Evaluation of a rapid detection method of clarithromycin resistance genes in *Mycobacterium avium* using the Amplification Refractory Mutation System-Loop-Mediated Isothermal Amplification method

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Abstract

Background: Clarithromycin (CLR) is the key drug in multidrug therapy for *Mycobacterium avium* complex (MAC) diseases and the only drug for which drug susceptibility is correlated with a clinical response in these diseases. In the case of CLR-resistant MAC, a point mutation is present at either position 2058 or 2059 of the peptidyl transferase active center in the domain V region of 23S rRNA at the macrolide binding site. Using conventional investigation, we clarified the correlation between drug susceptibility testing and mutation of drug resistance genes. In this study, we adapted a rapid detection method using the amplification refractory mutation system (ARMS)-loop-mediated isothermal amplification (LAMP) to identify a mutation in the 23S rRNA gene in *M. avium* isolates (Figure 1). Furthermore, we evaluated the usefulness as point-of-care testing (POCT) technology using clinical isolates.

Methods: Primers for ARMS-LAMP were designed using PrimerExplorerV5 software based on the nucleotide sequence data for 23S rRNA in *M. avium* strain 104 (Figure 2). Using the minimum inhibitory concentration of CLR, drug susceptibility was determined for 18 clinical *M. avium* isolates. Of these, eight CLR-susceptible and 10 CLR-resistant strains were analyzed by sequencing the 23S rRNA gene and ARMS-LAMP.

Results: Sequence analysis revealed that all eight CLR-sensitive strains tested were wild type, whereas all 10 CLR-resistant strains were mutants. Using ARMS-LAMP, no amplification with the mutant-type mismatch primer sets (MTPS) was observed in the eight wild-type strains, but amplification was observed with MTPS in the 10 mutant strains (Table 1).

Conclusion: The developed rapid detection method for the CLR resistance gene using ARMS-LAMP can determine drug resistance in a few hours without the need for special equipment. ARMS-LAMP may be a new clinically beneficial POCT technology for examination that is novel and extremely practical.

Introduction

Pulmonary MAC disease is effectively treated with multidrug therapy centered on CLR, but resistance to CLR is an important prognosticator for clinical aggravation. Therefore, a quick and easy method for detecting the presence of drug resistance to CLR is a clinically important issue. In the case of CLR-resistant MAC, a point mutation is present at either position 2058 or 2059 of the peptidyl transferase active center in the domain V region of 23S rRNA at the macrolide binding site. Using conventional investigation, we clarified the correlation between drug susceptibility testing and mutation of drug resistance genes[1)]. In this study, we adapted a rapid detection method using the ARMS-LAMP to identify a mutation in the 23S rRNA gene in *M. avium* isolates. Furthermore, we evaluated the usefulness as POCT technology using clinical isolates.

Methods

Strains	***M. avium*** **strain 104(reference strain), and 18 clinical *M. avium* isolates**
Drug susceptibility tests	**BrothMIC NTM (Kyokuto Pharmaceutical Industrial Co., Ltd.) was used to determine CLR susceptibility of the *M. avium* strains at pH 7.4**
Sequence analysis of DNA corresponding to domain V of 23S rRNA	**PCR was performed to amplify the region corresponding to domain V of the 23S rRNA gene according to the method described by Jamal et al.[2)].**
ARMS-LAMP method	**The judgment standard was checked for amplification, and if no amplification was observed, the strain was judged to be wild type. When amplification was present, samples were checked for the presence of amplified product using each primer set. When amplification was observed with GA-MTPS, the strain was judged to be A2058G mutant type. The strain was judged to be another mutant type at positions A2058T, A2058C, or A2059C of domain V of 23S rRNA when LAMP gave a product using TA-MTPS, CA-MTPS, or AC-MTPS, respectively.**

Methods

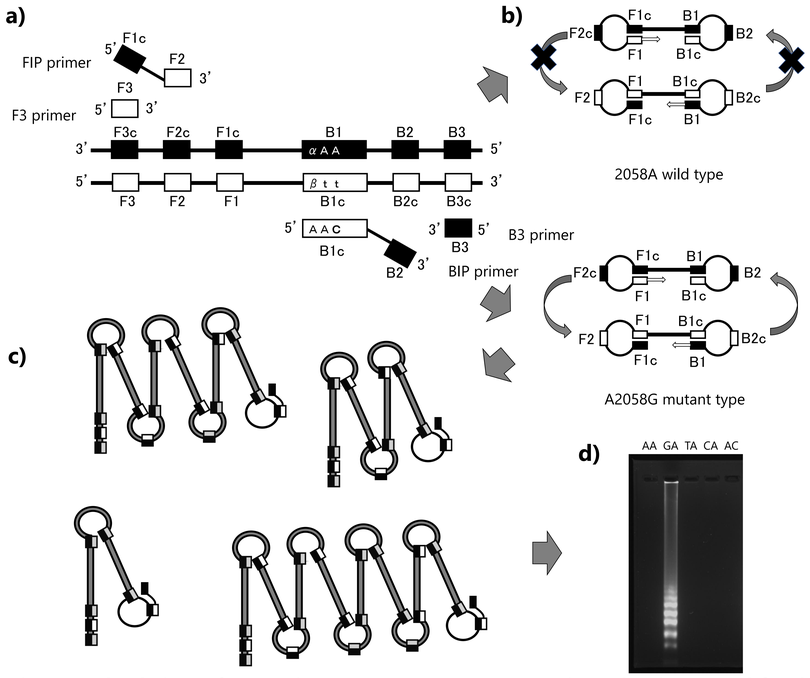

Figure 1. The designs of CLR resistance A2058G mutant-type mismatch primers used for the ARMS-LAMP assay. a) A strand-displacing DNA polymerase extends the DNA from FIP while separating from the DNA chain. The primer F3 binds to its complementary region on the DNA to displace the newly synthesized DNA. An analogous reaction is performed by BIP and B3. α (α = A, wild type; G, A2058G) and β (β = A, wild type; C, A2058G) are indicated by the point mutation at position 2058 of the 23S rRNA gene. The bold area indicates the mismatched base C (cytosine). b) The synthesized DNA self-anneals because of the complementary region at both ends and forms 'dumbbell' structures. c) After repeated rounds, a complementary region on the same chain is amplified. d) The products of ARMS-LAMP using the CLR-resistant A2058G mutant type mismatch primer set. AA: wild type, GA: A2058G mutant type, TA: A2058T mutant type, CA: A2058C mutant type, AC: A2059C mutant type.

Limitation

- ✔ **Only the primer set for detecting the mutant type could be created.**
- ✔ **We were unable to design a primer set corresponding to a clinical isolate of *M. intracellulare*.**

Conclusions

We developed a rapid detection method for the CLR resistance gene using ARMS-LAMP that can determine drug resistance in a few hours without the need for special equipment. This method may represent a new POCT technology that is clinically beneficial, novel, and extremely practical. In the future, *M. avium* should be directly detectable in sputum from patients who have been administered CLR multi-drug combination therapy for a long time after a definitive diagnosis of *M. avium* disease. This new ARMS-LAMP method may allow regular monitoring of CLR resistance.

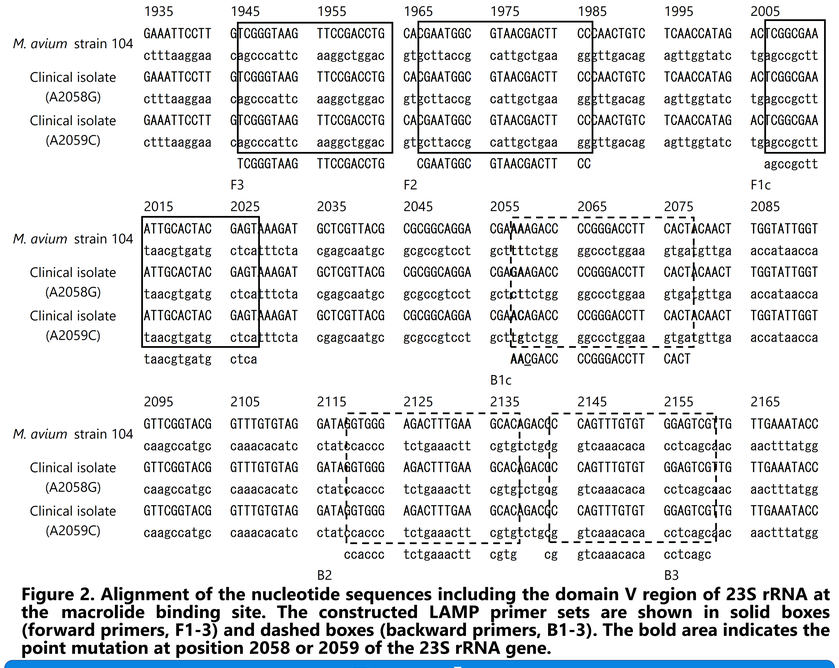

Figure 2. Alignment of the nucleotide sequences including the domain V region of 23S rRNA at the macrolide binding site. The constructed LAMP primer sets are shown in solid boxes (forward primers, F1-3) and dashed boxes (backward primers, B1-3). The bold area indicates the point mutation at position 2058 or 2059 of the 23S rRNA gene.

Results

Table 1. MICs of clarithromycin, mutations of nucleotides at positions 2058 and 2059 in the domain V region of the 23S rRNA gene and results of ARMS–LAMP using *M. avium* isolates.

Strains	Total number	MIC (μg/mL)	Genotype	ARMS-LAMP			
				GA-MTPS	CA-MTPS	TA-MTPS	AC-MTPS
Reference strain							
Mycobacterium avium 104	1	0.25	AA	-	-	-	-
Clinical isolates							
CLR susceptible strains	8	< 8	AA	-	-	-	-
CLR resistant strains	4	>32	GA	+	-	-	-
	2	>32	CA	-	+	-	-
	1	>32	TA	-	-	+	-
	3	>32	AC	-	-	-	+

CLR, clarithromycin. MIC, minimum inhibitory concentration. ARMS-LAMP, amplification refractory mutation system -loop-mediated isothermal amplification. GA-MTPS, A2058G mutant-type mismatch primer set. CA-MTPS, A2058C mutant-type mismatch primer set. TA-MTPS, A2058T mutant-type mismatch primer set. AC-MTPS, A2059C mutant-type mismatch primer set.

References

1) Inagaki T, et al. J Antimicrob Chemother. 66: 722-729. (2011)
2) Jamal MA, et al. Tuber Lung Dis. 80: 1-4. (2000)