JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2008, p. 527–532 Vol. 46, No. 2
0095-1137/08/$08.00+0 doi:10.1128/JCM.01741-07

High-Level Fluoroquinolone Resistance in Ophthalmic Clinical Isolates Belonging to the Species *Corynebacterium macginleyi*▽

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Received 31 August 2007/Returned for modification 17 October 2007/Accepted 21 November 2007

The clinical importance of nondiphtherial *Corynebacterium*, a ubiquitous member of the normal human microflora of the skin and mucous membrane, for ocular surface infections has been recognized recently. We performed an antimicrobial susceptibility test with Etest strips for three fluoroquinolones (ciprofloxacin, norfloxacin, and levofloxacin) and a taxonomic analysis on 21 isolates of *Corynebacterium* from ophthalmic samples. Of these, 16 isolates were identified as *C. macginleyi* at the species level on the basis of 16S rRNA gene sequence comparisons. The remaining five isolates were determined to be *C. mastitidis* (four) or *C. accolens* (one). Eleven of the *C. macginleyi* isolates showed high levels of resistance to all of the fluoroquinolones tested, and one isolate was resistant to norfloxacin alone. An analysis of the amplified quinolone-resistance-determining regions of the *gyrA* genes revealed that a single amino acid substitution in position 83 of the *gyrA* product was sufficient to generate the norfloxacin resistance phenotype, and double mutations leading to amino acid changes in positions 83 and 87 were necessary for high-level resistance against the other fluoroquinolones. We conducted the first example of multilocus sequence typing (MLST) analysis on *C. macginleyi*. The MLST analysis grouped the majority of *C. macginleyi* isolates into a single lineage, and another molecular strain typing by random amplified polymorphic DNA fragment patterns supported the finding, indicating that a particular lineage of *C. macginleyi* is dominant on the human ocular surface. This type of population might be particularly adaptable to the milieu on the human ocular surface.

Corynebacterium is a ubiquitous gram-positive, pleomorphic aerobe that colonizes the skin and mucous membrane in humans. Species of the genus *Corynebacterium* other than *C. diphtheriae* are components of the normal indigenous microflora, and they rarely have been linked to human infections. However, recent reports increasingly have described these microorganisms as causative agents for various infections, such as pneumonia (2, 24), vertebral osteomyelitis, septicemia (11, 19), and endocarditis (3, 16). In the ophthalmic area, a particular species of *Corynebacterium*, *C. macginleyi* (20), recently has been identified as the cause of bacterial conjunctivitis and corneal ulcer (6, 10). Interestingly, this species has been isolated only from eye specimens, and the findings seem to indicate tissue tropism in this species, like in other corynebacteria such as *C. auris* (ear samples) (5) and *C. glucuronolyticum* (genitourinary specimens from males) (4). In these reports, *C. macginleyi* has been described as susceptible to a wide spectrum of antimicrobials used for the treatment of conjunctivitis, including quinolones (6, 10).

Fluoroquinolone eye drops are frequently used worldwide for the treatment of bacterial conjunctivitis and corneal ulcer. Recently, we have become aware of the rising isolation rate of fluoroquinolone-resistant *Corynebacterium* species from ophthalmic specimens, including conjunctivitis cases. The aim of this study is to address the background of the emergence of fluoroquinolone resistance among the ophthalmic *Corynebacterium* isolates.

MATERIALS AND METHODS

Bacterial strains. This study included the 21 strains of *Corynebacterium* that were isolated from conjunctival swabs before ocular surgeries or discharges from conjunctivitis cases during the period from June 2005 to November 2006 at Tokushima University Hospital, Tokushima, Japan (Table 1). These isolates were identified as belonging to the *Corynebacterium* species by microbiological examinations in the clinical laboratory of Tokushima University Hospital. The species assignment of these isolates was made by the sequence analysis of the 16S rRNA genes, as described below. The type strain of *C. macginleyi* ATCC 51787 also was tested.

DNA isolation. The genomic DNA of each *Corynebacterium* strain grown in brain heart infusion broth containing 0.5% Tween 80 was purified in accordance with the method described by Morita et al. (17). The DNA concentration was calculated from the absorbance at 260 nm on a spectrophotometer (GE Healthcare Bio-Sciences K.K., Tokyo, Japan).

Test of susceptibility to fluoroquinolones. The MICs of ciprofloxacin (CFX), norfloxacin (NOR), and levofloxacin (LFX) against the 21 strains of *Corynebacterium* were individually determined by the Etest method (AB BIODISK, Solna, Sweden) on Mueller-Hinton sheep blood agar plates (BD Japan, Tokyo, Japan) in accordance with the manufacturer's instructions.

Amplification and sequencing of 16S rRNA genes. The 16S rRNA genes from all of the clinical *Corynebacterium* strains tested were amplified by TAKARA ExTaq (Takara Bio Inc., Otsu, Japan) using the eubacterial universal primers 27F and 1492R (12). PCR was performed with 200 ng of purified genomic DNAs under the following conditions. The PCR program used was preheating at 98°C for 1 min and then 35 cycles of 98°C for 10 s, 55°C for 30 s, and 72°C for 2 min, with a final extension at 72°C for 5 min. The nucleotide sequences of the purified amplicons were determined by BigDye Terminator chemistry on a Genetic An-

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▽ Published ahead of print on 12 December 2007.

TABLE 1. Profiles of the patients and the *Corynebacterium* isolates investigated in this study

Strain	Patient sex, age[a] (yr)	Clinical diagnosis[b]	Species identification[c] (% identity)
TBS-01	M, 58	Bacterial conjunctivitis*	*C. macginleyi* (99.3)
TBS-02	F, 72	Bacterial conjunctivitis*	*C. macginleyi* (99.4)
TBS-03	F, 58	Bacterial conjunctivitis*	*C. macginleyi* (99.4)
TBS-04	M, 68	Retinal detachment	*C. macginleyi* (99.9)
TBS-05	M, 83	Retinal detachment	*C. macginleyi* (99.4)
TBS-06	M, 54	Cataract	*C. mastitidis* (98.2)
TBS-07	M, 55	Diabetic retinopathy	*C. mastitidis* (98.2)
TBS-08	F, 89	Bacterial conjunctivitis	*C. macginleyi* (99.4)
TBS-09	F, 85	Cataract	*C. macginleyi* (99.4)
TBS-10	F, 67	Dry eye	*C. mastitidis* (98.2)
TBS-11	M, 78	Bacterial conjunctivitis*	*C. macginleyi* (99.4)
TBS-12	F, 74	Bacterial conjunctivitis	*C. accolens* (99.8)
TBS-13	M, 69	Retinal detachment	*C. macginleyi* (99.4)
TBS-14	M, 75	Cataract	*C. macginleyi* (99.4)
TBS-15	F, 69	Intact (discharge alone)	*C. macginleyi* (99.4)
TBS-16	M, 70	Retinal detachment	*C. macginleyi* (99.9)
TBS-17	F, 82	Cataract, limbal deficiency	*C. macginleyi* (99.4)
TBS-18	M, 78	Epiretinal membrane	*C. macginleyi* (99.4)
TBS-19	F, 74	Cataract	*C. mastitidis* (98.2)
TBS-20	F, 84	Cataract, high intraocular pressure	*C. macginleyi* (99.4)
TBS-21	F, 80	Cataract	*C. macginleyi* (99.4)

[a] M, male; F, female.
[b] The cases of bacterial conjunctivitis in which *C. macginleyi* was identified as the causative agent are indicated by asterisks.
[c] Species identification was performed on the basis of sequence similarities among the 16S rRNA genes by BLASTN (1).

alyzer 310 (Applied Biosystems Japan, Ltd., Tokyo, Japan). Homology searches of the obtained sequences against public databases were performed with the BLASTN program (1). The species assignment of the *Corynebacterium* strains was made according to the best-hit pairs, in which the nucleotide differences were less than 2%.

Amplification and sequencing of the QRDRs of the *gyrA* gene. The analysis of the quinolone-resistance-determining regions (QRDRs) of the *gyrA* gene was performed as described by Sierra et al. (23). The predicted amino acid sequences of the QRDRs were aligned and compared using the Clustal W program (25).

MLST. Multilocus sequence typing (MLST) had not yet been employed for any member of *Corynebacterium*. We therefore selected seven housekeeping genes (*adk*, *dnaA*, *fumC*, *gltA*, *gyrB*, *icd*, and *purA*) from the whole-genome sequences of four *Corynebacterium* species (*C. glutamicum*, *C. diphtheriae*, *C. efficiens*, and *C. jeikeium*) by referring to analyses of other species (http://www.mlst.net). We designed a pair of primers for each of the target genes based on the regions conserved among the four species (Table 2). Each gene was amplified under the same conditions as those of the 16S rRNA gene amplification, except that the extension step was 72°C for 1 min, and the nucleotide sequence of each product was determined as described above. After the primer sequences were removed, the nucleotide sequences obtained from the seven loci were concatenated. Concatenated nucleotide sequences containing a total of 3,284 bp were aligned and compared using the Clustal X program (9). A phylogenetic tree was constructed by the neighbor-joining method (22) with 1,000-replicate bootstrap analysis to assess the tree topology. An NJ plot (18) was used for drawing the phylogenetic tree.

Analysis of RAPD fragments. Random amplified polymorphic DNA (RAPD) analysis was performed in accordance with the method described by Martín et al. (15) for the molecular typing of *C. striatum* strains. The RAPD fragments generated by primers OPB17 (13) and A (14) from the 17 strains of *C. macginleyi*, including the reference strain ATCC 51787, were separated in 1.5% agarose gels and compared.

Nucleotide sequence accession numbers. All of the sequence data obtained in this study were deposited at DDBJ/EMBL/GenBank under accession numbers AB359242 to AB359410.

RESULTS

Characterization of *Corynebacterium* clinical isolates from eye specimens. We examined the 21 ophthalmic isolates that were suspected to be *Corynebacterium* species in our clinical laboratory. Each isolate was collected from independent patients (10 males and 11 females, 54 to 89 years of age), including six conjunctivitis cases (Table 1). The majority of isolates (16/21) were assigned to *C. macginleyi*, while the remaining ones were identified as belonging to two other species (four *C. mastitidis* and one *C. accolens*). Of the six cases of bacterial conjunctivitis, *C. macginleyi* had been diagnosed to be a causative agent in four cases. A history of antimicrobial therapy with fluoroquinolone eye drops was apparent for the five cases from which TBS-01, TBS-05, TBS-13, TBS-20, and TBS-21 were isolated, but this information was unclear in the other cases.

Susceptibility to fluoroquinolones. We measured the MICs of three fluoroquinolones, CFX, NOR, and LFX, against the *Corynebacterium* isolates using the Etest strip method. As shown in Table 3, half of the isolates tested (11 isolates) showed high-level resistance to all of the fluoroquinolones tested. Interestingly, one isolate, TBS-18, was unique in that it showed a high level of resistance to NOR alone, and the MICs of CFX and LFX against the isolate both showed only moderate elevation to 2 μg/ml. It also was noteworthy that all of the

TABLE 2. Primer pairs used for the MLST analysis of *Corynebacterium macginleyi* strains

Locus	Function of gene product	Primer	Nucleotide sequence[a] (5′ to 3′)	Size of amplicons (bp)
adk	Adenylate kinase	Coryn-adkF	CCCGGYGCYGGYAARGGCACCCA	395
		Coryn-adkR	CGGGTGCGGATSGTSTCYTCRTTGTC	
gyrB	β-Subunit of DNA gyrase	Coryn-gyrBF	GGCATTTCCGTGGTCAATGC	590
		Coryn-gyrBR	ACGGACTGCTTGTAGCCCTG	
fumC	Fumarate hydratase	Coryn-fumCF	AACATGGGICAGTCCTCCAA	581
		Coryn-fumCR	GGGTTGACCTTGCCYGGCATGATGGA	
purA	Adenylosuccinate synthetase	Coryn-purAF	CGCGGCATTGGGCCGACCTA	495
		Coryn-purAR	ACCGCCAGTGGTCTGCAGGTA	
dnaA	Replication initiation protein	Coryn-dnaAF	ACSCAGGARGAGTTCTTCCA	516
		Coryn-dnaAR	CTCRCGGCASAGRTACAT	
gltA	Citrate synthase	Coryn-gltAF	GGCCACCTGCCGGATGAGCAGGAA	411
		Coryn-gltAR	GGCCGGACAGGGCGTTGATACCG	
icd	Isocitrate dehydrogenase	Coryn-icdF	ATGATGAAGGTITCCGACCC	504
		Coryn-icdR	GAAGGTCTTRTCRTGGGAGCCGTACTC	

[a] Y = C or T; R = A or G; S = C or G; I = inosine.

TABLE 3. Relationship between mutations in the QRDRs of the *gyrA* genes and the MICs

Strains (species)[a]	GyrA amino acid sequence at[b]:		MIC (μg/ml)		
	83-S	87-D	CFX	NOR	LFX
ATCC 51787 (CMa)	S (TCG)	D (GAC)	0.125	1	0.125
TBS-04 (CMa)	S (TCG)	D (GAC)	0.047	0.380	0.064
TBS-09 (CMa)	S (TCG)	D (GAC)	0.032	0.500	0.032
TBS-15 (CMa)	S (TCG)	D (GAC)	0.064	0.500	0.125
TBS-17 (CMa)	S (TCG)	D (GAC)	0.064	0.500	0.064
TBS-06 (CMs)	S (AGC)	D (GAC)	0.094	1	0.125
TBS-07 (CMs)	S (AGC)	D (GAC)	0.023	0.500	0.064
TBS-10 (CMs)	S (AGC)	D (GAC)	0.125	2	0.125
TBS-19 (CMs)	S (AGC)	D (GAC)	0.125	2	0.064
TBS-12 (CAc)	S (TCG)	D (GAC)	0.125	1	0.125
TBS-01 (CMa)	L (TTG)	G (GGC)	>32	24	>32
TBS-02 (CMa)	L (TTG)	N (AAC)	>32	96	>32
TBS-03 (CMa)	L (TTG)	H (CAC)	>32	64	>32
TBS-05 (CMa)	L (TTG)	N (AAC)	>32	96	>32
TBS-08 (CMa)	L (TTG)	H (CAC)	>32	64	>32
TBS-11 (CMa)	L (TTG)	N (AAC)	>32	>256	>32
TBS-13 (CMa)	A (GCG)	Y (TAC)	>32	>256	>32
TBS-14 (CMa)	L (TTG)	N (AAC)	>32	32	>32
TBS-16 (CMa)	L (TTG)	Y (TAC)	>32	>256	>32
TBS-20 (CMa)	L (TTG)	E (GAA)	>32	>256	>32
TBS-21 (CMa)	L (TTG)	H (CAC)	>32	>256	>32
TBS-18 (CMa)	L (TTG)	D (GAC)	2	64	2

[a] The abbreviations in parentheses indicate the following species: CMa, *C. macginleyi*; CMs, *C. mastitidis*; and CAc, *C. accolens*.
[b] The sequences in parentheses indicate the codons for each amino acids.

isolates exhibiting high levels of fluoroquinolone resistance were *C. macginleyi*, and the isolates of the other species were susceptible to each of the three fluoroquinolones examined.

Mutations in the *gyrA* gene. To date, alterations in the targets and decreased accumulation due to impermeability of the membrane and/or overexpression of efflux pump systems are widely accepted as mechanisms leading to quinolone resistance. Of these, amino acid substitutions within DNA gyrase and topoisomerase IV due to point mutations in so-called QRDRs of *gyrA* and *purC*, respectively, mainly are responsible for the resistance to quinolones in most microorganisms (8, 21). Since the *Corynebacterium* species for which the whole-genome sequences have been determined to date lack *purC* homologues on their genomes (23), mutations in *gyrA* mainly are responsible for the quinolone resistance in those species.

The deduced amino acid sequence alignment of the QRDRs of *gyrA* from the 21 isolates showed five variable positions within the regions. Of these, position 87 was the most variable, and six kinds of amino acids (Asp, His, Asn, Tyr, Glu, and Gly) were observed there (Table 3). The next variable position was 83, in which 11, 9, and 1 isolate possessed Leu, Ser, and Ala, respectively. These two positions correspond to the mutation hot spots leading to quinolone resistance for most microorganisms (8, 21). Considering the MICs of the three fluoroquinolones examined, these mutations showed a complete correlation with the susceptibilities of the isolates to the fluoroquinolones. There was no correlation between amino acid substitutions in the other three variable positions and the susceptibilities to the fluoroquinolones. All of the high-level fluoroquinolone-resistant isolates possessed double mutations, generating changes in Ser-83 and Asp-87, that are not seen in the susceptible isolates. The amino acid substitution pattern of TBS-18 was of particular interest. In the isolate TBS-18, which showed a unique susceptibility pattern for the fluoroquinolones, Asp-87 was conserved, as it was in the susceptible isolates, and the only difference from the susceptible strains was a change from Ser-83 to Leu, suggesting that resistance to NOR was established by the single amino acid substitution of Ser-83 to Leu and did not depend on an additional change in Asp-87. This interpretation also was supported by the finding that the mutation in position 87 did not increase the MICs of NOR. On the other hand, the high-level resistance of the *C. macginleyi* isolates to CFX and LFX likely was acquired by a double mutation in positions 83 and 87.

In almost all of the fluoroquinolone-resistant *C. macginleyi* isolates, replacements of Ser-83 with other amino acids were generated by transitions in the second codon for Ser (TCG to TTG). Only in one isolate (TBS-13) was transversion observed in the first codon (TCG to GCG). In position 87, transitions and transversions were evenly observed, suggesting that the amino acid changes in this position originated from the high selective pressure exerted by the fluoroquinolones.

Population analysis of the *C. macginleyi* isolates. To address the background of the increasing isolation rate of *C. macginleyi* from eye specimens and the widely spreading fluoroquinolone resistance within the species, we conducted MLST analysis on the 16 isolates and a type strain of *C. macginleyi* in this study.

As shown in Fig. 1, the *C. macginleyi* isolates examined in this study were divided into three lineages, and 13 of the 16 isolates were grouped into a single cluster. The isolates TBS-02 and TBS-08 showed sequences identical to those of TBS-13 and TBS-21, respectively, in all of the loci. This finding indicates that one particular lineage of *C. macginleyi* is dominant on the human ocular surface.

Fluoroquinolone resistance was observed in each of the three lineages, meaning that fluoroquinolone resistance was

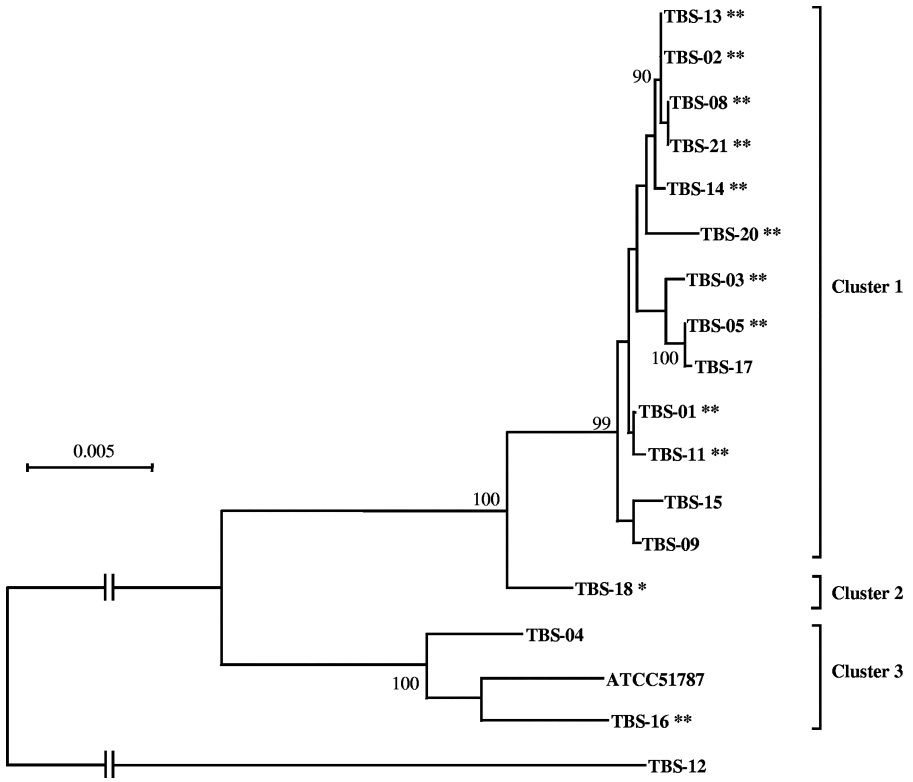

FIG. 1. MLST analysis of the *C. macginleyi* isolates. The concatenated sequences obtained from seven loci were aligned and compared. *C. accolens* TBS-12 was included in this analysis. The tree was constructed by the neighbor-joining method (22), and the numbers indicate the significant bootstrap values (%) for 1,000 replicates. **, Isolate resistant to CFX, NOR, and LFX; *, isolate resistant to NOR alone.

widely spread among the *C. macginleyi* isolates, undoubtedly due to the frequent exposure to this type of antimicrobial. Another finding of interest was that all of the *C. macginleyi* isolates from conjunctivitis cases (TBS-01, TBS-02, TBS-03, TBS-08, and TBS-11) belonged to the same lineage. Although the isolates examined in this study showed a deviated population structure (and, thus, a larger number of isolates must be tested to yield a definitive conclusion), the findings indicate that the isolates of this lineage might have higher pathogenic potential or adaptability to the human ocular surface than do other populations.

Molecular typing of *C. macginleyi* isolates by RAPD analysis. To validate the results of the MLST analysis, we conducted a RAPD analysis of the *C. macginleyi* isolates (Fig. 2). We tested two RAPD primers, A (14) and OPB17 (13). Consistently with the clustering yielded by the MLST analysis, the RAPD fragment pattern with primer A clearly divided the isolates into two groups, each corresponding to cluster 3 and clusters 1 and 2, respectively, as shown in Fig. 1. Primer OPB17 generated RAPD fragments with better discriminatory power on these isolates than primer A, and none of the isolates showed identical patterns except for isolates TBS-02 and TBS-13 (Fig. 2B, lanes 16 and 17). Although TBS-08 and TBS-21 formed another pair showing an identical sequence in the MLST analysis, there was a slight difference in the fragment pattern. The overall fragment patterns with primer OPB17 also were similar among the isolates belonging to the same cluster, as determined by the MLST analysis. These findings indicate that our

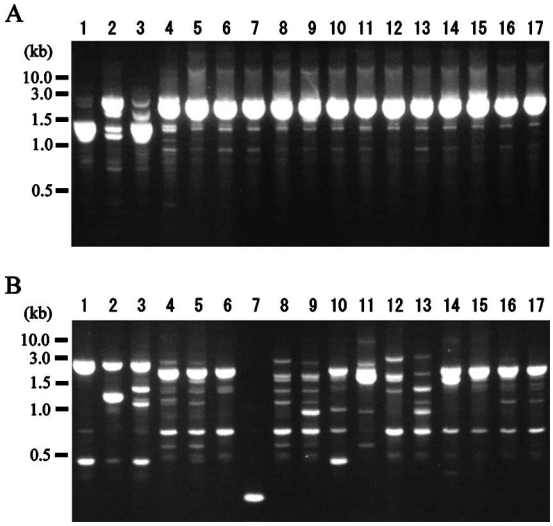

FIG. 2. Analysis of the genomic DNAs of *C. macginleyi* by RAPD typing with primers A (A) and OPB17 (B). The lanes were arranged according to the MLST analysis results shown in Fig. 1. Lanes: 1, ATCC 51787; 2, TBS-16; 3, TBS-04; 4, TBS-18; 5, TBS-09; 6, TBS-15; 7, TBS-11; 8, TBS-01; 9, TBS-17; 10, TBS-05; 11, TBS-03; 12, TBS-20; 13, TBS-14; 14, TBS-21; 15, TBS-08; 16, TBS-02; and 17, TBS-13.

MLST analysis described here had a discriminatory power equivalent to that of RAPD analysis for the molecular typing of *C. macginleyi* strains.

DISCUSSION

In the ophthalmology area, *C. macginleyi* has been reported to be isolated exclusively from eye specimens (6, 10, 20). In agreement with this finding, *C. macginleyi* was the most frequently encountered *Corynebacterium* species in the eye specimens collected at our hospital (16 of 21 isolates in this study). Joussen et al. (10) have reported that this species was isolated from 12.1% of 107 culture-positive conjunctivitis cases, and it should be regarded as the etiology of ocular surface infections, especially in patients with compromised conjunctiva and/or cornea. Accordingly, we also have increasingly experienced conjunctivitis cases in which *C. macginleyi* is the only bacterium recovered from culture examinations. In these cases, the Gram staining of the discharges showed many phagocytosis images of polymorphonuclear leukocytes engulfing gram-positive pleomorphic bacteria, suggesting that *C. macginleyi* should be recognized as an opportunistic pathogen for conjunctivitis as well as the predominant commensal bacterium on the human ocular surface.

Another important point is that the majority of *C. macginleyi* isolates (12/16) in our study had acquired high levels of resistance to fluoroquinolones. In fact, the conjunctivitis patients from which TBS-01 and TBS-03 were isolated had prolonged eye discharge and were refractory to topical treatment with fluoroquinolone eye drops for several months. The exchange of fluoroquinolone with aminoglycosides or chloramphenicol quickly improved the clinical symptoms in these cases. We diagnosed these patients as having bacterial conjunctivitis caused by fluoroquinolone-resistant *C. macginleyi*. However, in previous reports by Funke et al. (6) and Joussen et al. (10), *C. macginleyi* was described as uniformly susceptible to a wide range of topical antibiotics commonly used in ophthalmology. We confirmed that strain ATCC 51787, which is the type strain of *C. macginleyi* described by Riegel et al. in 1995 (20), is susceptible to CFX, NOR, and LFX (Table 3). A recent report of a farmer with bilateral conjunctivitis in Italy in 2002 also mentioned that the *C. macginleyi* strain isolated from conjunctival swabs showed good susceptibility to a large panel of antibiotics, including quinolones (7). Considering this worldwide trend of *C. macginleyi* susceptibility to quinolones, the rate of fluoroquinolone resistance in *C. macginleyi* in Japan might be much higher than in other countries. It is unlikely that the emergence of fluoroquinolone resistance in *C. macginleyi* is an event restricted to our hospital or that it originated from the recent nosocomial transmission of that particular clone through ophthalmologic instruments, since we detected no isolate showing identical profiles in all of the four examinations (i.e., susceptibility test to fluoroquinolone, analyses for *gyrA* mutation, MLST analysis, and RAPD analysis). Although many isolates of geographically different origins should be examined to resolve this question, the wide penetration of fluoroquinolone resistance among the *C. macginleyi* isolates observed in this study is undoubtedly the result of the heavy use of fluoroquinolone eye drops in Japan.

Recently, the mechanisms of quinolone resistance in *C. amycolatum* and *C. striatum* have been elucidated (23). That study revealed that the double mutations within the QRDRs of *gyrA* leading to amino acid changes in positions 83 (Ser to Arg) and 84 (Pro to Ala) for *C. amycolatum* and in positions 83 (Ser to Phe) and 87 (Asp to Ala) for *C. striatum* were responsible for the high levels of resistance to CFX and LFX. Likewise, in all of the fluoroquinolone-resistant *C. macginleyi* isolates examined in this study, the double mutation generating the amino acid substitutions in positions 83 and 87 was observed in all isolates except TBS-18, in which the amino acid substitution occurred only in position 83 and induced high-level resistance to NOR alone. It is evident that the *gyrA* mutation plays a primary role in the acquisition of fluoroquinolone resistance in *Corynebacterium* as well, and double mutations within the QRDRs of *gyrA* increase the level of fluoroquinolone resistance, as described for other microorganisms. The characteristic susceptibility pattern of TBS-18 to CFX, NOR, and LFX indicates that these fluoroquinolones show different modes of interaction with the QRDRs of the *C. macginleyi gyrA* product.

Another intriguing aspect arising from the population analysis by MLST is that the predominant *Corynebacterium* species on the human ocular surface is *C. macginleyi* of one particular lineage. Although we must examine a larger number of isolates of geographically different origins, this indicates intraspecies differences in the adaptability to the human ocular milieu and/or pathogenic potential in *C. macginleyi*. The emergence of fluoroquinolone resistance probably emphasizes this point by increasing the population level of *C. macginleyi* on the ocular surface of patients treated with fluoroquinolone eye drops. It remains to be elucidated by DNA-DNA hybridization how this lineage should be classified taxonomically. In relation to this point, the isolates belonging to clusters 1 and 2 might be a *C. macginleyi* subgroup intrinsically resistant to fluoroquinolones. We did not examine isogenic resistant mutants constructed in vitro; however, the fluoroquinolone resistance likely has emerged from originally susceptible strains, since both phenotypes coexist within the same cluster (TBS-09 and TBS-15).

In this study, we conducted the first example of an MLST analysis on corynebacteria. We used the *gyrB* gene, encoding the DNA gyrase B subunit, as one of the housekeeping genes. Although DNA gyrase is the target molecule of quinolones, the topology of the *gyrB* tree is similar to that constructed from concatenated sequences and showed no correlation with fluoroquinolone resistance. The MLST analysis result showed a good correlation to the RAPD analysis result, indicating that MLST is a useful tool for the molecular typing of corynebacteria as well. It is expected that the information on the target genes described here will contribute to the development and refinement of the MLST method for corynebacteria.

In summary, we report herein the emergence of fluoroquinolone resistance in the ophthalmic commensal *C. macginleyi*. In ophthalmology clinics, special attention must be paid to the potential virulence of *C. macginleyi* and its antimicrobial susceptibility patterns. We are now conducting a large-scale survey on several corynebacteria isolated from eye specimens at independent institutes and hospitals in various areas of Japan. This survey is expected to establish the isolation rate of *C. macginleyi* and its trend of antimicrobial sensitivity. It also will give answers to the important questions concerning *C. macginleyi* raised in this study.

ACKNOWLEDGMENT

This study was supported by Grants-in-Aid for Scientific Research on the Priority Area "Applied Genomics" from the Ministry of Education, Science and Technology of Japan.

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