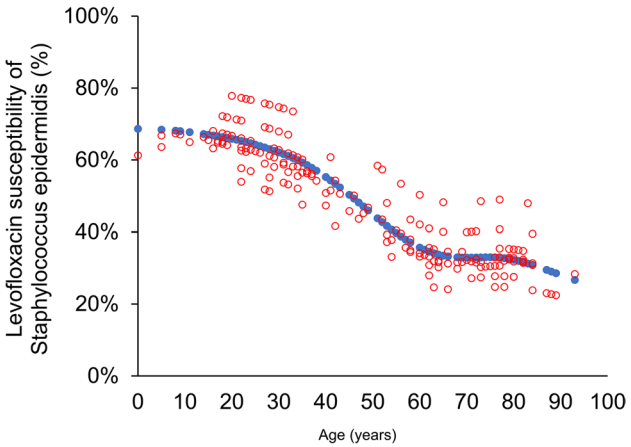

Fig. 4 Age-dependent changes in levofloxacin (LVFX) susceptibility of *Staphylococcus epidermidis* isolated from corneal lesions. The non-adjusted smoothed curve (closed blue circle) indicates LVFX susceptibility of GPC with respect to patient age using a generalized additive model with three degrees of freedom. Open red circle indicated onset-date-adjusted average LVFX susceptibility of GPC, adjusted to the year of isolation. Both plots show a corresponding decrease with the increase in age

Table 3 Correlation between susceptibility of *Staphylococcus epidermidis* isolates obtained during 2014–2016 to levofloxacin (LVFX), moxifloxacin (MFLX), and gatifloxacin (GFLX)

Year(s)	MFLX	LVFX		Reproducibility	Kappa coefficient	
		I+R	S		Mean	95% confidence interval
2014	I+R	9	0	95.0	0.900	0.710–1.000
	S	1	10			
2015	I+R	13	0	100.0	1.000	1.000
	S	0	8			
2016	I+R	9	0	100.0	1.000	1.000
	S	0	5			
2014–2016	I+R	31	0	98.2	0.963	0.891–1.000
	S	1	23			
Year(s)	GFLX	LVFX		Reproducibility	Kappa coefficient	
		I+R	S		Mean	95% confidence interval
2014	I+R	10	0	100.0	1.000	1.000
	S	0	10			
2015	I+R	13	0	100.0	1.000	1.000
	S	0	8			
2016	I+R	9	0	100.0	1.000	1.000
	S	0	5			
2014–2016	I+R	32	0	100.0	1.000	1.000
	S	0	23			
Year(s)	GFLX	MFLX		Reproducibility	Kappa coefficient	
		I+R	S		Mean	95% confidence interval
2014	I+R	9	1	95.0	0.900	0.710–1.000
	S	0	10			
2015	I+R	13	0	100.0	1.000	1.000
	S	0	8			
2016	I+R	9	0	100.0	1.000	1.000
	S	0	5			
2014–2016	I+R	31	1	98.2	0.963	0.891–1.000
	S	0	23			

of fluoroquinolones to infants under 16 years of age is restricted due to its toxicity to bone and cartilage. Diseases requiring systemic administration of fluoroquinolones are not generally prevalent in patients under 40 years of age. Therefore, it is possible that fluoroquinolone resistance has not developed in younger patients. A recent large cross-sectional study using longitudinal data collected in the USA revealed low fluoroquinolone susceptibility of staphylococci strains isolated from elderly patients with ocular infections [11]. Differences in susceptibility of staphylococci isolated from elderly patients may be explained by a history of previous exposure to fluoroquinolones.

The predominant isolates from corneal lesions were *S. aureus*, *S. epidermidis*, and coagulase-negative staphylococci, all of which ubiquitously colonize the ocular surface [7]. Use of topical fluoroquinolones within 3 months prior to bacteriological examination significantly increased fluoroquinolone-resistant staphylococci on the ocular surface [12]. Administration of topical fluoroquinolone after cataract surgery for one month also transiently increased fluoroquinolone-resistant staphylococci after 6–9 months [13].

Fluoroquinolone susceptibility of some bacteria, including GPC and *S. aureus*, decreased after extensive systemic use of fluoroquinolones [14–17]. However, we could not find any reports of ocular infection by fluoroquinolone-resistant bacteria after systemic use of fluoroquinolone. In addition, fluoroquinolone use is a risk factor for nosocomial fluoroquinolone-resistant MRSA infection [18] and colonization of the nasal mucosa by fluoroquinolone-resistant MRSA [19]. Systemic fluoroquinolone might influence the ocular surface

because of the high similarity between the nasal and ocular surface flora [20].

The mean age of patients increased, and the fluoroquinolone susceptibility of GPC isolates significantly decreased during the study period. There was a significant correlation between the patient's age and fluoroquinolone susceptibility (odds ratio 0.893, $p = 0.0055$) (Table 2). This study was performed at an eye hospital situated in a rural area of Japan. During the study period, according to the city hall of Miyakonojo City, Miyazaki Prefecture, the region's population decreased from 172,405 (2008) to 167,487 (2016), the percentage of residents under the age of 15 decreased from 14.3 to 13.9%, and that of residents over the age of 64 increased from 25.4 to 29.4%. This demographic change appeared to affect the mean age of patients with bacterial keratitis. In addition, a safety campaign for contact lens users carried out in 2008 and 2009 may have led to a decrease in the incidence of contact lens–associated keratitis.

Limitations of this study include its retrospective study design, and that it was only conducted in a single hospital. When we analyzed the bacteriological profiles of the isolates and the age of patients, some information about the medical history of patients was absent, specifically regarding their history of topical and systemic use of prescription fluoroquinolones. We hope that the future studies on the prescription use of fluoroquinolones would elucidate the relationship between usage and dosage of these medications and fluoroquinolone susceptibility of various bacterial species.

In conclusion, this study revealed that the prevalence of fluoroquinolone-resistant GPC was higher among the isolates of bacterial keratitis from elderly patients than those from younger patients.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00417-021-05351-5.

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Author contribution The authors who contributed to the design and conduct of the study were K.U., T.I., T.O., and A.Y.; to the collection, management, analysis, and interpretation of data, K.U., T.I., J.L., R.N., Y.M., Y.N., and A.Y.; and to the preparation, review, and approval of the manuscript, T.I. and K.M.

Data availability The data that support the findings of this study are available on request from the corresponding author.

Code availability None.

Declarations

Ethics approval This clinical study was approved by the Institutional Research Ethics Committee of the Miyata Eye Hospital.

Consent to participate Written informed consent was obtained from all patients.

Consent for publication Written informed consent was obtained from all patients.

Conflict of interest The authors declare no competing interests.

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