

〈Original Article〉

Annual changes in drug susceptibility of *Staphylococcus epidermidis* isolated from the conjunctival sac of pre-operative ophthalmological patients

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Summary In this study, we investigated changes in the levofloxacin (LVFX) susceptibility of *Staphylococcus epidermidis* isolated from the conjunctival sac of patients before undergoing ophthalmic surgery at Miyata Eye Hospital from 2008 to 2015. A total of 3084 strains of methicillin-resistant *S. epidermidis* and 3307 strains of methicillin-sensitive *S. epidermidis* were isolated. For both types of strains, susceptibility to LVFX decreased over time. Since LVFX is one of the most widely used antibiotic ophthalmic solutions, continuous monitoring of changes in drug susceptibility of LVFX will be crucial for preventing drug resistance.

Key words: Drug susceptibility, Levofloxacin, *Staphylococcus epidermidis*, Ophthalmic solution

1. Introduction

The Japanese Society of Chemotherapy, the Japanese Association for Infectious Diseases, and the Japanese Society for Clinical Microbiology conducted a joint study for analysis of the drug susceptibility of microorganisms (the Three Academic Societies Joint Antimicrobial Susceptibility Surveillance Program) by sampling of gram-positive bacteria, targeting *Staphylococcus aureus*, *Enterococcus faecalis*, and *Streptococcus pneumoniae*¹⁻³. In the field of ophthalmology, *Staphylococcus epidermidis* is considered one of the

main causative bacteria of ocular surface infections and bacterial endophthalmitis after cataract surgery (endophthalmitis)^{4,5}. Indeed, although a study of postoperative endophthalmitis carried out by the Japanese Society of Ophthalmic Surgery also revealed the presence of *S. aureus* and *E. faecalis*, *S. epidermidis* showed the highest frequency (46%)⁶, highlighting the need for targeted analyses of this microorganism in the treatment and prevention of ocular infection.

S. epidermidis is a species of coagulase-negative *Staphylococcus* (CNS) and a member of the resident bacterial flora of the conjunctival sac, along with *Corynebacterium spp.* and *Propionibacterium*

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acnes. Importantly, bacterial strains isolated in patients with postoperative endophthalmitis have previously been reported to be genetically identical to the resident microbiota isolated in the conjunctival sac^{7,8}. Thus, prophylactic administration of antibacterial ophthalmic solution, primarily fluoroquinolone-based solutions, is commonly carried out during the peri-operative period after an ophthalmic surgery⁹⁻¹¹. However, the fluoroquinolone susceptibility of CNS has recently been reported to be decreasing annually among isolates from ocular surface infections and endophthalmitis after cataract surgery^{12,13}. Additionally, the fluoroquinolone susceptibility of CNS from the conjunctival sac before cataract surgery has also decreased^{14,15}, although annual follow-up studies have not been conducted.

Therefore, in this study, we examined annual changes in the drug susceptibility of *S. epidermidis* isolated from the conjunctival sac of pre-operative ophthalmological patients.

2. Materials and Methods

This study was conducted in accordance with the tenets of the Declaration of Helsinki with preoperative comprehensive consent to use the patients' data. We evaluated the rate of susceptibility of *S. epidermidis* to levofloxacin (LVFX), a representative fluoroquinolone antibiotic, in isolates from the conjunctival sac of patients aged 20 years or older before undergoing operation at Miyata Eye Hospital from 2008 to 2015. Bacterial culture, identification, and susceptibility tests were carried out at Miyakonojo Healthcare Service Center (Miyakonojo, Japan). Topical anesthesia was applied using oxybutyprocaine hydrochloride, and samples from the inferior conjunctival fornix were then collected by scraping with sterilized cotton swabs. The specimens were stored in transport medium (Becton Dickinson Japan Co. Ltd., Tokyo, Japan) and were sent to the Miyakonojo Healthcare Service Center. Bacterial culture was carried out for 48 h at 36°C, using 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% CO₂.

Drug susceptibility was determined by the disk diffusion method with KB disks (Eiken Chemical Co., Ltd, Tokyo, Japan), according to the Kirby-Bauer antibiotic testing method¹⁶. Determination of susceptibility was carried out in accordance with the methods of the Clinical and Laboratory Standards Institute (CLSI). Three levels of susceptibility were determined: susceptible (S), intermediate (I), and resistant (R), and the ratio of susceptible (S) among all samples (S / [S + I + R]) was considered the drug susceptibility rate.

Mann-Whitney U tests were performed to estimate the percentage of methicillin-resistant *S. epidermidis*, and logistic regression was used to estimate the LVFX sensitivity rates and odds ratios.

3. Results

The number of strains of *S. epidermidis* isolated between 2008 and 2015 was 6391; methicillin-resistant *S. epidermidis* (MRSE) accounted for 3084 strains (48.3%), and methicillin-sensitive *S. epidermidis* (MSSE) accounted for 3307 strains (51.7%). The percentage of MRSE per year ranged from 42.4% to 56.7%, and there were no significant differences between the detection periods (Table 1). The age distribution of the patients was as follows: 1.3% of patients were in their 20s, 1.8% of patients were in their 30s, 1.9% of patients were in their 40s, 7.2% of patients were in their 50s, 20.3% of patients were in their 60s, 41.6% of patients were in their 70s, and 25.8% of patients were 80 years old or older. Moreover, 87.7% of patients were 60 years old or older. Additionally, there were no changes in age distributions during different years for patients age 60 years or older (86.3% in 2008, 84.1% in 2009, 83.3% in 2010, 87.6% in 2011, 90.1% in 2012, 90.1% in 2013, 92.2% in 2014, and 88.5% in 2015; Fig. 1).

For the LVFX sensitivity of all strains, a significant decreasing trend was observed from 2008 to 2015, with an odds ratio of 0.944 [95% confidence interval (CI): 0.924–0.965; $p < 0.0001$; Fig. 2]. For

Table 1. Numbers of isolated *Staphylococcus epidermidis* strains from 2008 through 2015

Strains	No. of isolated strains (%)							
	2008	2009	2010	2011	2012	2013	2014	2015
MRSE	384 (46.2)	425 (51.3)	459 (56.7)	313 (46.1)	355 (51.6)	417 (47.5)	375 (42.4)	356 (45.0)
MSSE	448 (53.8)	404 (48.7)	350 (43.3)	366 (53.9)	333 (48.4)	461 (52.5)	510 (57.6)	435 (55.0)
Total	832 (100.0)	829 (100.0)	809 (100.0)	679 (100.0)	688 (100.0)	878 (100.0)	885 (100.0)	791 (100.0)

MRSE: methicillin-resistant *Staphylococcus epidermidis*

MSSE: methicillin-sensitive *Staphylococcus epidermidis*

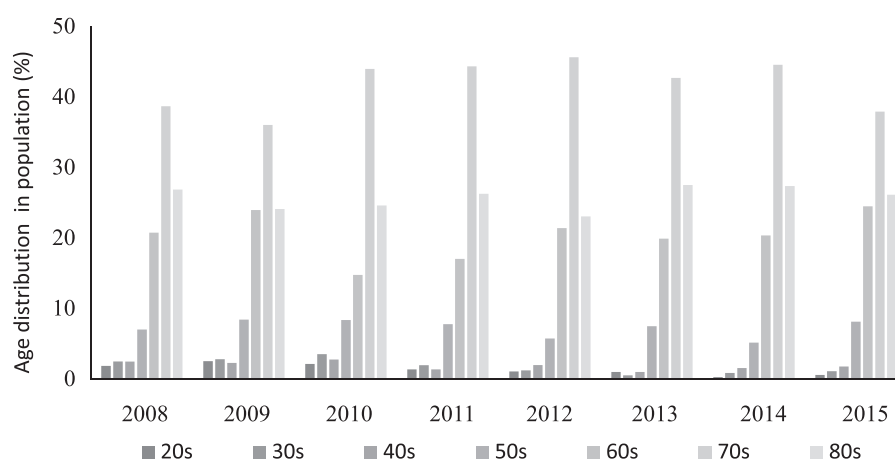


Fig. 1 Histogram comparing annual changes according to patient age.

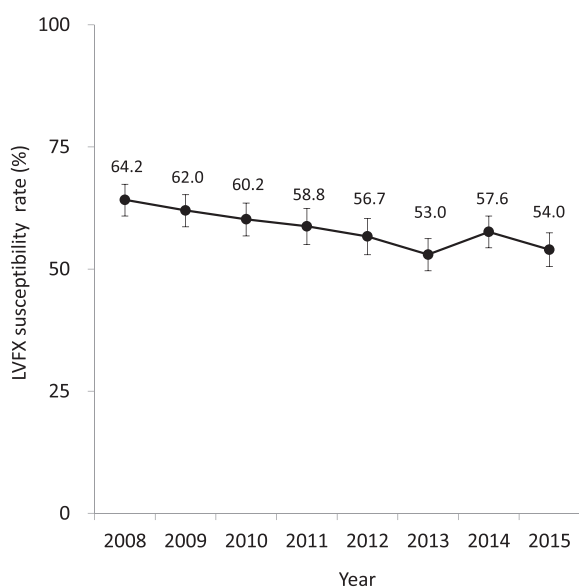


Fig. 2 Changes in the susceptibility of *Staphylococcus epidermidis* to LVFX (all 6391 strains). Susceptibility rates \pm 95% CIs are shown.

MRSE and MSSE, a significant decreasing trend was also observed, with odds ratios of 0.899 (95% CI: 0.870–0.92; $p < 0.0001$) and 0.923 (95% CI: 0.890–0.957; $p < 0.0001$; Figs. 3 and 4).

4. Discussion

In this study, we examined the drug susceptibility of 6391 strains of *S. epidermidis* isolated from 2008 to 2015. Our findings showed that the LVFX susceptibility of these isolates decreased annually. The age distributions of the patients from which the bacterial strains were isolated showed that elderly individuals age 60 years or older accounted for more than 80% of the patient population, regardless of the year. This finding could be explained by the observation that 80% of ophthalmic surgeries conducted at Miyata Eye Hospital consisted of senile cataract surgery, followed by vitreous surgery, for which the

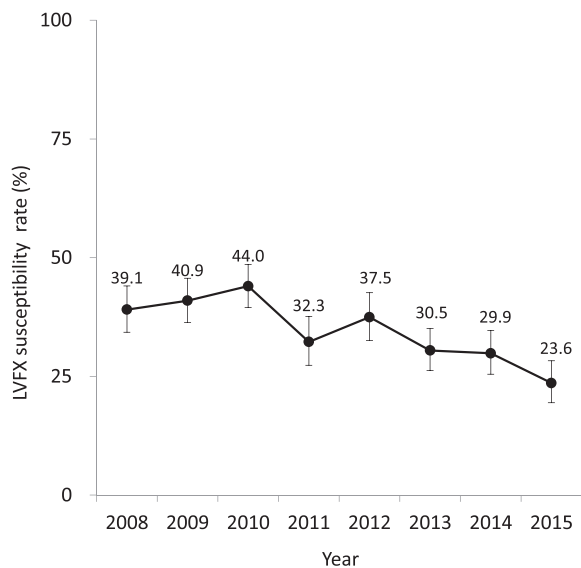


Fig. 3 Changes in the susceptibility of MRSE to LVFX (n = 3307 strains). Susceptibility rates ± 95% CIs are shown.

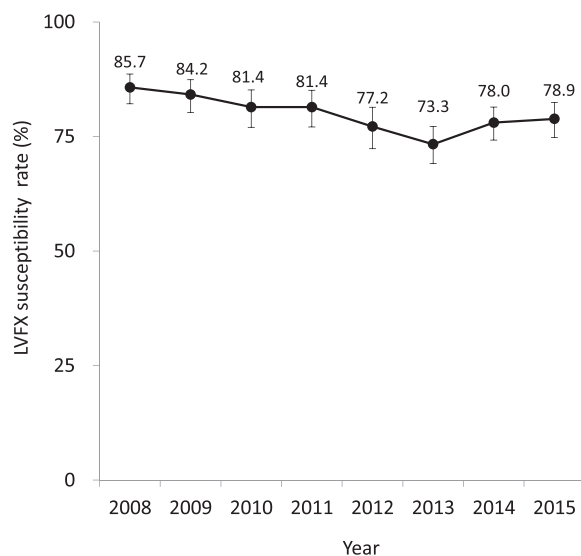


Fig. 4 Changes in the susceptibility of MSSE to LVFX (n = 3084 strains). Susceptibility rates ± 95% CIs are shown.

majority of patients are elderly.

In a study conducted at Miyata Eye Hospital from December 2004 to February 2005, Kataoka et al¹⁷. reported that for 67 strains of *S. epidermidis* isolated in the conjunctival sac of patients before cataract surgery, the rate of susceptibility to LVFX was 77.6%; this was significantly higher than the 64.2% susceptibility rate found in our study in 2008 ($p = 0.0288$, GEE trend analysis based on the logistic

model). Thus, these results also suggested that the rate of susceptibility to LVFX decreased annually. In addition, Minato et al¹⁶. have shown that for the CNS isolated in the conjunctival sac of patients scheduled for cataract surgery, the minimal inhibitory concentration (MIC) of fluoroquinolones was bimodal, suggesting that the number of fluoroquinolone-resistant strains may have increased, consistent with the findings of our current study.

In our study, there were no major differences in annual data for MSSE and MRSE; however, in both groups, susceptibility to LVFX decreased each year. Analysis of changes in the susceptibility of MSSE to LVFX showed that the susceptibility rate was 85.7% (highest) in 2008 and 73.3% (lowest) in 2013; however, the susceptibility rate increased to 78.9% in 2015, suggesting that the overall susceptibility was good. The rate of susceptibility of MRSE to LVFX decreased from 39.1% in 2008 to 23.6% in 2015, and LVFX-resistant strains accounted for more than 70% of all MRSE strains. Similarly, the rate of LVFX susceptibility of methicillin-sensitive CNS including MSSE collected from the normal conjunctival sac of elderly patients was reported to be 87% in 2009 according to Hori et al¹⁸. and 86% in 2010 according to Hoshi et al¹⁴., similar to the LVFX susceptibility rates observed from 2008 to 2010 in our study. Notably, there are few reports of the susceptibility of MSSE with similar backgrounds; therefore, careful monitoring of drug susceptibility will be important in the future.

Regarding MRSE, Seki et al. reported in 2003 that methicillin-resistant CNS (MRCNS) accounted for 44% of CNS isolated in elderly individuals and that susceptibility to LVFX was 65.6%¹⁹. In addition, in the aforementioned reports published by Hori et al. and Hoshi et al., MRCNS accounted for 38% and 37% of CNS, respectively, and their susceptibilities to LVFX were 18.2% and 29.4%, respectively^{14,18}. Notably, these results were from studies conducted at different facilities during a single year and did not necessarily show chronological changes over time; however, based on the findings of our study, the susceptibility of MRCNS to LVFX decreased in the elderly. Therefore, drug

susceptibility may need to be confirmed before conducting eye surgery, and the patient's condition also needs to be managed with suitable antimicrobial agents.

Staphylococcus spp. becomes a methicillin-resistant bacterium by acquiring the Staphylococcal cassette chromosome mec (SCC mec). As a result of the SCC mec genotype, this organism displays diversity in terms of the degree of resistance and the location of acquisition, namely community-acquired or hospital-associated methicillin-resistant *Staphylococcus aureus* or MRCNS. *Staphylococcus spp.* is also likely to become multidrug-resistant when under pressure from antimicrobial agents. In previous findings from the Three Academic Societies Joint Antimicrobial Susceptibility Surveillance Program and the Japan Nosocomial Infections Surveillance (JANIS) program, the rates of susceptibility of *S. aureus* to LVFX were 88–94% for methicillin-susceptible *S. aureus* (MSSA) and 7–14% for methicillin-resistance *S. aureus* (MRSA), and a high rate of resistance to LVFX was found among methicillin-resistant bacteria. However, the rate of resistance did not show any chronological changes over the years^{3,20}. Similarly, *S. epidermidis*, which was examined in our study, and the aforementioned CNS collected from the conjunctival sac of elderly patients were also methicillin-resistant bacteria with a high rate of resistance to LVFX. However, in our study, we did observe changes in the LVFX susceptibility rates over time. Interestingly, in a study conducted by JANIS from January to September 2015, the proportion of MRSE among *Staphylococcus epidermidis* isolates showed regional disparities, namely, 76–78% throughout Japan, 77–81%, in Tokyo, and 38–51% in Miyazaki Prefecture. Thus, the proportion of MRSE among the strains found in our study was the same as that from other organs in patients in Miyazaki Prefecture.

In 2012, the World Health Organization (WHO) published an article, titled “The evolving threat of antimicrobial resistance: options for action”²¹, in which they proposed measures against multidrug-resistant bacteria. One risk factor for the development of MRCNS in the conjunctival sac is a

single administration of antimicrobial agents, including ophthalmic solutions, within 30 days²²; therefore, in addition to proper use of antibacterial ophthalmic solutions, monitoring the susceptibility of *S. epidermidis* to LVFX in the field of ophthalmology is an extremely important measure against resistant bacteria. In the field of ophthalmology, fluoroquinolones account for more than 90% of peri-operative prophylactic antibiotics¹⁰ and are the first choice for the treatment of infectious keratitis²³. This frequent use of fluoroquinolones is considered to have contributed to the increase in resistant strains.

In the future, careful observation of the trends shown by drug-resistant bacterial strains will need to be carried out not only at individual hospitals but also at the regional and national levels and for other medical specialties. Moreover, a careful follow-up of multidrug-resistant bacteria is also needed.

In conclusion, we found that the LVFX susceptibility of *S. epidermidis* has been linearly decreasing in recent years. However, because we applied the disk diffusion method to determine drug susceptibility, the minimum inhibitory concentrations are still unknown. Additional studies are needed for continuous monitoring of these organisms and to determine the minimum inhibitory concentrations.

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Conflicts of interest:

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