Analysis of *cyp51A* polymorphisms of *Aspergillus fumigatus* in Japan

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Background

- *Aspergilllus fumigatus* is the most important *Aspergillus* species that causes chronic pulmonary aspergillosis (CPA) and invasive pulmonary aspergillosis (IPA). Azoles are first line antifungal drug treatment for CPA and IPA.
- However, azole resistance in *Aspergillus fumigatus* isolates is increasingly recognized as a cause of treatment failure recently.
- Azole resistance mechanisms are mostly correlated with *cyp51A* mutations.
  - ✓ In Europe, azole resistance is mostly caused by tandem repeat(TR) in the *cyp51A* promoter region.
  - ✓ In Japan, azole resistance is mostly caused by *cyp51A* point mutations, such as G54, G138, M220, and G448.

Tashiro M, et al. Antimicrob Agents Chemother. 2012 Jan;56(1):584-7.

- On the other hand, some azole-susceptible strains have *cyp51A* mutations: polymorphisms.
- The polymorphisms in Europe consist mainly of two combinations of mutations: *cyp51A*-5SNPs and *cyp51A*-3SNPs.

P. Escribano, et al. Antimicrobial Agents and chemotherapy 2011; 55(5): 2460-2462

- Strains with 5SNPs and 3SNPs have elevated azole MICs, although they are not azole resistant on the basis of the azole susceptibility breakpoints.

R. Garcia-Rubio, et al. Antimicrobial Agents and chemotherapy 2018; (5): 2460-2462

- little is known about the prevalence of *cyp51A* polymorphisms among azole-susceptible *A. fumigatus* strains isolated in Japan.

Materials and Methods

- We analyzed
  - ✓ 118 environmental isolates
  - ✓ 102 clinical azole-susceptible isolates.
- They are preserved at Chiba University Medical Mycology Research Center (Chiba City, Japan).
- For genotyping, microsatellite typing was conducted among all strains. We amplified and sequenced 6 loci (3A, 3B, 3C, 4A, 4B, and 4C) and counted the repeat numbers of each region from the sequence. We regarded the strains with the same combination of STRs, as the probably same strains and excluded them.
- The isolates were screened for *cyp51A* point mutations with Surveyor Nuclease (Figure 2). They are also screened for *cyp51A* TR with electrophoresis (Figure 3).
- We sequenced the *cyp51A* region of the strains which probably have mutations.
- When we found *cyp51A* mutations, we also conducted MIC tests based on Clinical and Laboratory Standards Institute (CLSI) .

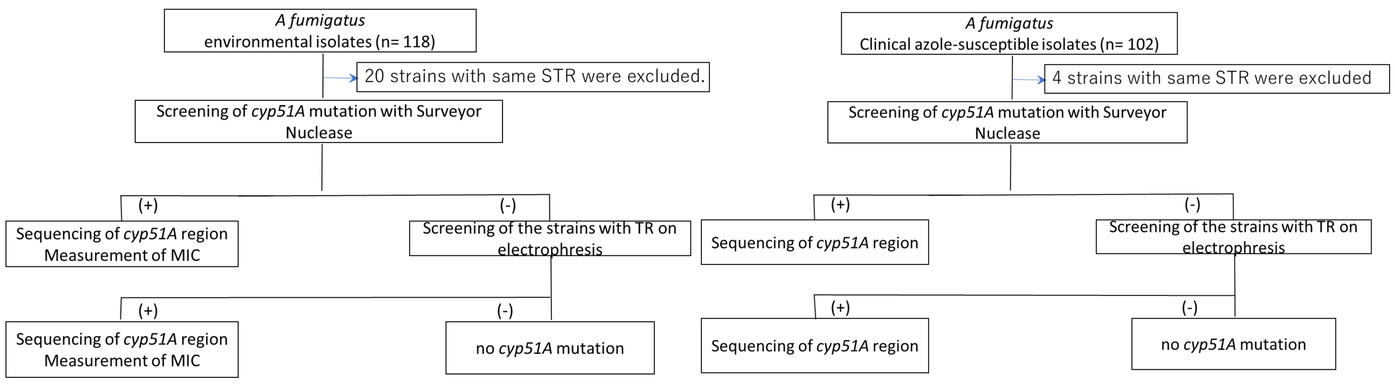
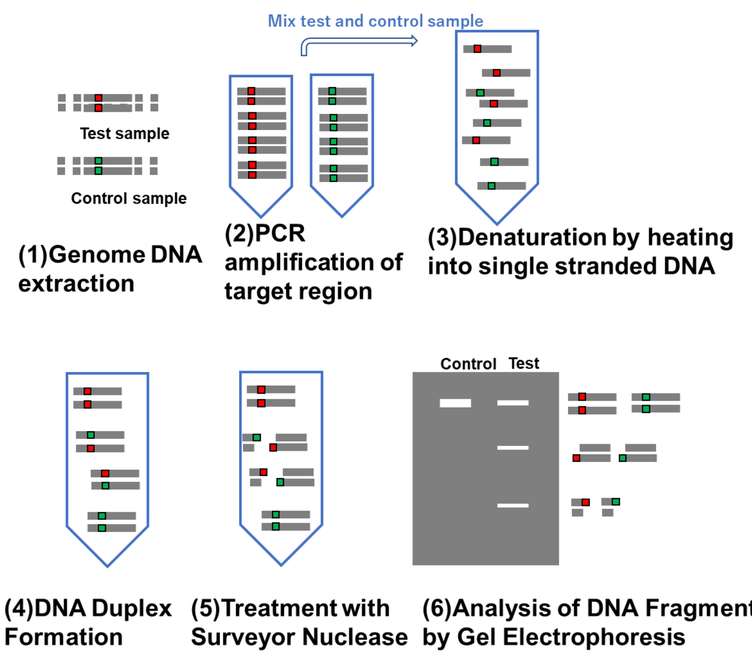
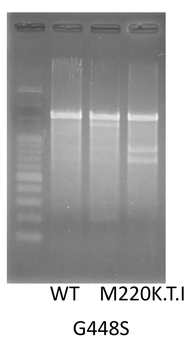


Figure 1 Analysis of *cyp51A* of environmental and clinical isolates



e.g.)



cyp51A mutation	digested fragments (bp)
G448S	1434/227
M220K.T.I.V	750/911 751/910 752/909

Figure 2 Screening for *cyp51A* mutation with Surveyor Nuclease

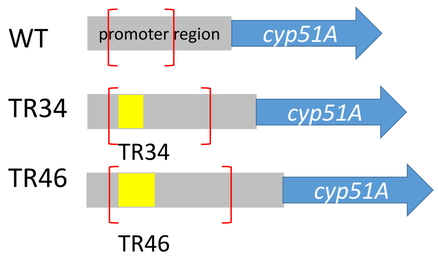


Figure 3 Screening for tandem repeat (TR)

Results

IFM No.	*cyp51A* seq	TR	sourse	MIC (μg/ml) ITCZ	MIC (μg/ml) VRCZ
62345	D343N	-	soil	1	1
62628	F46Y M172V N248T D255E E427K	-	soil	2	2
62400	N248K	-	soil	0.5	0.5
60748	N248K	-	air	0.5	0.5
63915	g1673c	-	air	0.5	0.5
60765	V396A	-	air	0.25	1
62432	N248K	-	soil	0.5	1
63953	L464 insertion	-	soil	0.5	0.5

Table 1 Environmental isolates with *cyp51A* mutations

IFM No.	cyp51A seq	TR	year	Hospital	MIC μg/mL ITCZ	MIC μg/mL VRCZ
49895	N248K	-	2000	Tokyo	0.5	1
53927	N248K	-	2004	Tokyo	0.5	0.5
61407	N248K	-	2012	Chiba	0.25	0.25
51977	M39I	-	2002	Hokkaido	0.25	0.25
51748	N248K	-	2002	Tokyo	0.5	0.5
63311	N248K	-	2015	Tokyo	1	0.5
60369	N248K	-	2011	Iwate	0.5	0.5
62521	N248K	-	2014	Tokyo	1	0.5
62241	N248K	-	2013	Aichi	0.5	0.5
62054	N248K	-	2013	Tokyo	0.25	0.125
60994	N248K	-	2011	Chiba	0.5	1
63141	N248K	-	2014	Osaka	1	1
64302	N248K	-	2016	Tokyo	0.5	1
64563	N248K	-	2016	Tokyo	0.5	1
64666	N248K	-	2016	Tokyo	1	0.5

Table 2 Clinical isolates with *cyp51A* mutations

			Isolate source		MIC	
IFM	**Clinical form**	**underlying diseases**	**Year**	**Hospital**	**ITCZ**	**VRCZ**
49895	SPA	unknown	2000	Tokyo	0.5	1
51748	ABPA	-	2002	Tokyo	0.5	0.5
53927	CPA	IP	2004	Tokyo	0.5	0.5
60369	IPA	near-drowning	2011	Iwate	0.5	0.5
60994	colonization	lung cancer	2011	Chiba	0.5	1
61407	CPA	COPD	2012	Chiba	0.25	0.25
62054	CPA	NTM	2013	Tokyo	0.25	0.125
62241	IPA	ALL	2013	Aichi	0.5	0.5
62521	SPA	Asthma	2014	Tokyo	1	0.5
63141	SPA	Old Tb	2014	Osaka	1	1
63311	CPA	NTM	2015	Tokyo	1	0.5
64302	CPA	NTM	2016	Tokyo	0.5	1
64563	CPA	COPD, BA	2016	Tokyo	0.5	1
64666	CPA	Old Tb, COPD	2016	Tokyo	1	0.5

Table 3 Clinical isolates with *cyp51A*^{N248K} mutation

- Only one environmental isolate had 5SNPs, which was the most prevalent polymorphisms in Europe.
- More N248K isolates were found in Japan than in Europe.
- All of the N248K isolates were azole susceptible.

Discussion

- Only one strain had 5SNPs.
- A substitution of N248K was the prevalent polymorphism in this research, although it was a rare polymophism in Europe.
- Strains in Japan may have different constitution of cyp51A polymorphisms from those in Europe.
- Clinical isolates may include more N248K strains than environmental isolates (Figure 4). It is possible that the prevalence of N248K strains among clinical isolates are related with their virulence.

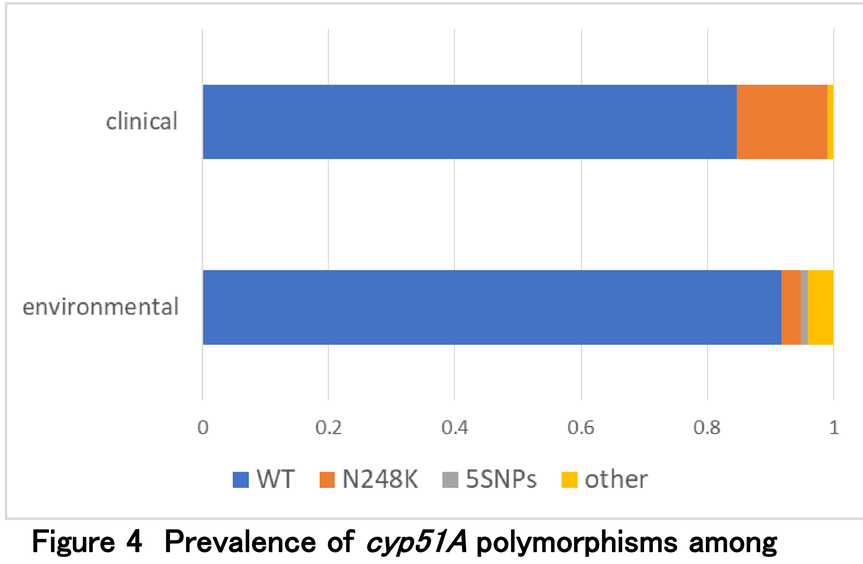


Figure 4 Prevalence of *cyp51A* polymorphisms among clinical and environmental isolates