

**Table S1: list of strains used in this study**

Strain	Name in this study	Parental strain	Genotype	Reference
DSY4606	P1	-	Wild type	[1]
DSY4593	P3	DSY4606	<i>MRR1</i> <sup>V668G</sup>	[1]
DSY5416	P3 <i>mrr1Δ</i>	DSY5241	<i>mrr1Δ::FRT</i>	[2]
DSY5437	P3 <i>mrr1Δ</i> + <i>MRR1</i>	DSY5416	<i>MRR1::NAT1</i>	[2]
DSY5439	P3 <i>mrr1Δ</i> + <i>MRR1</i> <sup>V668G</sup>	DSY5416	<i>MRR1</i> <sup>V668G</sup> :: <i>NAT1</i>	[2]
DSY5639	P3 <i>mrr1Δ</i> + <i>MRR1</i> <sup>G654A</sup>	DSY5416	<i>MRR1</i> <sup>G654A</sup> :: <i>NAT1</i>	This study
DSY5247	P3- <i>mfs7Δ</i>	DSY4593	<i>mfs7Δ::NAT1</i>	[2]
DSY5248	P1- <i>mfs7Δ</i>	DSY4606	<i>mfs7Δ::NAT1</i>	[2]
DSY5241	P3- <i>mrr1Δ</i>	DSY4593	<i>mrr1Δ::NAT1</i>	[2]
DSY5658	P1- <i>mrr1Δ</i>	DSY4606	<i>mrr1Δ::NAT1</i>	[2]
DSY5635	P1- <i>cdr1Δ</i>	DSY4606	<i>cdr1Δ::HygR</i>	This study
DSY5607	P3- <i>cdr1Δ</i>	DSY4593	<i>cdr1Δ::HygR</i>	This study
DSY5609	P3- <i>mfs7Δcdr1Δ</i>	DSY5247	<i>mfs7Δ::NAT1/cdr1Δ::HygR</i>	This study
DSY5611	P1- <i>mfs7Δcdr1Δ</i>	DSY5248	<i>mfs7Δ::NAT1/cdr1Δ::HygR</i>	This study
DSY5613	P3- <i>mrr1Δcdr1Δ</i>	DSY5241	<i>mfs7Δ::NAT1/cdr1Δ::HygR</i>	This study
DSY5664	P1- <i>mrr1Δcdr1Δ</i>	DSY5242	<i>mfs7Δ::NAT1/cdr1Δ::HygR</i>	This study
DSY5643	<i>MRR1-cdr1Δ</i>	DSY5437	<i>MRR1::NAT1/cdr1Δ::HygR</i>	This study
DSY5645	<i>MRR1</i> <sup>V668G</sup> - <i>cdr1Δ</i>	DSY5439	<i>MRR1</i> <sup>V668G</sup> :: <i>NAT1/cdr1Δ::HygR</i>	This study
DSY5644	<i>MRR1</i> <sup>G654A</sup> - <i>cdr1Δ</i>	DSY5639	<i>MRR1</i> <sup>G654A</sup> :: <i>NAT1/cdr1Δ::HygR</i>	This study

**Table S2: primers used in this study**

Primer	Sequence (5' to 3')
CICDR1-P1	AGGATGAATGGGTTAGCCAAATCG
CICDR1-TEF-5R	ATTTATTCCGAGCGCTATACAGTATAGTGCTTGCTGTTTCGAT
CICDR1-P2	GACGGCAATGGGAATCAAAATGC
CICDR1-ACT1-3f	ATTCATCCCATTTCATTCATCGTAATACCCCGCAAAGACCTT
CLCDR1-TEF-5f	ATTTATTCCGAGCGCTATACAGTATAGTGCTTGCTGTTTCGAT
CICDR1-ACT1-3r	AAGGTCTTTGCGGGGTATTACGATGGAATGAATGGGATGAAT
CICDR1-P3	CACACATTCCATTGGAGGCTCTT
CICDR1-P4	CAATGTTGCCCCACTCTAAGACCA
Hygro_1949R	ACCATCAGCACAGAATTA ACTCT
CICDR1-3-verif	GTTGAAGTATTTGGACTCGGAGTT
ACT1-pYM70	CCACCCAAGGCATTTCTATATCTT
pDS1918-P1	CGGCCAGTGAGCGCGCGTAA
V654A-R	AGTCCCTGTCAATCTGAGCGTCGTAGAAAGTCAAACCCAA
V654A-F	TTGGGTTTGACTTTCTACGACGCTCAGATTGACAGGGAGCT
CIMRR1-SacI	TAGGAAGAGCTCACAATAAGTTTTTCATTG
CIMRR1-Apa	TTGTGGGCCCAGAGATCTGTTCTAATGA
MRR1-3_rev_new	GATGAGGATGGCGAGAAGATCAATTCCATCTCCTCTACTCTTGGAACAAAGAGAATTGTCA ATCCTAACTGGTACCCACTTGTTGTTTTCAACGATGACGAGACAGCAGTATAGCGACCAGC
CIMRR1_F	CATTGCACAAAAGCAAAGC
CI_MRR1_3377_R	TGTGTTGTTGCGAAAAGACAGAAAG
CICDR1_5_Cas9	TGCTTTGTACAAGATGGCGG
CICDR1_3_Cas9	AAAGACTATATTGCCAAGGC
CIACT1-P <sup>a</sup>	TCTCCTTGCCTCACGGTATCTTG
CIACT1-F	CACGTTGTCCCAATTTAC
CIACT1-R	CTTGCTCAAAGTCCAAAG
MFS7-P <sup>a</sup>	TCTTCATTCCATTCTACAGGCGA
MFS7-F	GTTGCTATTATGATTGGTATC
MFS7-R	AGGCATAAGGATAGAACC
CICDR1-P <sup>a</sup>	TCTATCGGTTATGTCCAGCAGCAA
CICDR1-F	AACGGTCATGGATTAGAC
CICDR1-R	CCTTGATACAGAATATGGTTG

<sup>a</sup>: Taqman probes with FAM and TAMRA modifications

**Table S3: MICs of clinical isolate DSY4941**

Antifungal drugs	MIC (µg/ml)
Anidulafungin	0.12
Micafungin	0.12
Caspofungin	0.12
5-Flucytosine	0.06
Posaconazole	0.12
Voriconazole	0.12
Itraconazole	0.25
Fluconazole	16
Amphotericin B	0.5

**Table S4: Antifungal MICs of *C. lusitaniae* isolates and mutant derivatives**

Antifungal drugs	MIC (µg/ml)					
	<i>MRR1</i>	<i>MRR1</i> <i>cdr1Δ</i>	<i>MRR1</i> <sup>V688G</sup>	<i>MRR1</i> <sup>V688G</sup> <i>cdr1Δ</i>	<i>MRR1</i> <sup>V654A</sup>	<i>MRR1</i> <sup>V654A</sup> <i>cdr1Δ</i>
Anidulafungin	0.12	0.12	0.12	0.12	0.25	0.25
Micafungin	0.06	0.06	0.06	0.06	0.12	0.12
Caspofungin	0.25	0.25	0.5	0.5	0.5	0.5
5-Flucytosine	2	4	>64	>64	>64	>64
Posaconazole	0.03	0.015	0.5	0.015	0.5	0.06
Voriconazole	0.008	<0.008	0.25	0.015	0.5	0.25
Itraconazole	0.12	0.03	0.5	0.06	1	0.06
Fluconazole	0.5	0.12	32	1	64	32
Amphotericin B	0.25	0.25	0.5	0.25	0.5	0.5

**References**

1. Asner, S.A.; Giulieri, S.; Diezi, M.; Marchetti, O.; Sanglard, D. Acquired Multidrug Antifungal Resistance in *Candida Lusitaniae* during Therapy. *Antimicrobial Agents and Chemotherapy* 2015, *59*, AAC.02204-15-8, doi:10.1128/aac.02204-15.

2. Kannan, A.; Asner, S.A.; Trachsel, E.; Kelly, S.; Parker, J.; Sanglard, D. Comparative Genomics for the Elucidation of Multidrug Resistance in *Candida Lusitaniae*. *mBio* 2019, *10*, 1227–21, doi:10.1128/mbio.02512-19.