

Sporothrix brasiliensis Treatment Failure without Initial Elevated Itraconazole MICs in Felids at Border of Brazil

Appendix

Additional Materials and Methods

For short tandem repeat (STR) genotyping, nine markers were amplified with three multiplex PCRs and subsequently analyzed with a 3500 XL genetic analyzer (Applied Biosystems, Foster City, CA, USA) (1). Copy numbers were determined using GeneMapper 5 (Applied Biosystems) and the genetic relatedness between the current and previously genotyped isolates was assessed with BioNumerics v7.6.1 (Applied Maths NV, Sint-Martens-Latem, Belgium).

For antifungal susceptibility testing (AFST) in the mycelial phase incubation was performed at 30°C and minimum inhibitory concentrations (MICs) were read after 72 hours instead of the usual 48 hours (2). For inoculum preparation, isolates were suspended in 0.9% NaCl with 0.05% Tween 20 and the transmission of the suspension was measured at 530 nm using Spectrophotometer IV Control Genesys 20. Transmission range of 80%–82% ($0.9 - 5.0 \times 10^6$ CFU/mL) was used. The suspension was diluted (1:50) in RPMI 1640 (0.2% glucose) with a final conidial concentration of 0.4×10^4 to 5×10^4 CFU/mL. *Aspergillus flavus* ATCC 204304 was used as quality control. Amphotericin B (Bristol Myers Squib, Woerden, The Netherlands), fluconazole (Merck, Darmstadt, Germany), itraconazole (Janssen Cilag, Breda, The Netherlands), voriconazole (Pfizer Central Research, New York, USA), posaconazole (Merck), isavuconazole (Basilea Pharmaceutica, Basel, Switzerland), terbinafine (VWR, Leicestershire, UK), anidulafungin (Merck) and micafungin (Astellas Pharma, Tokyo, Japan) were tested. For amphotericin B, itraconazole, voriconazole, posaconazole, and isavuconazole, the MIC endpoint was the lowest concentration that produced complete inhibition of growth. For fluconazole, the

MIC was defined as the lowest concentration at which there was $\leq 50\%$ growth reduction as compared to growth control. For echinocandins, it was the lowest concentration producing a visual change in the appearance of the growth, defined as the minimum effective concentration (MEC). For terbinafine, the MIC was defined as the lowest concentration at which there was $\leq 80\%$ growth reduction when compared to the growth control. For mycelial phase, tentative epidemiologic cutoff values (ECVs) at 97.5% as per CLSI M57 recommendation, according to Espinel-Ingroff were implemented for amphotericin B, itraconazole, voriconazole, posaconazole and terbinafine (3).

For inoculum preparation of AFST in the yeast phase, isolates were suspended in 0.9% NaCl equal to 0.5 McFarland. The transmission of the suspension was measured at 530 nm using Spectrophotometer IV Control Genesys 20. A transmission range of 75%–77% ($1.0 - 5.0 \times 10^6$ CFU/mL) was used. The suspension was diluted 100 times in RPMI 1640. *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 were used as quality controls. The same antifungals as for the mycelial phase were tested. The MICs were read visually after 96–120 hours of incubation at 35°C. For amphotericin B, fluconazole and terbinafine, the MIC endpoint was identical as used for mycelial phase and for itraconazole, voriconazole, posaconazole, isavuconazole, anidulafungin and micafungin, the MIC was defined as the lowest concentration at which there was a $\leq 50\%$ growth reduction.

References

1. Spruijtenburg B, Bombassaro A, Meijer EFJ, Rodrigues AM, Grisolia ME, Vicente VA, et al. *Sporothrix brasiliensis* genotyping reveals numerous independent zoonotic introductions in Brazil. J Infect. 2023;86:610–3. PubMed <https://doi.org/10.1016/j.jinf.2023.02.034>
2. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. CLSI standard M38. 3rd edition. Wayne (PA): The Institute; 2017.
3. Espinel-Ingroff A, Abreu DPB, Almeida-Paes R, Brilhante RSN, Chakrabarti A, Chowdhary A, et al. Multicenter, international study of MIC/MEC distributions for definition of epidemiological cutoff values for *Sporothrix* species identified by molecular methods. Antimicrob Agents Chemother. 2017;61:e01057–17. PubMed <https://doi.org/10.1128/AAC.01057-17>

Appendix Table 1. Accession numbers (NCBI), date of sampling and geographic data of the animals with proven sporotrichosis in the triple border region*

CMRP	Accession no.	Date	Neighborhood	City	Country
CMRP6060	PQ741666	07/07/2021	Jardim Alice I	Foz do Iguaçu	Brazil
CMRP5585	PQ741608	09/07/2021	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP6061	PQ741667	09/07/2021	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP6062	PQ741668	09/07/2021	Jardim Copacabana	Foz do Iguaçu	Brazil
CMRP5586	PQ741609	30/07/2021	Parque Residencial Morumbi	Foz do Iguaçu	Brazil
CMRP6063	PQ741669	30/07/2021	Parque Residencial Morumbi	Foz do Iguaçu	Brazil
CMRP6064	PQ741670	09/08/2021	Portal da Foz	Foz do Iguaçu	Brazil
CMRP6065	PQ741671	09/08/2021	Portal da Foz	Foz do Iguaçu	Brazil
CMRP6066	PQ741672	16/08/2021	Portal da Foz	Foz do Iguaçu	Brazil
CMRP6067	PQ741673	16/08/2021	Portal da Foz	Foz do Iguaçu	Brazil
CMRP6068	PQ741674	16/08/2021	Portal da Foz	Foz do Iguaçu	Brazil
CMRP6069	PQ741675	16/09/2021	Parque Residencial Santa Rita	Foz do Iguaçu	Brazil
CMRP5587	PQ741610	16/09/2021	Jardim Alice I	Foz do Iguaçu	Brazil
CMRP6070	PQ741676	24/09/2021	Jardim Europa	Foz do Iguaçu	Brazil
CMRP6071	PQ741677	24/09/2021	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP6072	PQ741678	24/09/2021	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP6073	PQ741679	24/09/2021	Parque Residencial Morumbi	Foz do Iguaçu	Brazil
CMRP5588	PQ741611	29/12/2021	Beverli Falls Park	Foz do Iguaçu	Brazil
CMRP5589	PQ741612	03/01/2022	Jardim das Flores	Foz do Iguaçu	Brazil
CMRP5590	PQ741613	18/01/2022	Jardim das Flores	Foz do Iguaçu	Brazil
CMRP5591	PQ741614	18/01/2022	Jardim das Flores	Foz do Iguaçu	Brazil
CMRP5592	PQ741615	01/02/2022	Jardim Curitiba	Foz do Iguaçu	Brazil
CMRP5593	PQ741616	04/02/2022	Conjunto C	Foz do Iguaçu	Brazil
CMRP5594	PQ741617	04/02/2022	Conjunto C	Foz do Iguaçu	Brazil
CMRP6074	PQ741680	10/03/2022	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP6075	PQ741681	16/03/2022	Jardim Nacional	Foz do Iguaçu	Brazil
CMRP6077	PQ741682	05/04/2022	Cidade Nova II	Foz do Iguaçu	Brazil
CMRP6078	PQ741683	19/04/2022	Conjunto C	Foz do Iguaçu	Brazil
CMRP6079	PQ741684	19/04/2022	Cidade Nova II	Foz do Iguaçu	Brazil
CMRP6080	PQ741685	27/04/2022	Parque Residencial Santa Rita	Foz do Iguaçu	Brazil
CMRP6082	PQ741686	02/06/2022	Conjunto C	Foz do Iguaçu	Brazil
CMRP6083	PQ741687	02/06/2022	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP6084	PQ741688	07/06/2022	Residencial Morumbi III	Foz do Iguaçu	Brazil
CMRP5785	PQ741618	07/06/2022	Parque Residencial Italia	Foz do Iguaçu	Brazil
CMRP6085	PQ741689	08/06/2022	Vila São Sebastião	Foz do Iguaçu	Brazil
CMRP6086	PQ741690	14/06/2022	Vila Borges	Foz do Iguaçu	Brazil
CMRP6087	PQ741691	14/06/2022	Vila Borges	Foz do Iguaçu	Brazil
CMRP6088	PQ741692	20/06/2022	Jardim São Paulo II	Foz do Iguaçu	Brazil
CMRP6089	PQ741693	21/06/2022	Jardim São Paulo II	Foz do Iguaçu	Brazil
CMRP6090	PQ741694	21/06/2022	Porto Belo	Foz do Iguaçu	Brazil
CMRP5786	OR501573	22/06/2022	NA	Ciudad del Este	Paraguay
CMRP6091	PQ741695	07/07/2022	Portal da Foz	Foz do Iguaçu	Brazil
CMRP6092	PQ741696	07/07/2022	Portal da Foz	Foz do Iguaçu	Brazil
CMRP6093	PQ741697	14/07/2022	Portal da Foz	Foz do Iguaçu	Brazil
CMRP6094	PQ741698	14/07/2022	Portal da Foz	Foz do Iguaçu	Brazil
CMRP6095	PQ741699	28/07/2022	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP6096	PQ741700	11/08/2022	Vila Borges	Foz do Iguaçu	Brazil
CMRP6097	PQ741701	11/08/2022	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP6098	PQ741702	19/08/2022	Vila São Sebastião	Foz do Iguaçu	Brazil
CMRP5787	OR501574	19/08/2022	NA	Ciudad del Este	Paraguay
CMRP6099	PQ741703	24/08/2022	Conjunto C	Foz do Iguaçu	Brazil
CMRP6100	PQ741704	26/08/2022	Conjunto C	Foz do Iguaçu	Brazil
CMRP6101	PQ741705	26/08/2022	Conjunto C	Foz do Iguaçu	Brazil
CMRP6102	PQ741706	26/08/2022	Conjunto C	Foz do Iguaçu	Brazil
CMRP6103	PQ741707	06/09/2022	Jardim Residencial São Roque	Foz do Iguaçu	Brazil
CMRP6104	PQ741708	09/09/2022	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP6105	PQ741709	20/09/2022	Profilurb I	Foz do Iguaçu	Brazil
CMRP6106	PQ741710	05/10/2022	Jardim Bela Vista de Itaipu III	Foz do Iguaçu	Brazil
CMRP6107	PQ741711	05/10/2022	Jardim Oriente	Foz do Iguaçu	Brazil
CMRP6108	PQ741712	05/10/2022	Profilurb I	Foz do Iguaçu	Brazil
CMRP5791	PQ741619	30/11/2022	NA	Hernandarias	Paraguay
CMRP5792	PQ741620	10/12/2022	NA	Hernandarias	Paraguay
CMRP5915	PQ741621	19/01/2023	NA	Hernandarias	Paraguay
CMRP5916	PQ741622	19/01/2023	NA	Hernandarias	Paraguay
CMRP5917	PQ741623	12/05/2023	Portal da Foz	Foz do Iguaçu	Brazil
CMRP5918	PQ741624	12/05/2023	Portal da Foz	Foz do Iguaçu	Brazil
CMRP5919	PQ741625	25/05/2023	Portal da Foz	Foz do Iguaçu	Brazil

CMRP	Accession no.	Date	Neighborhood	City	Country
CMRP5940	PQ741640	25/05/2023	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP5941	PQ741641	25/05/2023	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP5921	PQ741626	01/06/2023	Vila Guarani	Foz do Iguaçu	Brazil
CMRP5922	PQ741627	08/06/2023	NA	Ciudad del Este	Paraguay
CMRP5923	PQ741628	12/06/2023	Jardim Novo Horizonte	Foz do Iguaçu	Brazil
CMRP5924	PQ741629	12/06/2023	Jardim Novo Horizonte	Foz do Iguaçu	Brazil
CMRP5925	PQ741630	22/06/2023	Centro	Foz do Iguaçu	Brazil
CMRP5942	PQ741642	22/06/2023	Profilurb I	Foz do Iguaçu	Brazil
CMRP5926	PQ741631	28/06/2023	Jardim São Luiz	Foz do Iguaçu	Brazil
CMRP5927	PQ741632	28/06/2023	Jardim São Luiz	Foz do Iguaçu	Brazil
CMRP5943	PQ741643	30/06/2023	Vila A	Foz do Iguaçu	Brazil
CMRP5944	PQ741644	30/06/2023	Jd. Califórnia II	Foz do Iguaçu	Brazil
CMRP5928	PQ741633	06/07/2023	Jardim Guaira	Foz do Iguaçu	Brazil
CMRP5945	PQ741645	06/07/2023	Bubas	Foz do Iguaçu	Brazil
CMRP5946	PQ741646	11/07/2023	Profilurb I	Foz do Iguaçu	Brazil
CMRP5929	PQ741634	11/07/2023	Cohapar III	Foz do Iguaçu	Brazil
CMRP5947	PQ741647	13/07/2023	Conjunto C	Foz do Iguaçu	Brazil
CMRP5948	PQ741648	13/07/2023	Loteamento Bela Vista de Itaipu II	Foz do Iguaçu	Brazil
CMRP5930	PQ741635	13/07/2023	Jardim Cedro	Foz do Iguaçu	Brazil
CMRP5931	PQ741636	20/07/2023	Conjunto C	Foz do Iguaçu	Brazil
CMRP5949	PQ741649	20/07/2023	Portal da Foz	Foz do Iguaçu	Brazil
CMRP5932	PQ741637	26/07/2023	Jardim Duarte	Foz do Iguaçu	Brazil
CMRP5950	PQ741650	28/07/2023	Jardim Novo Horizonte	Foz do Iguaçu	Brazil
CMRP5933	PQ741638	28/07/2023	Jardim Novo Horizonte	Foz do Iguaçu	Brazil
CMRP5935	PQ741639	28/07/2023	Jardim Eliza II	Foz do Iguaçu	Brazil
CMRP5952	PQ741651	16/08/2023	Jardim California	Foz do Iguaçu	Brazil
CMRP5953	PQ741652	16/08/2023	Centro	Foz do Iguaçu	Brazil
CMRP5955	PQ741653	19/08/2023	NA	Ciudad del Este	Paraguay
CMRP5956	PQ741654	24/08/2023	Jardim das Flores	Foz do Iguaçu	Brazil
CMRP5957	PQ741655	24/08/2023	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP5958	PQ741656	04/09/2023	Jardim São Paulo II	Foz do Iguaçu	Brazil
CMRP5959	PQ741657	04/09/2023	Jardim São Paulo II	Foz do Iguaçu	Brazil
CMRP5960	PQ741658	04/09/2023	Conjunto Habitacional Fernanda	Foz do Iguaçu	Brazil
CMRP5961	PQ741659	06/09/2023	Jardim Tropical	Foz do Iguaçu	Brazil
CMRP5962	PQ741660	06/09/2023	Parque Ouro Verde	Foz do Iguaçu	Brazil
CMRP5963	PQ741661	12/09/2023	Vila Guarani	Foz do Iguaçu	Brazil
CMRP5964	PQ741662	12/09/2023	Parque Residencial Morumbi III	Foz do Iguaçu	Brazil
CMRP5965	PQ741663	13/09/2023	Cohapar III	Foz do Iguaçu	Brazil
CMRP5966	PQ741664	15/09/2023	Loteamento Bela Vista de Itaipu II	Foz do Iguaçu	Brazil
CMRP5967	PQ741665	15/09/2023	Loteamento Bela Vista de Itaipu II	Foz do Iguaçu	Brazil
CMRP6111	PQ741713	25/10/2023	Jardim São Luiz	Foz do Iguaçu	Brazil

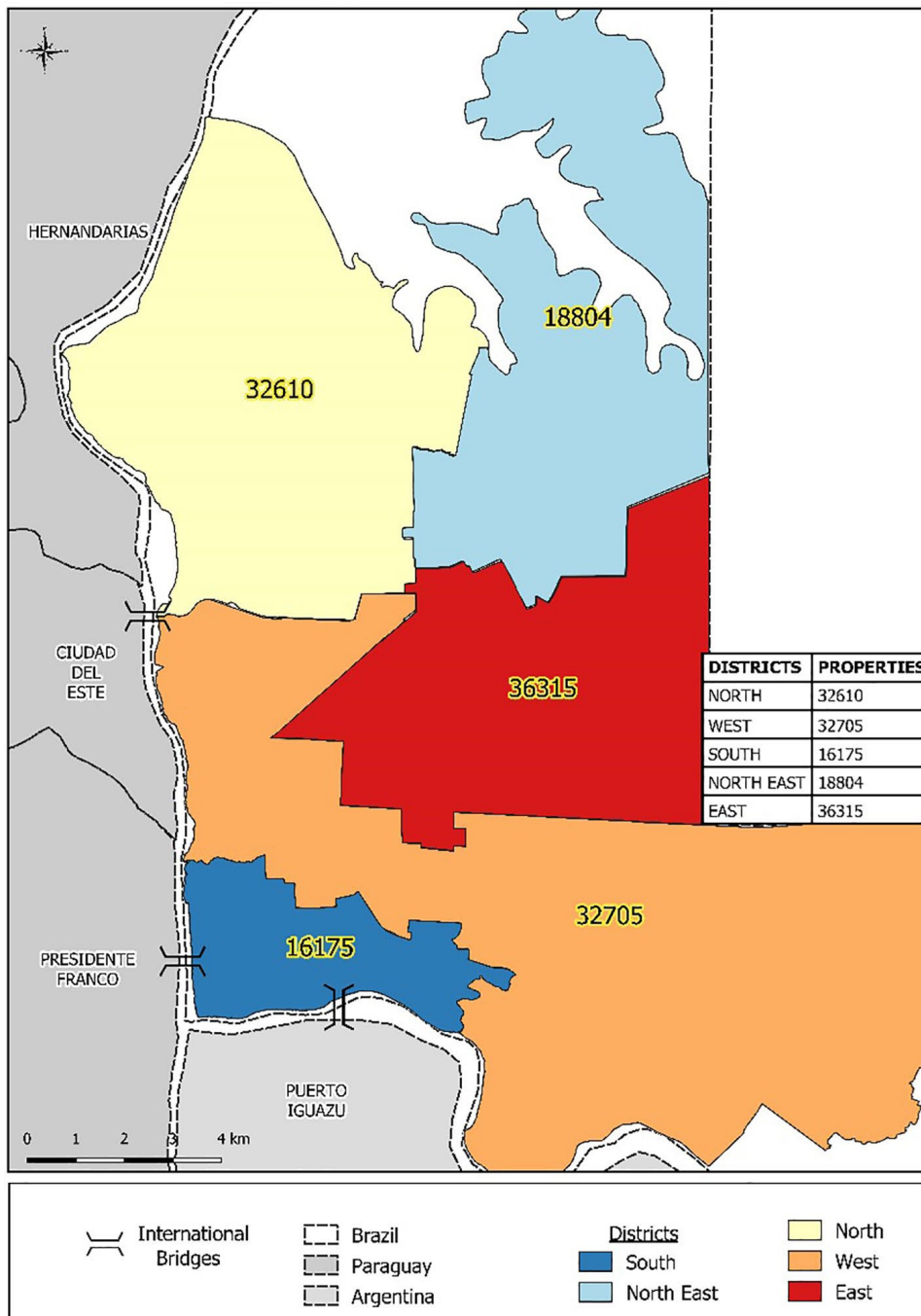
*NA, not applicable.

Appendix Table 2. Comparison between clinical outcome and geometric mean MIC*

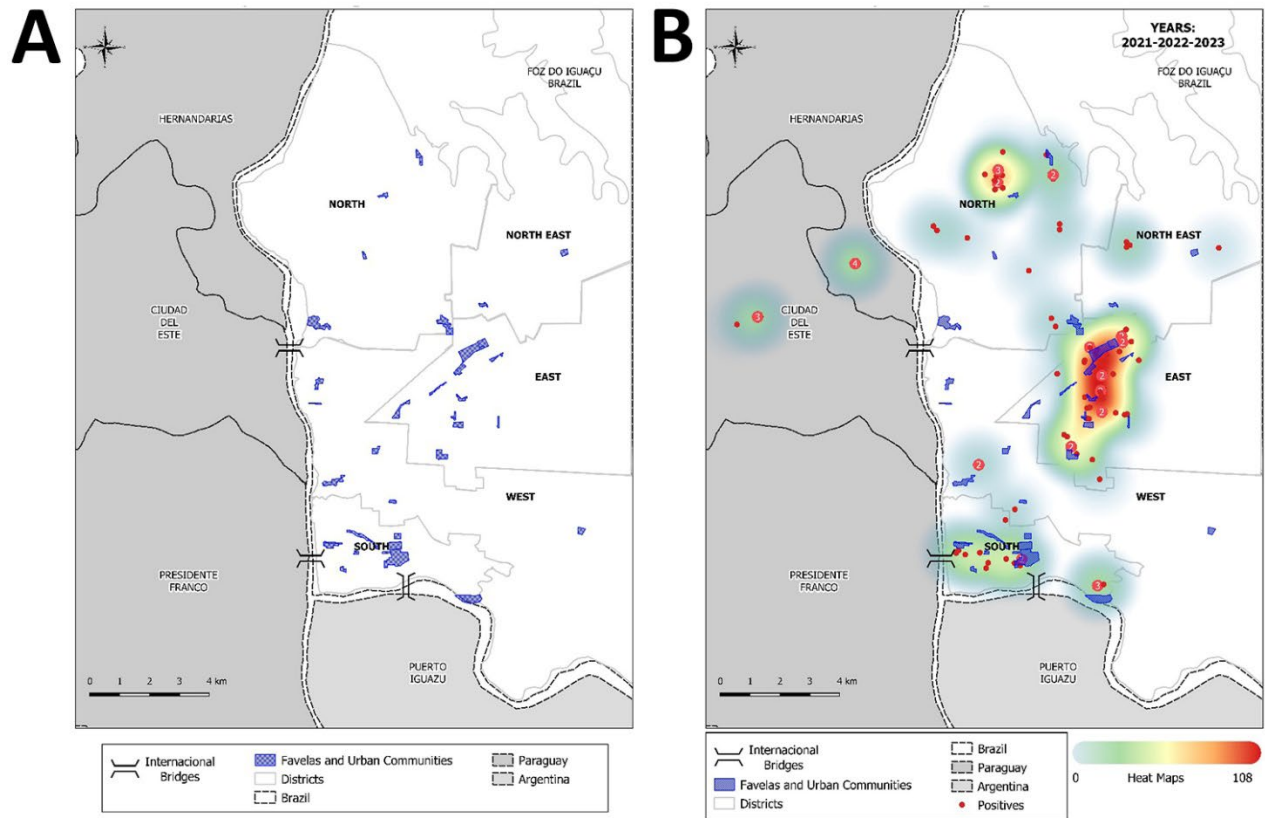
Outcome	Geometric mean MIC																	
	AMB		FLU		ITC		VOR		POS		ISA		TRB		AFG		MCF	
	M	Y	M	Y	M	Y	M	Y	M	Y	M	Y	M	Y	M	Y	M	Y
Cutaneous disseminated disease																		
Death	1	0.7	64	20	0.3	0.05	15	0.3	0.4	0.05	8	0.07	0.09	0.14	0.008	0.05	0.008	0.04
Clinical cure	1	0.8	64	21	0.4	0.04	16	0.4	0.4	0.06	8	0.06	0.08	0.13	0.008	0.07	0.008	0.06
Lost to follow up	1	0.7	64	25	0.5	0.09	16	0.6	0.5	0.12	8	0.19	0.07	0.07	0.008	0.07	0.008	0.06
Euthanized	1	0.5	64	28	0.3	0.03	14	0.5	0.3	0.04	5	0.06	0.08	0.04	0.008	0.05	0.008	0.12
Fixed cutaneous disease																		
Clinical cure	1	0.6	64	15	0.3	0.04	15	0.2	0.4	0.05	7	0.05	0.08	0.14	0.008	0.06	0.008	0.06
Lost to follow up	1	0.4	64	32	0.28	0.07	12	0.3	0.3	0.06	6	0.08	0.14	0.12	0.01	0.09	0.01	0.05
Euthanized	1	1	64	25	0.3	0.02	16	0.3	0.5	0.03	6	0.02	0.09	0.12	0.01	0.03	0.008	0.02
Extracutaneous disease																		
Clinical cure	2	1	64	18	0.37	0.05	16	0.6	0.4	0.06	10	0.04	0.14	0.18	0.008	0.02	0.008	0.04

*AMB, amphotericin B; AFG, anidulafungin; FLU, fluconazole; ISA, isavuconazole; ITC, itraconazole; M, mycelial phase; MFG, micafungin; POS, posaconazole; TRB, terbinafine; VOR, voriconazole; Y, yeast phase.

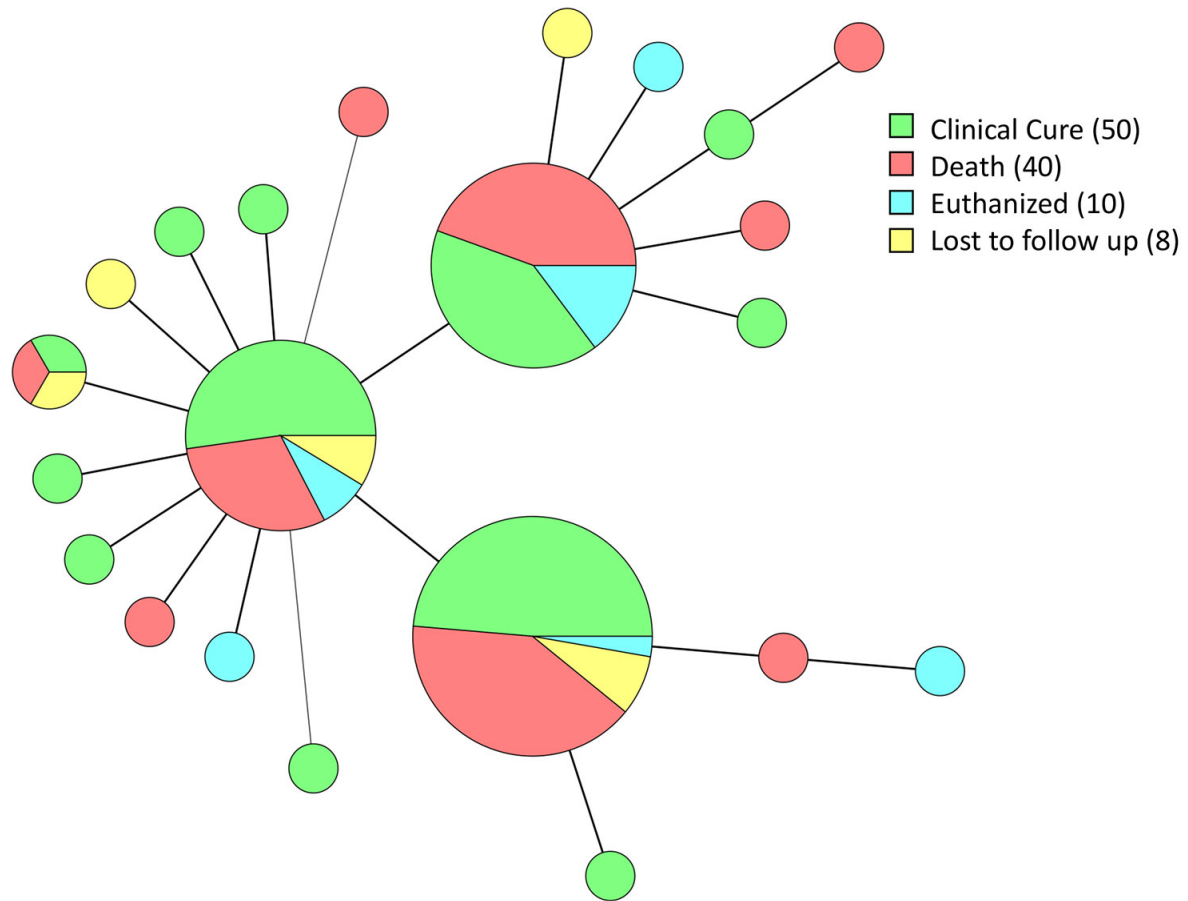
Residences Per District



Appendix Figure 1. Number of residences per district of Foz do Iguaçu, Brazil.



Appendix Figure 2. Location of the favelas and poor urban communities (FPUC) in Foz do Iguaçu, Brazil (A). Map with FPUC + all sporotrichosis cases 2021-2023 (B).



Appendix Figure 3. Minimum-spanning tree of 108 *Sporothrix brasiliensis* isolates based on nine microsatellite markers. Isolates are colored after the clinical outcome and branch lengths indicate the genetic relatedness, with thick solid lines (variation in one marker) and thin solid lines (variation in two markers).