Annual changes in drug susceptibility of \textit{Staphylococcus epidermidis} isolated from the conjunctival sac of pre-operative ophthalmological patients

Yukari Noguchi\(^1\), Ryohei Nejima\(^1\), Kenji Marume\(^2\), Akiko Yagi\(^1\), Yosai Mori\(^1\), Takashi Ono\(^1\), Takuya Iwasaki\(^1\), Eiichiro Sakoda\(^2\) and Kazunori Miyata\(^1\)

**Summary** In this study, we investigated changes in the levofloxacin (LVFX) susceptibility of \textit{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 \textit{S. epidermidis} and 3307 strains of methicillin-sensitive \textit{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, \textit{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 \textit{Staphylococcus aureus}, \textit{Enterococcus faecalis}, and \textit{Streptococcus pneumoniae}\(^1\textsuperscript{-3}\). In the field of ophthalmology, \textit{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 \textit{S. aureus} and \textit{E. faecalis}, \textit{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.

\textit{S. epidermidis} is a species of coagulase-negative \textit{Staphylococcus} (CNS) and a member of the resident bacterial flora of the conjunctival sac, along with \textit{Corynebacterium} spp. and \textit{Propionibacterium}...
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. Thus, prophylactic administration of antibacterial ophthalmic solution, primarily fluoroquinolone-based solutions, is commonly carried out during the peri-operative period after an ophthalmic surgery. 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. Additionally, the fluoroquinolone susceptibility of CNS from the conjunctival sac before cataract surgery has also decreased, 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 pre-operative 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 oxybuprocaine 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.) 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 method. 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. 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

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<tbody>
<tr>
<td>MRSE</td>
<td>384</td>
<td>425</td>
<td>459</td>
<td>313</td>
<td>355</td>
<td>417</td>
<td>375</td>
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<td></td>
<td>(46.2)</td>
<td>(51.3)</td>
<td>(56.7)</td>
<td>(46.1)</td>
<td>(51.6)</td>
<td>(47.5)</td>
<td>(42.4)</td>
<td>(45.0)</td>
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<tr>
<td>MSSE</td>
<td>448</td>
<td>404</td>
<td>350</td>
<td>366</td>
<td>333</td>
<td>461</td>
<td>510</td>
<td>435</td>
</tr>
<tr>
<td></td>
<td>(53.8)</td>
<td>(48.7)</td>
<td>(43.3)</td>
<td>(53.9)</td>
<td>(48.4)</td>
<td>(52.5)</td>
<td>(57.6)</td>
<td>(55.0)</td>
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<tr>
<td>Total</td>
<td>832</td>
<td>829</td>
<td>809</td>
<td>679</td>
<td>688</td>
<td>878</td>
<td>885</td>
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<td></td>
<td>(100.0)</td>
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MRSE: methicillin-resistant *Staphylococcus epidermidis*

MSSE: methicillin-sensitive *Staphylococcus epidermidis*

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 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

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majority of patients are elderly.

In a study conducted at Miyata Eye Hospital from December 2004 to February 2005, Kataoka et al. 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 al. 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 al. and 86% in 2010 according to Hoshi et al., 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%. 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. 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 years20. 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 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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References