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Molecular epidemiology of *Candida tropicalis* isolated from urogenital tract infections

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Abstract

Candida tropicalis is a common human pathogenic yeast, and its molecular typing is important for studying the population structure and epidemiology of this opportunistic yeast, such as epidemic genotype, population dynamics, nosocomial infection, and drug resistance surveillance. In this study, the antifungal susceptibility test and multilocus sequence typing (MLST) analysis were carried out on *C. tropicalis* from central China. Among 64 urogenital isolates, 45 diploid sequence types (DST) were found, of which 20 DSTs (44.4%) were new to the central database. The goeBURST analysis showed that CC1 (clonal complex) was the only azole-resistant (100%, 10/10) cluster in Wuhan, which was composed of DST546, DST225, DST376, and DST506, and most of the strains (90%, 9/10) were isolated from the urinary tract. Potential nosocomial infections were mainly caused by CC1 strains. The azole resistance rate of urinary isolates (50.0%, 21/42) was higher than that of vaginal isolates (27.3%, 6/22). The genotype diversity and novelty of vaginal isolates were higher than those of urinary isolates. *C. tropicalis* population in Wuhan was genetically diverse and divergent from that seen in other countries. In this study, there were significant differences in genotype and azole susceptibility between urine and vaginal strains. The azole-resistant cluster (CC1) found in urine is of great significance for the clinical treatment and prevention of nosocomial infection. The newly discovered DSTs will contribute to further study the similarity, genetic relationship, and molecular epidemiology of *C. tropicalis* worldwide.

KEYWORDS
azole resistance, *Candida tropicalis*, epidemiology, multilocus sequence typing, urogenital tract infection

1 | INTRODUCTION

Candida spp. are important components of the microflora of human skin, oral and vaginal mucosa, and gastrointestinal tract. They are important opportunistic pathogens that can cause superficial and invasive infections in human hosts (Falagas et al., 2010). Over the past decade, infections caused by *Candida* have risen significantly and become an important cause of nosocomial infections, leading to serious public health problems (Gajdács et al., 2019; Scordino et al., 2018; Wu et al., 2015). Among the *Candida* spp, *C. tropicalis* is considered to be the first or second most common pathogenic yeast in some geographical regions such as East Asia and Latin America (Arrua et al., 2015; Kumar et al., 2014; Pahwa et al., 2014).

In recent years, the proportion of urinary tract infections caused by *Candida* has increased, especially in critically ill patients (Bongomin et al., 2017; Fisher, 2011; Gharanfoli et al., 2019). The risk factors for *Candida* urinary tract infection have been well documented, including age, female sex, long-term hospitalization, admission to intensive care unit, immunosuppressive therapy, radiotherapy, recent use of broad-spectrum antibiotics, and use of urinary tract instruments (Hollenbach, 2008; Sobel et al., 2011). Many studies have shown that hospitalized patients with candiduria have a higher mortality rate than patients without candiduria (Behzadi et al., 2015; Bougnoux et al., 2008; Gajdács, & Urbán, 2019; Muñoz et al., 2011; Negri et al., 2012). Vaginal microflora helps to maintain and protect the genitourinary system from infection (Kamińska & Gajecka, 2017). However, asymmetric microflora and its proportional imbalance may lead to infections caused by opportunistic resident pathogens (Brotman et al., 2010; Kamińska & Gajecka, 2017). *Candida* is one of the most common causes of vaginitis (Jacob et al., 2018). Although *Candida albicans* is the main source of urogenital tract infections, other *non-albicans Candida* species such as *C. tropicalis* are increasingly recognized as common yeast pathogens (Tan et al., 2015; Wu, Liu, et al., 2017). Fluconazole is the first choice for the treatment of *C. tropicalis* infection in the urogenital tract (Donders et al., 2018; Fisher, 2011), but the problem of drug resistance has become increasingly prominent, which brings difficulties to clinical treatment (Fan et al., 2017; Fernández-Ruiz et al., 2015; Huang et al., 2014; Morris et al., 2018; Tan et al., 2016; Teo et al., 2017; Wu, Liu, et al., 2017). The prevalence of *C. tropicalis* and the increasing resistance to fluconazole have attracted the attention of more and more researchers, prompting a large number of studies on the epidemiological and biological characteristics of *C. tropicalis* (Al-Obaid et al., 2017; Chen et al., 2019; Chew et al., 2017; Choi et al., 2016; Fan et al., 2017; Magri et al., 2013; Scordino et al., 2018; Wang et al., 2016; Wu, Guo, et al., 2017; Wu et al., 2012).

The prevalence and drug resistance rates of *C. tropicalis* vary between and within geographical regions, and local epidemiological data are essential for management (Fernández-Ruiz et al., 2015; Scordino et al., 2018; Tan et al., 2016; Wu et al., 2019). The molecular epidemiological study of *C. tropicalis* can explore the epidemic genotype, potential route of transmission, the genetic background of drug-resistant strains, biological niches, and population structure (Wu, Guo, et al., 2017; Wu et al., 2019; Zuza-Alves et al., 2017). At present, several genetic typing methods have been used to study the molecular epidemiology of *C. tropicalis*. The *C. tropicalis* multilocus sequence typing (MLST) system with standardized six housekeeping genes (*ICL1*, *MDR1*, *SAPT2*, *SAPT4*, *XYR1*, and *ZWF1a*) initiated by Tavanti et al. (2005) was the most widely used, which can better discriminate isolates and has high reproducibility. Strains and populations from different laboratories and different geographical regions can be compared through the online MLST database to explore their different geographical sources, anatomical sources, acquisition and transmission of drug resistance, and genetic variation patterns (Wu et al., 2019; Zuza-Alves et al., 2017).

In this study, we report, for the first time, the MLST genotype data of *C. tropicalis* isolates recovered from the urogenital tract in central China, explored the relationship between susceptibility to azole and DSTs, and described the existence of a specific cluster of DSTs that may be closely related to nosocomial transmission.

2 | MATERIALS AND METHODS

2.1 | Isolates and identification

All *C. tropicalis* isolates from the urogenital tract were consecutively collected from the clinical laboratory in Renmin Hospital of Wuhan University, China, during 1 year (between December 2018 and November 2019). Patients corresponding to the included isolates had clinical symptoms, and duplicate isolates of each patient during one hospitalization were removed. All isolates were stored at −80°C in 15% glycerol. During the study, the isolates were maintained on Sabouraud dextrose agar. Microbial identification of clinical isolates was conducted by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonics). Protein was extracted by the formic acid/acetonitrile (FA/ACN) method (Lee et al., 2017; Mizusawa et al., 2017). In short, mix 2–3 colonies with 300 µl distilled water and 600 µl 100% ethanol. After centrifugation at 15,000 *g* for 2 min, the precipitate was dried at room temperature, reconstituted in 20 µl 70% FA and 20 µl ACN, fully mixed, and centrifuged at 15 000 *g* for 2 min. 1 µL supernatant was dried at room temperature on the 96-spot steel plate, and then, 1 µL HCCA matrix was added. Each sample was tested in duplicate, and the threshold for correct species identification was defined as ≥1.70 (Lee et al., 2017).

2.2 | Antifungal susceptibility testing

The in vitro susceptibility of *C. tropicalis* isolates to fluconazole (FLC), voriconazole (VRC), and itraconazole (ITC) was determined by the broth microdilution method following the M27-E4 standard (CLSI, 2017a) proposed by the Clinical and Laboratory Standards Institute (CLSI). Antifungal standard powders (Solarbio Life Sciences) were dissolved with DMSO as stock solutions. The medium used in the experiment was RPMI 1640 containing glucose L-glutamine but no bicarbonate (Genom, Hangzhou, China). Quality control strains during susceptibility testing were *C. albicans* ATCC 90028, *C. parapsilosis* ATCC 22019, and *C. krusei* ATCC 6258 (National Clinical Testing Center). The minimum inhibitory concentrations (MICs) of FLC and VRC were determined at 24 hr according to CLSI M60 guideline (CLSI, 2017b) (for FLC: ≤2 µg/ml, susceptible; 4 µg/ml, intermediate; ≥8 µg/ml, resistant. for VRC: ≤0.12 µg/ml, susceptible; 0.25–0.5 µg/ml, intermediate; ≥1 µg/ml, resistant). WT distribution limit epidemiological cutoff values of ITC were determined at 24 hr according to CLSI M59 guideline (≤0.5 µg/ml, wild-type; >0.5 µg/ml, non-wild-type) (CLSI, 2018).

2.3 | Multilocus sequence typing

The total genomic DNA extracted from yeast cells using the Yeast Genomic DNA Extraction Kit (Solarbio Life Sciences). MLST was performed based on the six housekeeping genes (*ICL1*, *MDR1*, *SAPT2*, *SAPT4*, *XYR1*, and *ZWF1a*). The primers used for amplification and sequencing have been described previously (Tavanti et al., 2005). PCRs were carried out in 25 μl volumes containing 2.5 μl template DNA, 1.5 μl forward/reverse primers, 12.5 μl 2*Taq PCR PreMix (Innovagene Biotechnology), and 8.5 μl double-distilled water. Amplification conditions were as follows: initial denaturation at 94°C for 7 min; 30 cycles at 94°C (60 s), 53°C (60 s) and 72°C (65 s); and a final 10-min extension step at 72°C. Amplification products were purified using the PCR Purification Kit (Omega Biotek). Bi-directional DNA sequencing was done on an ABI 3730XL automatic sequencer (Applied Biosystems). Sequencing results were spliced with Geneious 4.8 software (https://www.geneious.com/), and polymorphic sites were confirmed by visual examination of the chromatograms. The heterozygous sites in the chromatograms were defined by the heterozygous data (K, M, R, S, W, and Y) from the International Union of Pure and Applied Chemistry (https://iupac.org/) nomenclature. The allele numbers and DSTs of isolates were defined by comparing the sequences with those available in the *C. tropicalis* MLST Databases (https://pubmlst.org/ctropicalis). The new alleles and new DSTs were named by the database curator after scrutiny.

2.4 | Phylogenetic and population structure analysis

The MLST data were analyzed with the goeBURST algorithm in PHILOVIZ 2.0 software (http://www.phyloviz.net/) to identify clonal complexes (CCs). Isolates were considered the same CC if sharing five out of six alleles. The phylogenetic relationship of 64 isolates was determined through cluster analysis using UPGMA (unweighted pair group method with arithmetic averages) of the MEGA 7 software (http://www.megasoftware.net/). Since the software could not recognize the merge base symbols, the sequence was first processed as follows (Tavanti et al., 2005): Nucleotide sequences from the six sequenced loci were concatenated, and then the homozygous base site was copied into two corresponding homozygous bases, and the heterozygous base site was replaced with its corresponding two different bases. The significance of UPGMA cluster nodes was determined by bootstrapping with 1,000 replications. Bootstrap values of ≥70% were defined as statistically significant. The genetic relationship between the 64 isolates from Wuhan with 1,090 isolates in the central *C. tropicalis* MLST database (as of 24 February 2020) was studied by using the goeBURST algorithm in PHILOVIZ 2.0 software and minimal spanning tree algorithm of the BioNumerics 7.6 software (https://www.applied-maths.com/).

3 | RESULTS

3.1 | Description of clinical isolates and azole susceptibility

A total of 64 strains of *C. tropicalis* were isolated from the urogenital tract of 64 patients during the study period (Table 1), 42 strains were isolated from the urine and 22 strains were isolated from the vaginal swabs. Among these patients, 23 (35.9%) were men and 41 (64.1%) were women. The majority of patients were from the urological ward (12) and gynecology ward (12), followed by reproductive ward (11) and intensive care unit (ICU) ward (6), respiratory intensive care unit (RICU) ward (5), geriatric ward (4), neurology ward (4), and other wards (10).

The azole susceptibility results of 64 *C. tropicalis* isolates were as follows: For FLC: susceptible, 36 (56.2%); intermediate, 1 (1.6%); resistant, 27 (42.2%). For VRC: susceptible, 30 (46.9%); intermediate, 6 (9.4%); resistant, 28 (43.7%). For ITC: wild-type, 35 (54.7%); non-wild-type, 29 (45.3%). The fluconazole resistance rate of urine isolates (50.0%, 21/42) was much higher than that of vaginal isolates (27.3%, 6/22). Isolates from the urology ward and reproductive center were generally susceptible to fluconazole (87.0%, 20/23), whereas isolates from the oncology ward, geriatrics ward, and neurology ward were mostly fluconazole-resistant (81.8%, 9/11).

3.2 | *C. tropicalis* strain differentiation by MLST

In the sequencing analysis, DNA sequences from the coding regions of six housekeeping genes (ranging in size from 370 bp to 525 bp) were concatenated to obtain a dataset of 2,677 bp for each isolate. A total of 129 cases of heterozygosity were detected: Y = C + T (45.8%, 59/129), R = A + G (25.6%, 33/129), W = A + T (14.7%, 19/129), K = G + T (8.5%, 11/129), M = A + C (3.1%, 4/129), and S = G + C (2.3%, 3/129). In the six analyzed gene fragments, 129 (4.8%) polymorphic sites were identified, including 10 in *ICL1*, 25 in *MDR1*, 40 in *SAPT2*, 13 in *SAPT4*, 31 in *XYR1*, and 10 in *ZWF1a*. The *ZWF1a* gene presented the highest typing efficiency, distinguishing 1.50 genotypes per polymorphism, whereas *SAPT2* presented the lowest efficiency, distinguishing 0.20 genotypes per polymorphism (Table 2).

The concatenated sequences of 64 isolates were classified into 45 DSTs, with 20 (44.4%) newly identified DSTs (Table 1). Eight new alleles were identified in searches of the *C. tropicalis* MLST database: two in *SAPT4* (alleles 107, 108), three in *XYR1* (alleles 169, 170, and 171), and three in *ZWF1a* (alleles 62, 64, and 65). All new DSTs and alleles have been submitted to the *C. tropicalis* MLST database. Of the 45 DSTs identified, DST546 (n = 4), DST331 (n = 3), DST346 (n = 3), DST506 (n = 3), DST525 (n = 3), and DST615 (n = 3) were common types. Interestingly, most isolates (78.9%, 15/19) of these types had high MICs for antifungal azoles, and most were isolated from urine (84.2%, 16/19). There was clear segregation of DSTs between urine and vaginal specimens, and only six DSTs (DST169, DST225, DST331, DST343, DST525, and DST615) were shared between

TABLE 1 Isolate sources, antifungal susceptibility, and MLST analyses of 64 *C. tropicalis* isolates

Isolate	Collection date	Source	Ward	MIC (μg/ml)			DST	Clonal complex
				FLC	VRC	ITC		
CTR-1	10/12/2018	Urine	Pancreatic	>64	16	1	506	1
CTR-2	24/01/2019	Urine	RICU	4	<0.0313	0.25	980[a]	Singleton
CTR-3	26/01/2019	Urine	Neurosurgery	32	16	4	615	Singleton
CTR-4	04/02/2019	Urine	Geriatric	>64	8	16	525	5
CTR-5	12/02/2019	Urine	RICU	2	0.25	0.25	987[a]	Singleton
CTR-6	05/03/2019	Vaginal swab	Gynecology	64	16	16	982[a]	Singleton
CTR-7	07/03/2019	Urine	Geriatric	0.5	0.125	0.125	330	Singleton
CTR-8	09/03/2019	Urine	Neurosurgery	2	0.125	0.25	437	Singleton
CTR-9	23/03/2019	Vaginal swab	Reproductive	0.5	0.0313	0.125	994[a]	Singleton
CTR-10	24/03/2019	Vaginal swab	Gynecology	1	0.0625	0.25	343	3
CTR-11	27/03/2019	Urine	Urology	0.5	0.0625	0.125	169	Singleton
CTR-12	31/03/2019	Urine	Urology	0.5	0.0625	0.25	346	7
CTR-13	02/04/2019	Vaginal swab	Gynecology	32	16	16	615	Singleton
CTR-14	06/04/2019	Urine	ICU	0.5	0.0313	0.125	730	4
CTR-15	07/04/2019	Urine	ICU	32	4	8	615	Singleton
CTR-16	14/04/2019	Vaginal swab	Gynecology	0.125	0.0313	0.25	169	Singleton
CTR-17	21/04/2019	Urine	RICU	64	16	16	482	Singleton
CTR-18	26/04/2019	Urine	Neurology	64	16	2	331	2
CTR-19	27/04/2019	Vaginal swab	Gynecology	32	>16	>16	525	5
CTR-20	27/04/2019	Urine	Cardiovascular	8	16	8	331	2
CTR-21	16/05/2019	Vaginal swab	Reproductive	8	16	>16	983[a]	Singleton
CTR-22	20/05/2019	Vaginal swab	Reproductive	0.25	0.0313	0.25	984[a]	Singleton
CTR-23	27/05/2019	Vaginal swab	Gynecology	0.125	0.0313	0.25	723	3
CTR-24	30/05/2019	Vaginal swab	Reproductive	0.125	0.0313	0.25	995[a]	Singleton
CTR-25	04/06/2019	Vaginal swab	Gynecology	1	<0.0313	0.25	901	Singleton
CTR-26	05/06/2019	Vaginal swab	Gynecology	32	16	16	996[a]	Singleton
CTR-27	11/06/2019	Urine	Neurology	0.5	0.0625	0.125	184	6
CTR-28	03/08/2019	Vaginal swab	Gynecology	32	4	1	225	1
CTR-29	07/08/2019	Vaginal swab	Reproductive	0.25	0.125	0.5	998[a]	3
CTR-30	15/08/2019	Urine	Oncology	>64	8	1	225	1
CTR-31	21/08/2019	Urine	Urology	16	8	8	525	5
CTR-32	23/08/2019	Urine	Nephrology	>64	2	4	376	1
CTR-33	23/08/2019	Vaginal swab	Reproductive	0.25	0.0625	0.25	331	2
CTR-34	24/08/2019	Vaginal swab	Reproductive	0.5	0.25	1	852	Singleton
CTR-35	24/08/2019	Vaginal swab	Reproductive	0.25	0.0313	0.5	139	6
CTR-36	29/08/2019	Urine	Urology	1	1	2	999[a]	Singleton
CTR-37	11/09/2019	Vaginal swab	Gynecology	0.125	0.125	0.25	1002[a]	8
CTR-38	11/09/2019	Vaginal swab	Reproductive	0.5	0.25	2	988[a]	Singleton
CTR-39	13/09/2019	Urine	Gynecology	16	16	4	976[a]	Singleton
CTR-40	28/09/2019	Vaginal swab	Reproductive	0.5	0.0313	0.5	985[a]	7
CTR-41	30/09/2019	Urine	Urology	0.25	0.125	0.25	403	Singleton
CTR-42	30/09/2019	Urine	Neurology	64	8	1	546	1
CTR-43	01/10/2019	Urine	ICU	0.25	0.0625	0.25	346	7
CTR-44	03/10/2019	Urine	Neurology	64	2	1	546	1

(Continues)

Table 1 (Continued)

Isolate	Collection date	Source	Ward	MIC (μg/ml)			DST	Clonal complex
				FLC	VRC	ITC		
CTR-45	03/10/2019	Urine	Oncology	64	2	2	546	1
CTR-46	04/10/2019	Urine	Urology	1	0.25	0.25	989[a]	Singleton
CTR-47	05/10/2019	Vaginal swab	Reproductive	0.25	0.0313	0.5	139	6
CTR-48	07/10/2019	Urine	RICU	0.25	0.0313	0.25	978[a]	5
CTR-49	08/10/2019	Urine	Urology	32	4	2	990[a]	Singleton
CTR-50	09/10/2019	Urine	ICU	>64	4	4	506	1
CTR-51	11/10/2019	Urine	Endocrinology	0.5	0.25	0.5	434	2
CTR-52	14/10/2019	Urine	Urology	0.25	0.0313	0.125	394	2
CTR-53	16/10/2019	Vaginal swab	Gynecology	0.25	0.0313	0.5	1000[a]	Singleton
CTR-54	23/10/2019	Urine	Geriatric	>64	2	1	506	1
CTR-55	23/10/2019	Urine	ICU	0.25	0.125	0.125	977[a]	4
CTR-56	29/10/2019	Urine	Geriatric	32	16	0.5	979[a]	Singleton
CTR-57	30/10/2019	Urine	Urology	1	0.0625	0.125	184	6
CTR-58	31/10/2019	Urine	Transplantation	>64	8	1	546	1
CTR-59	01/11/2019	Urine	Urology	0.5	0.25	0.5	489	8
CTR-60	01/11/2019	Urine	ICU	0.25	0.0625	0.25	343	3
CTR-61	05/11/2019	Urine	Urology	0.25	0.0625	0.25	346	7
CTR-62	07/11/2019	Urine	Urology	0.5	<0.0313	0.25	978[a]	5
CTR-63	11/11/2019	Urine	RICU	64	8	4	923	Singleton
CTR-64	30/11/2019	Urine	Oncology	64	16	16	181	Singleton

[a]New DSTs in *C. tropicalis* MLST databases (https://pubmlst.org/ctropicalis).

TABLE 2 Characters of six housekeeping genes used in multilocus sequence typing

Gene loci	Italy[a] (28 isolates) G/P (Ratio)	Kuwait[b] (63 isolates) G/P (Ratio)	Brazil[c] (61 isolates) G/P (Ratio)	Beijing, China[d] (58 isolates) G/P (Ratio)	Hainan, China[e] (116 isolates) G/P (Ratio)	Wuhan, China (64 isolates) G/P (Ratio)
ICL1	2/13 (0.15)	12/26 (0.46)	6/20 (0.30)	7/3 (2.33)	8/6 (1.33)	6/10 (0.60)
MDR1	4/18 (0.22)	31/36 (0.86)	17/27 (0.63)	15/6 (2.50)	37/21 (1.76)	21/25 (0.84)
SAPT2	5/38 (0.13)	11/40 (0.28)	8/39 (0.21)	5/2 (2.50)	11/7 (1.57)	8/40 (0.20)
SAPT4	5/38 (0.13)	19/19 (1.00)	8/34 (0.24)	18/13 (1.38)	21/18 (1.17)	19/13 (1.46)
XYR1	6/20 (0.30)	22/12 (1.83)	25/19 (1.32)	15/1 (15.00)	33/16 (2.06)	24/31 (0.77)
ZWF1a	3/10 (0.30)	11/11 (1.00)	8/15 (0.53)	10/3 (3.33)	14/11 (1.27)	15/10 (1.50)

G: Number of genotypes; P: number of polymorphic nucleotide sites; Ratio: the ratio of the number of genotypes over the number of polymorphic nucleotide sites.

[a]Scordino et al. (2018).

[b]Al-Obaid et al. (2017).

[c]Magri et al. (2013).

[d]Wu et al. (2012)

[e] Wu, Guo, et al. (2017)

them. The novelty and diversity of vaginal isolates were significantly higher than those of urine isolates. The proportion of new types of vaginal specimens (52.4%, 11/21) was also higher than that of urine specimens (30.0%, 9/30). There were eight repetitive DSTs in urine samples and only one repetitive DST in vaginal samples.

3.3 | Phylogenetic analysis of 64 *C. tropicalis* isolates in Wuhan

The genetic relationship among the DSTs of *C. tropicalis* isolates from Wuhan was evaluated by the construction of an unrooted

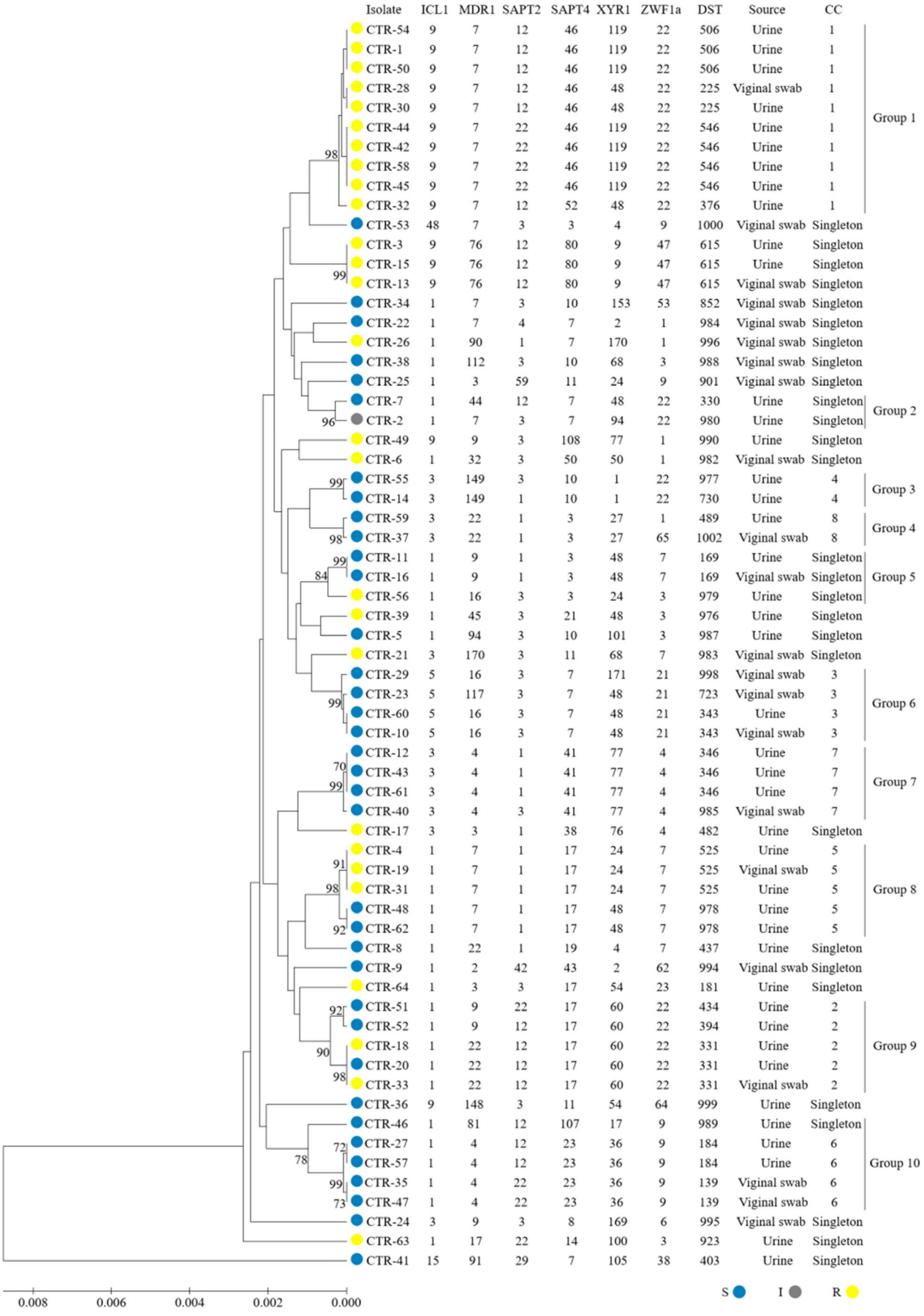
Isolate ICL1 MDR1 SAPT2 SAPT4 XYR1 ZWF1a DST Source CC
CTR-54 9 7 12 46 119 22 506 Urine 1
CTR-1 9 7 12 46 119 22 506 Urine 1
CTR-50 9 7 12 46 119 22 506 Urine 1
CTR-28 9 7 12 46 48 22 225 Viginal swab 1
CTR-30 9 7 12 46 48 22 225 Urine 1
CTR-44 9 7 22 46 119 22 546 Urine 1
CTR-42 9 7 22 46 119 22 546 Urine 1
CTR-58 9 7 22 46 119 22 546 Urine 1
CTR-45 9 7 22 46 119 22 546 Urine 1
CTR-32 9 7 12 52 48 22 376 Urine 1
CTR-53 48 7 3 3 4 9 1000 Viginal swab Singleton
CTR-3 9 76 12 80 9 47 615 Urine Singleton
CTR-15 9 76 12 80 9 47 615 Urine Singleton
CTR-13 9 76 12 80 9 47 615 Viginal swab Singleton
CTR-34 1 7 3 10 153 53 852 Viginal swab Singleton
CTR-22 1 7 4 7 2 1 984 Viginal swab Singleton
CTR-26 1 90 1 7 170 1 996 Viginal swab Singleton
CTR-38 1 112 3 10 68 3 988 Viginal swab Singleton
CTR-25 1 3 59 11 24 9 901 Viginal swab Singleton
CTR-7 1 44 12 7 48 22 330 Urine Singleton
CTR-2 1 7 3 7 94 22 980 Urine Singleton
CTR-49 9 9 3 108 77 1 990 Urine Singleton
CTR-6 1 32 3 50 50 1 982 Viginal swab Singleton
CTR-55 3 149 3 10 1 22 977 Urine 4
CTR-14 3 149 1 10 1 22 730 Urine 4
CTR-59 3 22 1 3 27 1 489 Urine 8
CTR-37 3 22 1 3 27 65 1002 Viginal swab 8
CTR-11 1 9 1 3 48 7 169 Urine Singleton
CTR-16 1 9 1 3 48 7 169 Viginal swab Singleton
CTR-56 1 16 3 3 24 3 979 Urine Singleton
CTR-39 1 45 3 21 48 3 976 Urine Singleton
CTR-5 1 94 3 10 101 3 987 Urine Singleton
CTR-21 3 170 3 11 68 7 983 Viginal swab Singleton
CTR-29 5 16 3 7 171 21 998 Viginal swab 3
CTR-23 5 117 3 7 48 21 723 Viginal swab 3
CTR-60 5 16 3 7 48 21 343 Urine 3
CTR-10 5 16 3 7 48 21 343 Viginal swab 3
CTR-12 3 4 1 41 77 4 346 Urine 7
CTR-43 3 4 1 41 77 4 346 Urine 7
CTR-61 3 4 1 41 77 4 346 Urine 7
CTR-40 3 4 3 41 77 4 985 Viginal swab 7
CTR-17 3 3 1 38 76 4 482 Urine Singleton
CTR-4 1 7 1 17 24 7 525 Urine 5
CTR-19 1 7 1 17 24 7 525 Viginal swab 5
CTR-31 1 7 1 17 24 7 525 Urine 5
CTR-48 1 7 1 17 48 7 978 Urine 5
CTR-62 1 7 1 17 48 7 978 Urine 5
CTR-8 1 22 1 19 4 7 437 Urine Singleton
CTR-9 1 2 42 43 2 62 994 Viginal swab Singleton
CTR-64 1 3 3 17 54 23 181 Urine Singleton
CTR-51 1 9 22 17 60 22 434 Urine 2
CTR-52 1 9 12 17 60 22 394 Urine 2
CTR-18 1 22 12 17 60 22 331 Urine 2
CTR-20 1 22 12 17 60 22 331 Urine 2
CTR-33 1 22 12 17 60 22 331 Viginal swab 2
CTR-36 9 148 3 11 54 64 999 Urine Singleton
CTR-46 1 81 12 107 17 9 989 Urine Singleton
CTR-27 1 4 12 23 36 9 184 Urine 6
CTR-57 1 4 12 23 36 9 184 Urine 6
CTR-35 1 4 22 23 36 9 139 Viginal swab 6
CTR-47 1 4 22 23 36 9 139 Viginal swab 6
CTR-24 3 9 3 8 169 6 995 Viginal swab Singleton
CTR-63 1 17 22 14 100 3 923 Urine Singleton
CTR-41 15 91 29 7 105 38 403 Urine Singleton
Group 1
Group 2
Group 3
Group 4
Group 5
Group 6
Group 7
Group 8
Group 9
Group 10
98
99
96
99
99
98
99
84
99
70
99
91
98
92
92
90
98
72
78
99
73
0.008 0.006 0.004 0.002 0.000
S I R

FIGURE 1 Dendrogram generated from multilocus sequence typing data for 64 *Candida tropicalis* isolates from urogenital tract infection in Wuhan. Phylogenetic analysis was performed using the unweighted pair group method with arithmetic averages (UPGMA). Strains are classified as fluconazole susceptible (blue), intermediate (gray), or resistant (yellow). The clonal complexes (CCs) determined by goeBURST were generally consistent with groups defined by UPGMA. CC1 was the most common and the only fluconazole-resistant cluster in Wuhan.

dendrogram based on MLST data. The UPGMA dendrogram (Figure 1) showed that the 64 isolates belonged to 10 groups and some singletons. The goeBURST analysis revealed 20 DSTs grouped into eight CCs; 25 DSTs were dispersed as unrelated singletons. The CCs determined by goeBURST were generally consistent with groups defined by UPGMA. CC1 was most common (15.6%, 10/64), correlating with the UPGMA group 1 with 100% similarity. CC1 included four types: DST506 (n = 3), DST546 (n = 4), DST225 (n = 2), and DST376 (n = 1). Most of the strains (90%, 9/10) were from urine. CC1 was the only fluconazole-resistant cluster in this study, and all strains had high MIC values against fluconazole and other azoles. Of the fluconazole-resistant isolates in this study, 37.0% (10/27) belonged to CC1, and the remaining 17 fluconazole-resistant isolates were scattered among CC2, CC5, and singletons. All strains of CC3, CC4, CC6, CC7, and CC8 were susceptible to fluconazole. All CCs except CC4 contained strains from urine and vaginal swabs. Strains from vaginal swabs were more dispersed than those from urine, with 54.5% (12/22) of the former classified as singletons and 38.1% (16/42) of the latter.

3.4 | Nosocomial infections

A comparison of clinical and epidemiological data revealed the presence of five DSTs concerning possible nosocomial infections (Table 3), most of which occurred in the neurology ward (3), oncology ward (2), ICU (2), and gynecology ward (2). Although most of the shared DST strains came from different wards, patients with the same DST had overlapping hospital stays, suggesting the possibility of nosocomial infection. Most of the infected strains were classified as CC1, the most prevalent CC in Wuhan. All strains in Table 3 were resistant to fluconazole, and most of them came from urine.

3.5 | Genetic evolution and population structure of *C. tropicalis*

We downloaded the allele profile data of 1,090 strains (as of 20 February 2020) from the *C. tropicalis* MLST database and analyzed the genetic relationship with 64 strains from Wuhan. Of the 1,154 strains, goeBURST analysis grouped 834 DSTs into 88 CCs and 283 singletons (Figure 2). Of the strains from Wuhan, 51 strains (32 DSTs) were distributed in 18 CCs, and 13 strains (13 DSTs) were singletons. CC4 in Figure 2 contains the most DSTs (DST225, DST376, DST506, DST546, and DST330) of Wuhan, followed by CC6 (DST394, DST331, and DST434) and CC53 (DST343, DST723, and DST998).

In the minimum spanning tree (Figure 3), it is clear that most of the strains from Wuhan were clustered with strains from other cities in mainland China (Beijing, Shanghai, Hainan, Shenzhen, Nanchang, Harbin, Chengdu, and Tianjin), followed by strains from Taiwan. Strains from Wuhan rarely clustered with strains from outside China. There is a cross-aggregation of strains among countries, for example, CC2 contains strains from China, the UK, the USA, Brazil, India, and South Korea. However, some countries with more DSTs have formed relatively independent CC. China, Britain, India, South Korea, Italy, Singapore, and Brazil all have unique CC. The strains between countries and regions are both conserved and shared.

4 | DISCUSSION

In this study, 45 DSTs were identified from 64 strains, and eight new alleles and 20 new DSTs were found. The new DSTs accounted for 44.4% (20/45) of the total DSTs, indicating the high diversity and novelty of *C. tropicalis* isolates in Wuhan. The six gene fragments analyzed showed the genetic variation within and among strains from

TABLE 3 Information on strains of possible nosocomial infection events

DST	Isolate	Date	Source	Ward	CC	FLC MIC (μg/ml)
225	CTR-28	03/08/2019	Vaginal swab	Gynecology	1	32
	CTR-30	15/08/2019	Urine	Oncology		>64
331	CTR-18	26/04/2019	Urine	Neurology	2	64
	CTR-20	27/04/2019	Urine	Cardiovascular		8
506	CTR-50	09/10/2019	Urine	ICU	1	>64
	CTR-54	23/10/2019	Urine	Geriatric		>64
546	CTR-42	30/09/2019	Urine	Neurology	1	64
	CTR-44	03/10/2019	Urine	Neurology		64
	CTR-45	03/10/2019	Urine	Oncology		64
615	CTR-13	02/04/2019	Vaginal swab	Gynecology	Singleton	32
	CTR-15	07/04/2019	Urine	ICU		32

Wuhan. The number of polymorphic sites in this study (129 of 64 isolates) was higher than that in other cities in China, such as Beijing (28 of 58 isolates) (Wu et al., 2012), Shanghai (93 of 82 isolates) (Wang et al., 2016), and Hainan (79 of 116 isolates) (Wu, Guo, et al., 2017). However, it was lower than other countries, such as Brazil (154 of 61 isolates) (Magri et al., 2013), Italy (137 of 28 isolates) (Scordino et al., 2018), and Kuwait (144 of 63 isolates) (Al-Obaid et al., 2017). The ratio of genotype number to polymorphic nucleotide number of *SAPT4* locus was higher than that of all geographical populations previously reported. The genetic variation of *C. tropicalis* in Wuhan is abundant in China.

In the past MLST studies on *C. tropicalis*, there were many studies on blood isolates and few studies on urogenital isolates. This study is the first molecular epidemiological analysis of *C. tropicalis* from the urogenital tract. We observed that the distribution of *C. tropicalis* genotypes between the vagina and urine sources was uneven. There were more shared DSTs among the urine isolates, while there were few duplicated DSTs among the vaginal isolates. The genetic diversity of vaginal isolates was higher. New alleles and new DSTs were easier to find in vaginal specimens. Almost all of the CCs analyzed by goeBURST contained strains from both two sources, but the strains of CC1, the largest local epidemic group, mainly came from urine. This phenomenon suggests that the vaginal and urethral environments may select for different *C. tropicalis* genotypes. In the study of *Candida glabrata* in Tanzania (Mushi et al., 2019), there were more repetitive DSTs in vaginal secretions and more novel DSTs in urine, which also indicated that there may be genotypic choices at different anatomical sites. Moreover, in the past studies of *C. albicans*, the isolates obtained from different body sites also showed different genotype distribution patterns (McManus, & Coleman, 2014; Wang et al., 2015). Although genotypes were not related to anatomical sites in Wu et al's analysis (Wu et al., 2019) of the global *C. tropicalis* MLST database, and most genotypes and genetic clusters can colonize in various human body sites and cause infections. The differences in genotype selection shown in this study may be endemic to this region.

Azole resistance of *C. tropicalis* makes clinical treatment difficult. In this study, the fluconazole resistance rate of urinary isolates was higher than that of vaginal isolates. Fluconazole is the first choice for the treatment of *Candida* urinary tract infection (Fisher, 2011). Other antifungal drugs have relatively large toxic and side effects (amphotericin B) or difficulty in achieving measurable concentrations in the urine (echinocandins and other azoles) (Fisher et al., 2011). Once *C. tropicalis* is resistant to fluconazole in urethral infection, clinical treatment will face huge pressure on drug selection, which will cause serious long-term damage to patients' health. Although the urology ward and reproductive ward in our hospital had more strains, mostly (87.0%, 20/23) were susceptible to fluconazole. *C. tropicalis* isolated from the geriatrics ward, neurology ward, and oncology ward were mostly (81.8%, 9/11) resistant to fluconazole. Thus, it can be seen that the azole resistance of *C. tropicalis* varies greatly in different departments. The possible nosocomial infections in our hospital were caused by azole-resistant strains, and most of them occurred in the above three wards (geriatrics ward, neurology ward, and oncology ward). Most infected patients had catheters and were in rooms on adjacent floors of a building. The research of Asticcioli et al. (2007) indicates that the shared use of medical equipment and instruments may be the cause of hospital transmission between different wards.

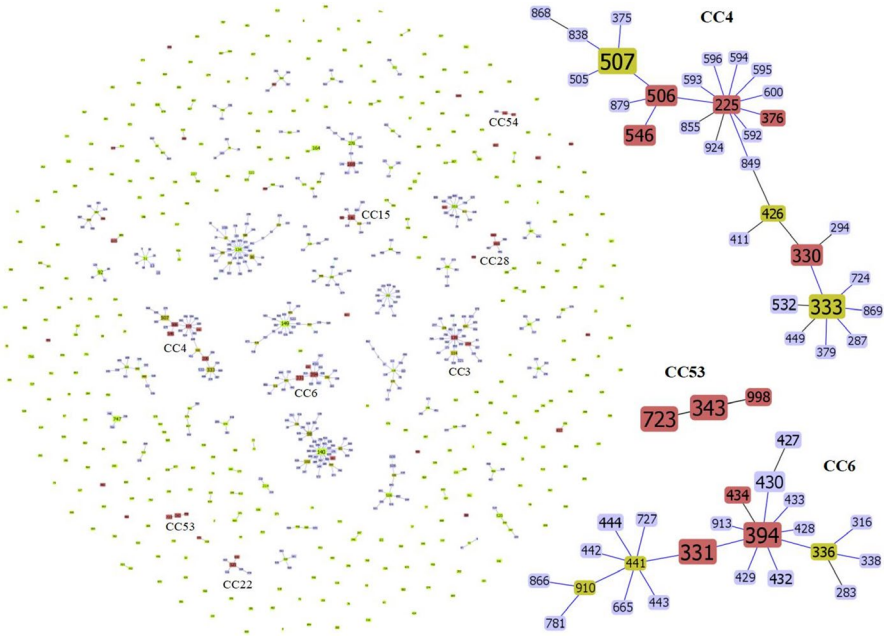

FIGURE 2 Genetic population structure of 1,154 *Candida tropicalis* isolates. Population snapshot obtained by goeBURST analysis using the 834 confirmed diploid sequence types (DSTs); DSTs are linked when they differ in one of the six loci used for multilocus sequence typing. Single DSTs represent singletons. CC4 (clonal complex), CC6, and CC53 all contain at least three DSTs from Wuhan. The size of each DST reflects the number of strains associated with that DST. The dark red ones contain the DSTs from Wuhan.

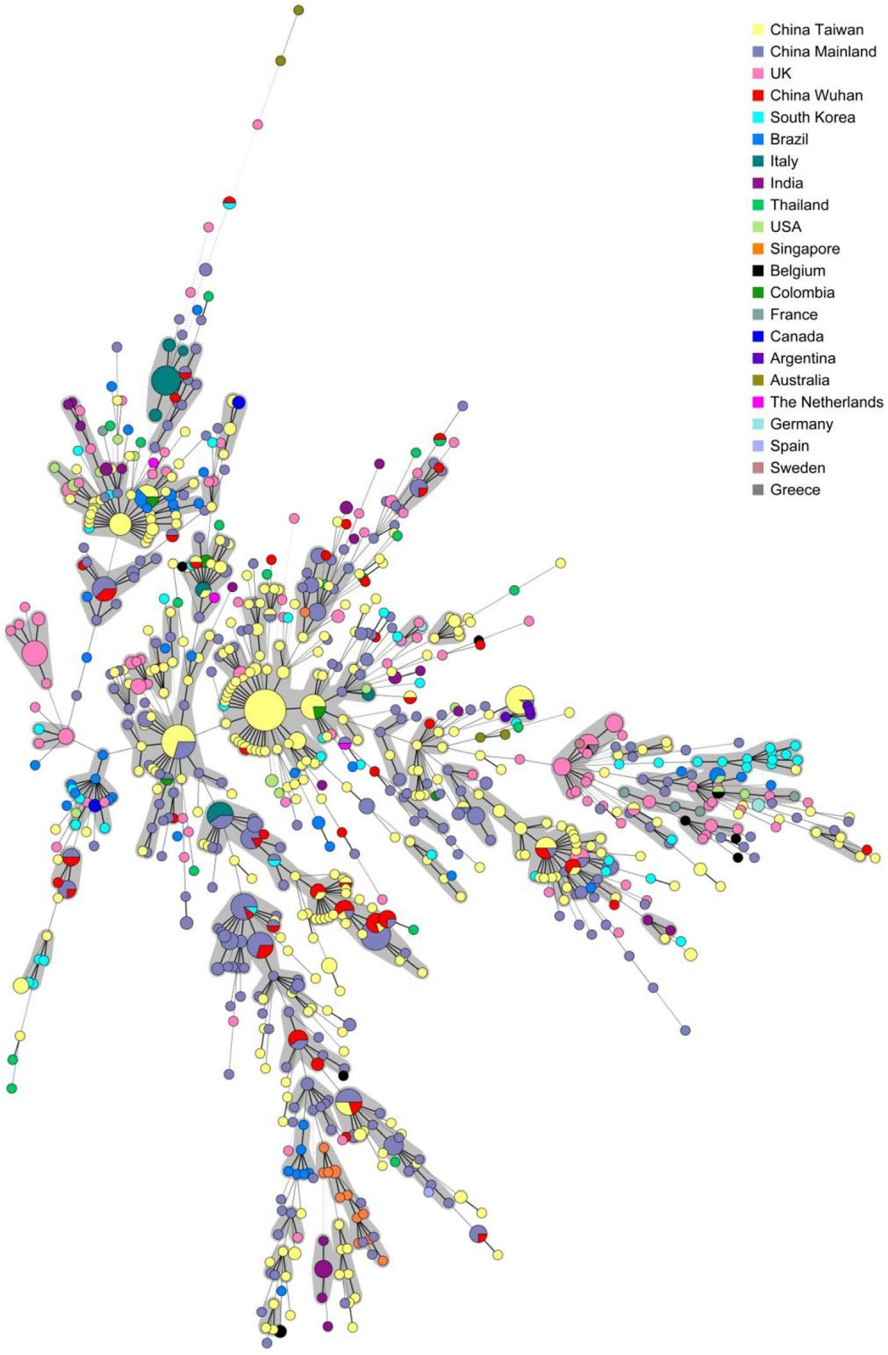

FIGURE 3 A minimum spanning tree illustrating the relationship between the 64 *Candida tropicalis* isolates from Wuhan and 1,090 isolates from other regions in the *C. tropicalis* MLST database as of 24 February 2020. Each circle corresponds to a specific DST. The size of the circle indicates the number of isolates belonging to a specific DST, and the color of the circle represents the country/region to which it belongs. Shaded areas indicate groups of target clonal complexes (CCs).

A study in Italy confirmed that hospital conditions and hand hygiene of medical staff can lead to nosocomial transmission (Scordino et al., 2018). In this study, it was found that the patients with the same type of *C. tropicalis* during the overlapping hospitalization could only indicate the possibility of in-hospital transmission. Unfortunately, we did not collect samples of the hospital environment and medical personnel to confirm this. CC1 was a community of azole-resistant *C. tropicalis* prevalent in our hospital, 90% of which came from urine. In CC1, DST225, DST506, and DST546 caused suspected nosocomial transmission. We found a cluster of azole-resistant strains and potential nosocomial transmission, both of which emphasize the importance of MLST in monitoring and preventing nosocomial infection transmission.

In the global analysis, four types (DST225, DST376, DST506, DST546) of CC1 in Wuhan (Figure 1) belonged to the global large CC4 (Figure 2), and all strains were highly resistant to azoles.

Many strains from other regions in CC4 were resistant to fluconazole. In Taiwan, strains of DST225, DST376, DST506, DST375, DST505, DST507, DST753, DST754, and DST838 were all resistant to fluconazole (Chen et al., 2019). In Shanghai, strains of DST376, DST505, DST506, and DST507 were also resistant to fluconazole (Wang et al., 2016). CC4 contained fluconazole-resistant DSTs from Wuhan, Taiwan, and Shanghai, and some DSTs were shared between the three cities, suggesting that these strains may have similar genetic backgrounds. DST225 was the putative founder of the fluconazole-resistant strains identified in CC4. In previous studies, DST225 can be isolated from fruits and soil, and resistant to fluconazole, supporting the transfer of azole-resistant *C. tropicalis* from agriculture to human hosts (Lo et al., 2017; Yang et al., 2012). The shared DSTs in Wuhan, Taiwan, and Shanghai may be related to human activities and the transportation of agricultural products. In the minimum spanning tree of the world, the Wuhan strains were mostly clustered with strains in China and rarely clustered with strains outside China. It can be seen that the gene flow of the strains detected in Wuhan is more frequent in China than in other countries. Unique CCs have been found in China, the UK, India, South Korea, Italy, Singapore, and Brazil. It reflects that the genetic background is closely related to the geography, and the strains in each region may have their unique origin.

In conclusion, this is the first study based on the molecular epidemiology of *C. tropicalis* in urogenital tract infection. The results showed that the genotype and azole susceptibility were quite different between urine and vaginal specimens. Importantly, we identified endemic azole-resistant cluster and detected suspected nosocomial infections, highlighting the importance of active molecular surveillance to understand the prevalence of azole-resistant *C. tropicalis* and to provide valuable information for the prevention and control of nosocomial infections.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTION

Qianyu Wang: Conceptualization (lead); Formal analysis (equal); Methodology (lead); Writing-original draft (lead); Writing-review & editing (lead). **Congrong Li:** Funding acquisition (supporting); Project administration (lead); Writing-original draft (supporting). **Dongling Tang:** Formal analysis (equal); Funding acquisition (lead). **Kewen Tang:** Conceptualization (supporting); Writing-original draft (supporting).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Clinical Research Ethics Committee of Renmin Hospital of Wuhan University (WDRY2020-K182). Written informed consent from the participants was not required to participate in this study following the national legislation and the institutional requirements.

DATA AVAILABILITY STATEMENT

All data are provided in full in the Results section of this article. The detailed sequencing results of 20 new diploid sequence types (DSTs) and 8 new alleles have been submitted to the *Candida tropicalis* MLST Databases (https://pubmlst.org/ctropicalis) and are available for download to all readers. Information on 1090 strains used to analyze the genetic evolution and population structure of *C. tropicalis* was also downloaded from https://pubmlst.org/ctropicalis.

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