

# Non-*cyp51A* Azole-Resistant *Aspergillus fumigatus* Isolates with Mutation in HMG-CoA Reductase

## Technical Appendix

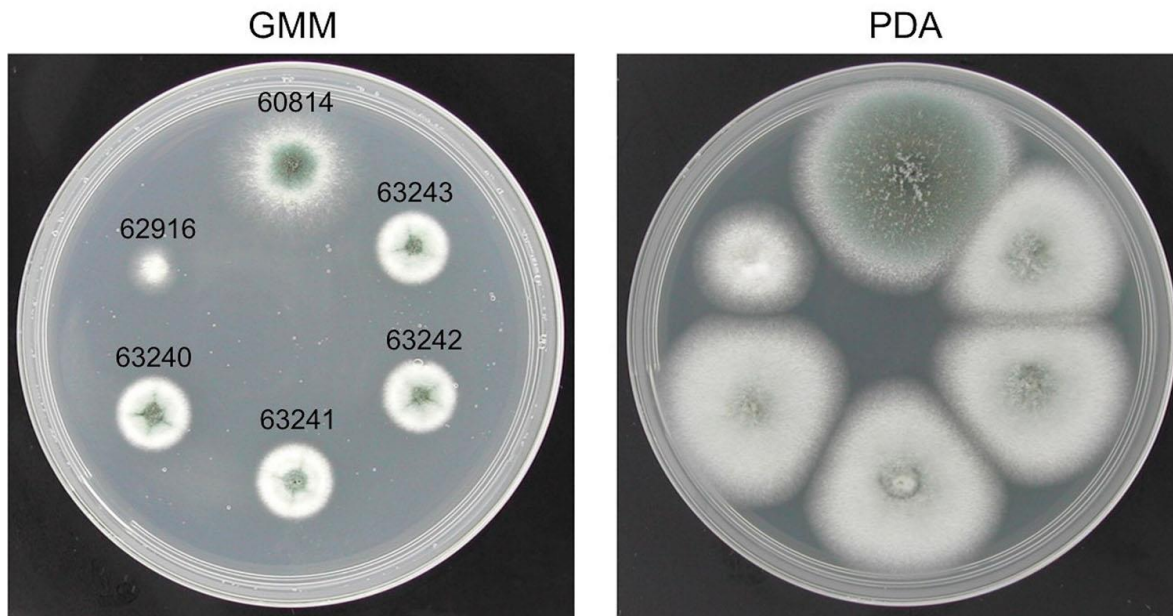
**Technical Appendix Table 1.** Primers used in study of non- *cyp51A* azole-resistant *Aspergillus fumigatus*

Primer name	Sequence (5' to 3')
<b>SQ(<i>cyp51A</i>)</b>	
<i>cyp51A</i> _SQ1F	GAAGCGGTTAAGGCGGGAATAGACG
<i>cyp51A</i> _SQ2F	GAATAATTTACTGTTCTCCTCTAG
<i>cyp51A</i> _SQ3F	GGTGCCGATGCTATGGCTTACGGCCTAC
<i>cyp51A</i> _SQ4F	CGGATCGGACGTGGTGTATGATTG
<i>cyp51A</i> _SQ5F	CTTGACGGTGACAAGGACTCTCAG
<i>cyp51A</i> _SQ6F	CGCGTGCTCCTTGCTTCACCTGGAG
<i>cyp51A</i> _M-R	GTAGATTGACCTCATGCGCGCATGAGCAGC
<i>cyp51A</i> -R	CAGGTTTTTCGCACGAGCTTCTCC
<b>SQ(<i>hmg1</i>)</b>	
<i>hmg1</i> _SQ1F	CATTCCTGAGATTTCTCAGCATCGA
<i>hmg1</i> _SQ2F	TGGACGTCGATACTCTGCTTCA
<i>hmg1</i> _SQ3F	AGATCCCGGACACCTCCTCCGAT
<i>hmg1</i> _SQ4F	ACTGCGGCAGCGGACAACAGGGGCA
<i>hmg1</i> _SQ5F	AGCAACAAGACTAGCATCTTTGGGAG
<i>hmg1</i> _SQ6F	CGTGTCATCGAGAGTCTTCTCAAGAGT
<i>hmg1</i> _SQ7F	GCGGTCAAGATCCGTCGAGCTGTT
<i>hmg1</i> _SQ8F	GGTCGAAGCATTTTGACTGCGGC
<i>hmg1</i> _SQ9F	GGAAGCGTGGGTGGGTTCAACGCT
<i>hmg1</i> _SQ10F	CGTCTCAGCAGCCGTGAGTGCTGCT
<i>hmg1</i> _SQR1	ACTCTTGAGAAGACTCTCGATGACACG
<i>hmg1</i> _SQR2	CCATGTGTATTTTCGACAGCCAGC
<b>SQ(<i>erg6</i>)</b>	
<i>erg6</i> _SQ1F	GACAGTTGAGATAAAAAAGGTGTAAG
<i>erg6</i> _SQ2F	GATCGGCGAGATCCTCGTTGTGCA
<i>erg6</i> _SQ3F	TGTTCAAGCCCGACCACATTGGCATC
<i>erg6</i> _SQ4F	TACTTACCGCGCGAGTCTCCTCT
<i>erg6</i> _SQR1	TACTCTCAATATTTCCACTTGGGCCA
<i>cyp51A</i> -F(pPTR-k)	GAATTCGAGCTCGGTAAGGCAATTCAGGGGATGAACAAG
<i>cyp51A</i> -R(pPTR-p)	GCTTGCATGCCTGCATTTAGACAGGATCTGAAGTTTTTC
<i>erg6</i> -F(pPTR-k)	GAATTCGAGCTCGGTACCTCCTTGAGCATCTGAACGTAAGG
<i>erg6</i> -R(pPTR-p)	GCTTGCATGCCTGCATGTTATTTTGAAGTCGAGCTGAATCC
<i>hmg1</i> -F(pPTR-k)	GAATTCGAGCTCGGTAAGGCAATTCAGGGGATGAACAAG
<i>hmg1</i> -N-R	CCAGCGAGCGGCATTGAACAAGTAGCCG
<i>hmg1</i> -C-F	CGGCTACTTGTTCAATGCCGCTCGCTGG
<i>hmg1</i> -R(pPTR-p)	GCTTGCATGCCTGCATGCCAGCTGTTGACATACGACAGAAGC

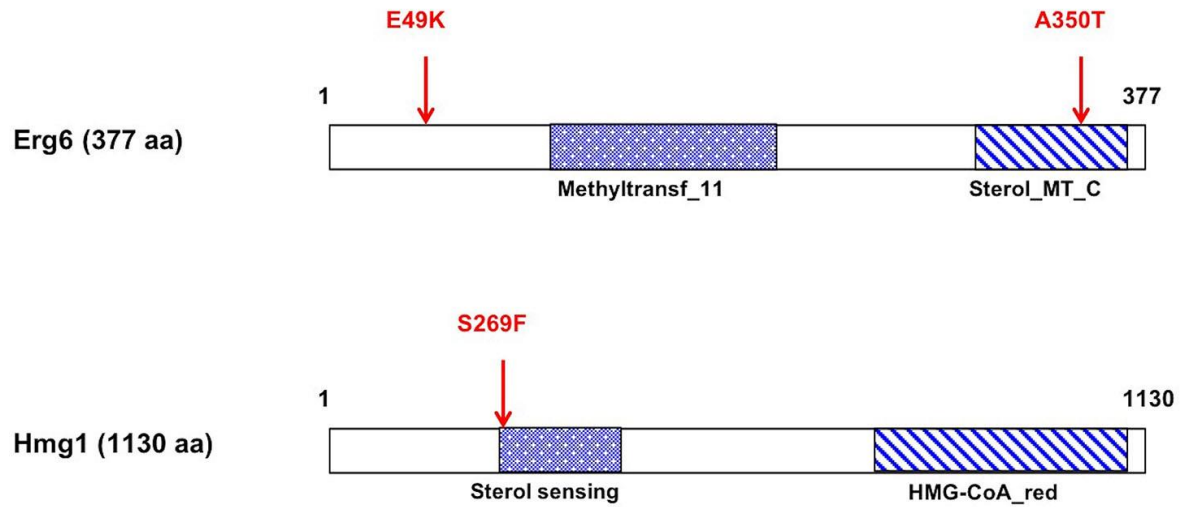
**Technical Appendix Table 2.** Sequence variants in the third strain (IFM 63240) of *Aspergillus fumigatus*

Gene ID	Type	Amino acid change	Length of the protein, amino acids	Gene name	Annotation
AFUA_2G08170	SNV	Glu118*	396	–	Has domain with predicted role in regulation of transcription
AFUA_2G11830	Deletion	Trp292fs†	333	–	Hypothetical protein
AFUA_4G03630	SNV	Ala350Thr	377	<i>erg6</i>	Sterol 24-C-methyltransferase with a predicted role in glycosylphosphatidylinositol (GPI)-anchor biosynthesis
AFUA_5G05850	SNV	Trp177*	465	–	Hypothetical protein
AFUA_5G12650	SNV	Asn195Asp	499	–	Hypothetical protein
AFUA_2G03700	SNV	Ser269Phe	1130	<i>hmg1</i>	Hydroxymethylglutaryl-CoA (HMG-CoA) reductase
AFUA_5G14660	SNV	Gln99Arg	529	–	GABA permease? amino acid transmembrane transporter?

\*Nonsense change, which generated termination codon  
 †fs, frame shift



**Technical Appendix Figure 1.** Observed colony morphology of *Aspergillus fumigatus* strains isolated from a single patient. GMM and PD agar plates were incubated at 37°C for 48 h.



**Technical Appendix Figure 2.** Mutation sites of Hmg1 and Erg6. Arrows indicate mutation sites. Methyltransf\_11, methyltransferase domain; Sterol\_MT\_C, sterol methyltransferase C-terminal domain; Sterol sensing, sterol sensing domain; HMG-CoA\_red, HMG-CoA reductase domain.