

Bacteremia Caused by *Corynebacterium amycolatum* with a Novel Mutation in *gyrA* Gene that Confers High-Level Quinolone Resistance

Seoyoung Yoon, M.D., Heejung Kim, M.D., Yangsoon Lee, M.D., and Sinyoung Kim, M.D.

Department of Laboratory Medicine, Yonsei University College of Medicine, Seoul, Korea

Although *Corynebacterium amycolatum* can cause opportunistic infections, it is commonly considered as contaminant. In this report, we present a case of bacteremia caused by *C. amycolatum* with a novel mutation in the *gyrA* gene that confers high-level quinolone resistance to the organism.

Key Words: *Corynebacterium amycolatum*, *gyrA* mutation, Quinolone resistance

INTRODUCTION

Corynebacterium species except *Corynebacterium diphtheriae* are part of the normal flora of the skin and mucous membrane and occur as common contaminants in diagnostic specimens. Therefore, physicians often tend to consider these organisms as contaminants. However, these microorganisms have increasingly been recognized as pathogens in immunocompromised patients [1]. *Corynebacterium amycolatum*, in particular, is a common clinical isolate that often shows multidrug resistance to commonly used antibiotics such as β -lactams, macrolides, and fluoroquinolones [2, 3]. In this report, we present a case of bacteremia caused by *C. amycolatum* possessing a novel mutation in the *gyrA* gene, which confers high-level quinolone resistance to the organism.

CASE REPORT

An 88-yr-old man with diabetes mellitus presented to our emergency department with chest pain. There was no defi-

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Corresponding author: Sinyoung Kim, M.D.

Department of Laboratory Medicine, Yonsei University College of Medicine,
712 Eonju-ro, Gangnam-gu, Seoul 135-720, Korea
Tel: +82-2-2019-3533, Fax: +82-2-2019-4822, E-mail: sykim@yuhs.ac

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nite evidence of acute coronary syndrome, but laboratory examination findings indicated impending end-stage renal disease. Hemodialysis was performed through a subclavian double-lumen catheter on alternate days. After 3 sessions of hemodialysis, on the 11th hospital day, the patient developed fever (temperature, 38.0 °C), and the blood profile showed a total white blood cell count of 8,800/ μ L with 74% neutrophils and an elevated C-reactive protein level of 134.2 mg/L. Hence, samples for blood culture were drawn in triplicate, and empiric antibiotic therapy was initiated with 200 mg of ciprofloxacin administered intravenously every 12 hr. On the 14th hospital day, after 72 hr of incubation, "diphtheroid-like organisms" were detected in all 3 aerobic blood-culture bottles. Although the patient had fever and elevated C-reactive protein level, which do indicate bacteremia, the positive blood culture was considered to show merely contaminants, and therefore, additional blood cultures were performed to identify the causative agent. The repeated blood cultures yielded the same results. Therefore, the antibiotic therapy was changed to vancomycin (1 g/day), and the indwelling double-lumen catheter was removed because of suspected catheter-related infection. Follow-up blood cultures were negative, and the patient recovered fully.

1. Identification

Initially, the "diphtheroid-like organisms" were identified as *C. diphtheriae* by an API Coryne strip (bioMerieux, Marcy l'Etoile, France). Later, 16S rRNA sequence analysis was performed to confirm the species, as described previously [4]. The isolate exhibited 99.1% identity with *C. amycolatum* CIP 103452^T (GenBank accession no.: X82057), and VITEK

Table 1. MIC values for *Corynebacterium amycolatum*

Antibiotics	MIC* (µg/mL)	MIC [†] (µg/mL)		
		range	MIC ₅₀	MIC ₉₀
Penicillin	0.19	≤ 0.12–> 4	1	> 4
Ampicillin	0.125	≤ 0.25–> 8	4	> 8
Imipenem	0.032	≤ 0.25–0.5	≤ 0.25	≤ 0.25
Ciprofloxacin	> 32	≤ 0.25–> 4	> 4	> 8
Gentamicin	8	≤ 1–> 8	4	> 4
Vancomycin	0.032	≤ 0.5–2	≤ 0.5	8

*in this case; [†]MIC values were determined in 60 strains of *C. amycolatum* by using the method described by Gómez-Garcés et al. [6].

Abbreviation: MIC, minimum inhibitory concentration

2 Anaerobe and *Corynebacterium* Identification Card (bio-Mérieux) analysis confirmed the presence of *C. amycolatum* with a probability of 99.0%.

2. Susceptibility testing

To study the multidrug resistance phenotype of *C. amycolatum*, we measured the minimum inhibitory concentrations (MICs) of penicillin, ampicillin, imipenem, ciprofloxacin, gentamicin, and vancomycin for this isolate by using Etest (AB bioMérieux, Solna, Sweden). The MIC values are shown in Table 1; ciprofloxacin had the highest MIC value of > 32 µg/mL. To elucidate the mechanism underlying the high-level ciprofloxacin resistance, we analyzed the sequence of the quinolone resistance-determining region of the *gyrA* gene, as described by Sierra et al. [5]. This isolate showed novel mutations in the *gyrA* gene, wherein the codon for Ser-87 mutated to form an Ile codon and the codon for Asp-91 mutated to a Gly codon (GenBank accession no.:EU835887, Table 2).

DISCUSSION

Although its pathogenic role was apparent only in few cases, *C. amycolatum*, like *Corynebacterium striatum*, can cause endocarditis, bacteremia, and catheter exit-site infection. In cases wherein clinically significant infection by any of the coryneform bacteria is suspected, species identification and antibiotic susceptibility determination are important to decide and administer the optimal antibiotic and treatment regimens. However, possibilities like misidentification of species or non-availability of adequate data for using automated kits with identification cards should be considered. Genotypic assays such as 16S rRNA sequencing may be required to confirm the identity of species. In this present case, the clinical isolate showed a high level of resistance to ciprofloxacin (MIC value of > 32 µg/mL), probably

Table 2. Mutations observed in the quinolone resistance-determining regions of the *gyrA* gene of *Corynebacterium amycolatum* isolates and their ciprofloxacin MIC values

Isolates	Amino acid sequence of <i>gyrA</i> *					Ciprofloxacin MIC (µg/mL)
	87	88	89	90	91	
<i>C. amycolatum</i> (#1008) [†]	Ser	Ala	Ile	Tyr	Asp	0.064
<i>C. amycolatum</i> (#1049) [†]	Arg	Ala	Ile	Tyr	Asp	> 32
<i>C. amycolatum</i> (#1009) [†]	Arg	Pro	Ile	Tyr	Asp	> 32
<i>C. amycolatum</i> (#1011) [†]	Arg	Ala	Val	Tyr	Asp	> 32
Isolate (EU835887)	Ile	Ala	Ile	Tyr	Gly	> 32

*The amino acid position number was obtained from the amino acid sequence of the *gyrA* gene of *Corynebacterium glutamicum*; [†]Data reproduced from Sierra et al. [2].

Abbreviations: MIC, minimum inhibitory concentration; Ser, serine; Ala, alanine; Ile, isoleucine; Tyr, tyrosine; Asp, aspartate; Arg, arginine; Pro, proline; Val, valine; Gly, glycine

due to the novel mutation in the *gyrA* gene, wherein the codon for Ser-87 mutated to form an Ile codon and the codon for Asp-91 mutated to a Gly codon [6]. Therefore, in cases of suspected *Corynebacterium* spp. infection, the physician and the microbiologists in laboratories should confirm the antibiotic susceptibility patterns of the clinical isolates to help decide the optimal antibiotic and treatment regimens.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

REFERENCES

- Funke G, von Graevenitz A, Clarridge JE 3rd, Bernard KA. Clinical microbiology of coryneform bacteria. Clin Microbiol Rev 1997; 10:125-59.
- Soriano F, Zapardiel J, Nieto E. Antimicrobial susceptibilities of *Corynebacterium* species and other non-spore-forming gram-positive bacilli to 18 antimicrobial agents. Antimicrob Agents Chemother 1995;39:208-14.
- Funke G and Bernard KA. Coryneform gram-positive rods. In: Murray PR, Baron EJ, et al. eds. Manual of clinical microbiology. 9th ed. Washington, DC: ASM Press, 2007:485-514.
- Greisen K, Loeffelholz M, Purohit A, Leong D. PCR primers and probes for the 16S rRNA gene of most species of pathogenic bacteria, including bacteria found in cerebrospinal fluid. J Clin Microbiol 1994;32:335-51.
- Sierra JM, Martinez-Martinez L, Vázquez F, Giralt E, Vila J. Relationship between mutations in the *gyrA* gene and quinolone resistance in clinical isolates of *Corynebacterium striatum* and *Corynebacterium amycolatum*. Antimicrob Agents Chemother 2005;49: 1714-9.
- Gómez-Garcés JL, Alos JI, Tamayo J. In vitro activity of linezolid and 12 other antimicrobials against coryneform bacteria. Int J Antimicrob Agents 2007;29:688-92.