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Expansion of a predominant azole-resistant *Candida tropicalis* genotype from 2012 to 2018: Evidence from orchard environments in Taiwan

Yin-Zhi Chen[1,‡], Kuo-Yun Tseng[1,‡], Min-Nan Tseng[2], Jyh-Nong Tsai[3], Ching-Ching Hsu[4], Yu-Chieh Liao[5], Chih-Chao Lin[1], De-Jiun Tsai[1], Feng-Jui Chen[1], Li-Yun Hsieh[1], Chiao-Mei Lin[1], Chi-Jung Wu[1,6], Huey-Kang Sytwu[1] and Hsiu-Jung Lo[1,7,8,*]

[1]Taiwan Mycology Reference Center, National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Miaoli County, Taiwan
[2]Kaohsiung District Agricultural Research and Extension Station, Pingtung County, Taiwan
[3]Plant Pathology Division, Taiwan Agricultural Research Institute, Council of Agriculture, Executive Yuan, Taichung, Taiwan
[4]Taichung District Agricultural Research and Extension Station, Council of Agriculture, Executive Yuan, Changhua County, Taiwan
[5]Institute of Population Health Sciences, National Health Research Institutes, Miaoli County, Taiwan
[6]Department of Internal Medicine, National Cheng Kung University Hospital and Medical College, Tainan, Taiwan
[7]Department of Biological Science and Technology, National Yang Ming Chiao Tung University, Hsinchu, Taiwan
[8]School of Dentistry, China Medical University, Taichung, Taiwan

*To whom correspondence should be addressed. Hsiu-Jung Lo, PhD. Taiwan Mycology Reference Centre, National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, 35 Keyan Road, Zhunan, Miaoli County 350401, Taiwan. Tel: 886-37-206-166; ext. 35516; Fax: 886-37-586-457. E-mail: hjlo@nhri.edu.tw
‡Y.Z.C. and K.Y.T. contributed equally to this article.

Abstract

A predominant fluconazole-resistant *Candida tropicalis* clade 4 genotype, based on MLST analysis, causing candidemia in humans in several tropical countries, was detected in the environment in a 2012 orchard survey in Taiwan, which is an emerging one health issue. This follow-up study investigated clade 4 azole-resistant *C. tropicalis* in orchards, comparing the 2012 survey data with the 2018 survey findings. We compared *C. tropicalis* isolated from the same 53 orchards, including 23 wax apple, 17 grape, and 13 papaya orchards, in both the 2012 and 2018 surveys. We collected samples of fruits, soils, and irrigation water from environment and swab samples from armpit and hand, as well as oral mouth rinses of the farmers. Overall, the rate of fluconazole-resistant *C. tropicalis* from the 2018 survey was significantly higher than that from the 2012 survey (27/55 vs. 9/46, $P = .003$). Furthermore, we found that the use of azole fungicides was associated with the detection of azole-resistant *C. tropicalis*. Notably, 77.8% (7/9) of the azole-resistant isolates in the 2012 survey and 92.6% (25/27) in the 2018 survey were genetically related and belonged to the clade 4 genotype. Our findings demonstrate that the rate of fluconazole-resistant *C. tropicalis* from orchards increased significantly and the clade 4 drug-resistant *C. tropicalis* spread widely in orchard environments, especially among grape ones. Our findings show that different types of crop had different cultivation habits. Hence, grape orchard environment is a priority to conduct intervention for cultivation habits of farmers, especially on azole fungicide use in Taiwan.

Lay summary

Drug-resistant *Candida tropicalis*, a human pathogen, was increasingly found in soils and fruits in orchards from the 2012 survey to the 2018 survey. This rise is linked to azole fungicide use, highlighting the important concept of 'One Health' and need for responsible agricultural practices.

Key words: drug resistance, environment, pathogenic yeasts, multilocus sequence typing.

Introduction

Fungal infections pose an increasingly significant threat to global health, agriculture, and biodiversity. Each year, over 6.5 million people suffer from invasive fungal infections, and approximately 3.8 million human deaths are associated with fungal diseases.[1] Such infections span both human and plant populations, underscoring the need for a 'one health' approach to address this complex challenge.[2,3]

The drug-resistant non-*albicans Candida* spp. are particularly troublesome for optimal health recovery of immunecompromised patients with candidemia.[4] *Candida tropicalis* is one of the leading non-*albicans Candida* spp. causing candidemia in human subjects, especially those residing in tropical Asia and Latin America.[5,6] Because of major differences in the geographic distribution of human-invasive *Candida* spp., each country or region must conduct its own surveillance program to assess the dominant species and emergence of drug-resistant strains.[7] Azole-resistant *C. tropicalis* clinical isolates have been reported worldwide, particularly in the Asia-Pacific region, in the past decades.[8–10] To track national trends in the distribution of pathogenic yeast species and their antifungal susceptibility, the National Health Research Institutes (NHRI) launched the Taiwan Surveillance of Antimicrobial Resistance of Yeasts (TSARY) in 1999. Subsequent surveys have been

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conducted every four years.[11–16] Recent TSARY data revealed that 91.1% (51/56) of fluconazole-resistant *C. tropicalis* isolates collected between 2014 and 2018 were classified as clade 4 genotypes. This classification was determined using multilocus sequence typing (MLST) analysis, which examined the sequences of six specific genetic fragments: *ICL1*, *MDR1*, *SAPT2*, *SAPT4*, *XYR1*, and *ZWF1a*.[16,17] Our research, along with other studies, has shown that tandem gene duplications in *ERG11*, combined with distinct mutations, are major factors driving azole-resistant phenotypes in clade 4 *C. tropicalis* isolates. In addition to Taiwan, these resistant strains have been identified in various regions, including China, Thailand, Singapore, and Australia.[16,18]

Beyond clinical environments, *C. tropicalis* has also been isolated from soil and aquatic environments.[19,20] Of particular concern, azole-resistant clade 4 genotypes of *C. tropicalis* have recently been found in fruits and soils from orchards,[19–21] as well as fruits purchased from supermarkets.[22] This shows the extensive distribution and potential transmission pathways of these resistant strains.

This follow-up study was designed to determine the distribution of the azole-resistant *C. tropicalis* clade 4 genotype in orchard environments and to compare the 2012 survey data with that obtained from the same set of orchards in the 2018 survey. We found that the rate of fluconazole-resistant *C. tropicalis* from orchards had increased significantly and that the majority of drug-resistant *C. tropicalis* belonged to the clade 4 genotype. In addition, the use of azole fungicides was positively associated with the detection of azole-resistant *C. tropicalis*.

Materials and methods

Study design

A follow-up orchard survey was designed to investigate whether the azole-resistant *C. tropicalis* clade 4 genotype persisted in the environment of orchards. We characterized 46 *C. tropicalis* isolated from the 53 orchards, including 23 wax apple, 17 grape, and 13 papaya orchards, of the 80 orchards that were surveyed in 2012.[23] Samples were collected from the same 53 orchards from July 2018 to May 2020. Since 40 of 55 isolates were collected from 2018, in short, we designated this survey 'Orchard 2018' after the year of its initiation. The sampling date of each isolate is listed in Supplementary Table 1. The sample collection was designed according to our previous Orchard 2012 survey.[23] Briefly, five samples of fruits and soils of each orchard were collected, as well as one sample of irrigation water. We also collected swab samples from armpit and hand, as well as oral mouth rinses of the farmers. The grape, papaya, and wax apple farms were labeled by starting with 'CGRF', CPAF', and 'SWAF', respectively, followed by a number. Thus, 265 samples of fruit and soil and 53 samples of water, armpit swab, hand swab, and mouth rinses were analyzed. The characteristics of *C. tropicalis* from the same 53 orchards in the 2012 and 2018 surveys were compared. Whether any azole fungicides were used within 30 days before sample collection was recorded. The orchard survey, titled 'Species distribution and drug susceptibility of yeast pathogens in farmers and fields,' was approved by NHRI's Human Experiment and Ethics Committee (EC1070117).

Microbiologic processing

We isolated yeasts from samples according to previously established procedures.[23] Briefly, all swabs were maintained at room temperature and transported to the laboratory within 24 h. The samples were streaked onto CHROMagar *Candida* medium (BBL, Becton Dickinson, Cockeysville, MD, USA). The isolates were identified by rDNA sequencing for internal transcribed spacer and/or D1/D2 regions.[24] The primers used in this study are listed in Supplementary Table 2. All novel rDNA sequences were submitted to the website of the National Centre for Biotechnology Information (Supplementary Table 1). One isolate per type of sample was further analyzed. The strains from the 2012 and 2018 surveys were labeled by starting with 'YFA12' and 'YFA18', respectively, followed by four numbers. The results of the same set of 53 orchards in the 2018 survey were compared with those of the 2012 survey.

Drug-susceptibility testing

Susceptibilities of the 87 *C. tropicalis* isolates to clinical-use fluconazole (2–64 mg/l) and three commonly used fungicides in agriculture in Taiwan, including difenoconazole (1–32 mg/l), tebuconazole (1–32 mg/l), and triadimenol (2–64 mg/l), were determined. Cultures were incubated at 35°C for 24 h in RPMI medium 1640 (31800-022, Gibco BRL). The growth of each isolate was measured using a Multiskan FC Microplate Photometer (Thermo Fisher Scientific Inc., USA). The minimum inhibitory concentrations (MICs) were defined as the concentration of drug capable of reducing the turbidity of cells by more than 50%. The procedures and the clinical breakpoints for strains were based on a recent publication by the Clinical and Laboratory Standards Institute, following the protocols outlined in M27 for antifungal susceptibility testing of yeasts and M60 for epidemiological cutoff values.[25,26] For fluconazole, the clinical breakpoints were as follows: MICs $\leq$ 2 mg/l were considered to be susceptible, $\geq$8 mg/l resistant, and 4 mg/l susceptible-dose dependent (SDD). The breakpoints for fungicides in agriculture were not defined. The MICs of 50% and 90% of the total population were defined as MIC_{50} and MIC_{90}, respectively, when MICs $\leq$ 2 mg/l were considered as 2 mg/l and MICs $\geq$ 64 mg/l were considered as 64 mg/l. The correlation between susceptibilities to fluconazole and to fungicides in agriculture was determined by Spearman's correlation coefficient analysis. The strength of the correlation was assigned using the following guide: an absolute value of 0.00–0.19 was very weak; 0.20–0.39 was weak; 0.40–0.59 was moderate; 0.60–0.79 was strong; and 0.80–1.0 was very strong.

Multilocus sequence typing

MLST was conducted as described in our previous report,[16] which was modified from an earlier report.[27] Briefly, the DNA fragments of six genes of *C. tropicalis*, including *ICL1*, *MDR1*, *SAPT2*, *SAPT4*, *XYR1*, and *ZWF1a*, were sequenced and included in the analyses. The resultant sequences were aligned with BioNumerics 3.0 (Applied Maths, Kortrijk, Belgium) and compared with those in the database of *C. tropicalis* (http://pubmlst.org/website) to determine the level of sequence identities (DST). The primers used are listed in Supplementary Table 2.

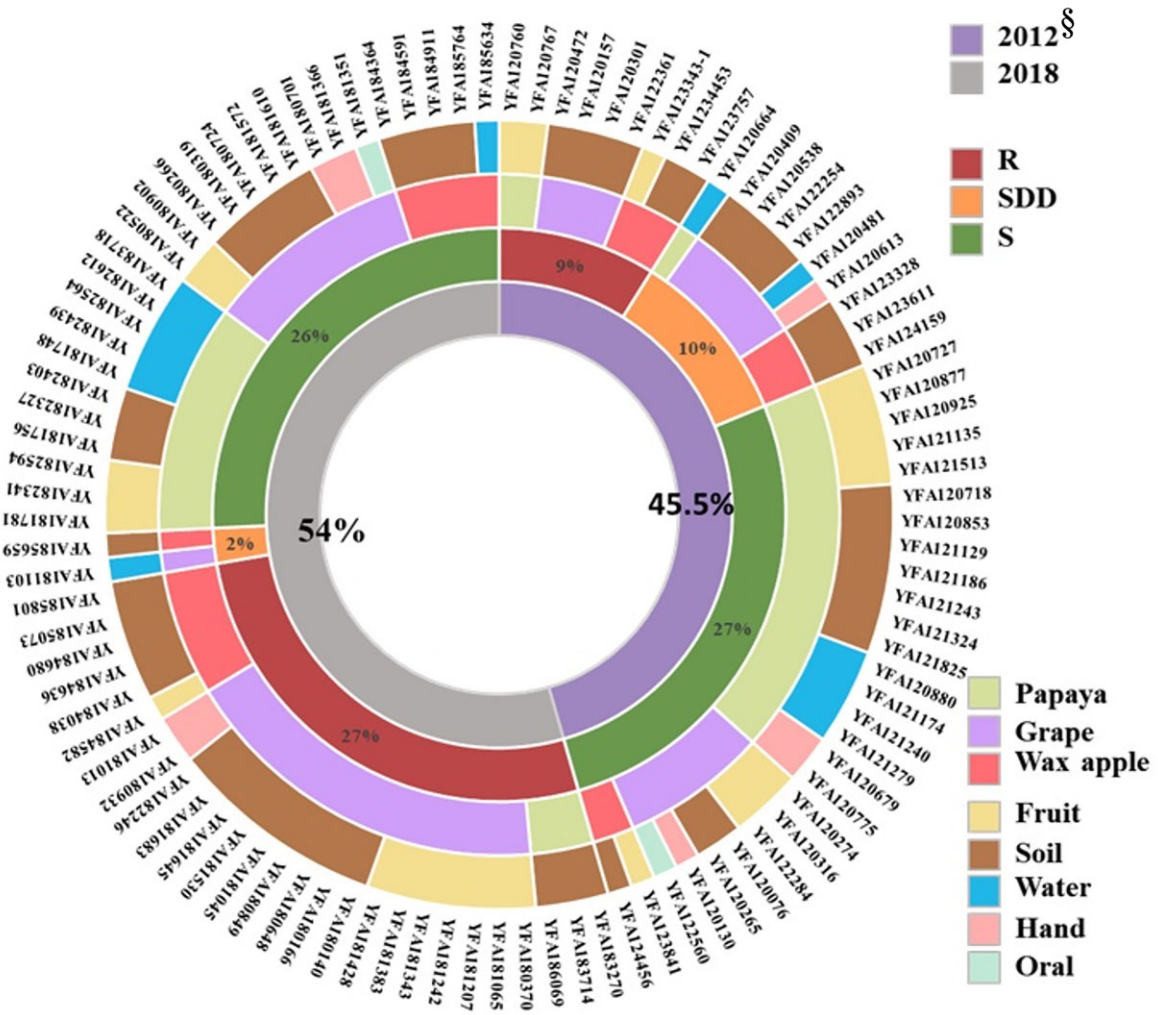

Figure 1. Characteristics of the 101 *Candida tropicalis* isolates. From inside to outside, the innermost ring represents isolates from different surveys, while the second ring indicates fluconazole susceptibilities: R (resistant), SDD (susceptible-dose dependent), and S (susceptible). The third ring refers to the orchards (papaya, grape, and wax apple). The fourth one represents the sources of the isolated strains (fruit, soil, water, hand, and mouth). The strains are labeled in the outermost ring. § 2012 survey indicates data previously published in Tseng et al. *Emerg Infect Dis*. 2024.[21]

Results

Distribution of *Candida tropicalis* from orchard environment and farmer

Candida tropicalis isolates from the same 53 orchards in both the 2012 and 2018 surveys were compared. A total of 46 *C. tropicalis* isolates were recovered from 28 orchards in the 2012 survey, including 11/13 (84.6%), 10/17 (58.8%), and 7/23 (30.4%) from papaya, grape, and wax apple orchards, respectively. A total of 55 were from 35 orchards in the 2018 survey, including 9/13 (69.2%), 15/17 (88.2%), and 11/23 (47.8%) from papaya, grape, and wax apple orchards, respectively (Fig. 1). The detailed information of each isolate, including sampling collection date, is listed in Supplementary Table 1. The wax apple had the lowest detection rate of *C. tropicalis*.

The rates of *C. tropicalis* detection in the same type of orchards did not differ significantly between the two surveys. There were 17, 21, and 8 isolates from orchards of grape, papaya, and wax apple, respectively, in the 2012 survey, and 29, 14, and 12 isolates from orchards of grape, papaya, and wax apple, respectively, in the 2018 survey (Fig. 1, Table 1 and Supplementary Table 1). We did not recover *C. tropicalis* from armpit in either survey. The distribution of the same type of source was not significantly different between the two surveys. The 46 individual yeasts in the 2012 survey were isolated from 22 soil, 13 fruit, 6 water, 4 hand, and 1 oral rinse samples. Those 55 in the 2018 survey were isolated from 30 soil, 13 fruit, 7 water, 4 hand, and 1 oral rinse samples (Fig. 1 and Supplementary Table 1).

Susceptibility of *Candida tropicalis* to fluconazole

In the 2012 survey, approximately 19.6% (9), 21.7% (10), and 58.7% (27) of the 46 isolates were fluconazole-resistant, fluconazole-SDD, and fluconazole-susceptible, respectively. In 2018, approximately 49.1% (27), 3.6% (2), and 47.3% (26) of the 55 isolates were fluconazole-resistant, fluconazole-SDD, and fluconazole-susceptible, respectively (Fig. 1, Table 1, and Supplementary Table 1). MIC_{50} and MIC_{90} of the total population and of fluconazole in the 2012 survey were 2 and 32 mg/l, respectively, and those in the 2018 survey were 4 and 64 mg/l, respectively.

The fluconazole-resistant rate of *C. tropicalis* in the 2018 survey was significantly higher than that in the 2012 survey

Table 1. The distribution of 101 *Candida tropicalis* isolates in orchard surveys.

	Orchard				Environment				Farmer		
	Grape	Papaya	Wax apple	Fruit	Soil	Water	Subtotal	Hand	Oral	Subtotal	Total
The 2012 survey[§]											
R	4	2	3	4	5	0	9	0	0	0	9
SDD	6	1	3	0	7	2	9	1	0	1	10
S	7	18	2	9	10	4	23	3	1	4	27
Subtotal	17	21	8	13	22	6	41	4	1	5	46
The 2018 survey											
R	18	3	6	8	17	0	25	2	0	2	27
SDD	1	0	1	0	1	1	2	0	0	0	2
S	10	11	5	5	12	6	23	2	1	3	26
Subtotal	29	14	12	13	30	7	50	4	1	5	55
The 2012 survey and the 2018 survey											
R	22	5	9	0	12	22	0	34	0	2	0	36
SDD	7	1	4	0	0	8	3	11	0	1	0	12
S	17	29	7	0	14	22	10	46	0	5	2	0	53
Total	46	35	20	0	26	52	13	91	0	8	2	0	101

R, resistant; SDD, susceptible-dose dependent; S, susceptible.
[§]The 2012 survey indicates data selected from the 53 orchards of the previously published in Tseng et al. *Emerg Infect Dis.* 2024.

(49.1% vs. 19.6%, $P = .003$). In the 2012 survey, approximately 37.5% (3/8), 23.5% (4/17), and 9.5% (2/21) of *C. tropicalis* from orchards of wax apple, grape, and papaya, respectively, were resistant to fluconazole. In the 2018 survey, approximately 50% (6/12), 62.1% (18/29), and 21.4% (3/14) of *C. tropicalis* from orchards of wax apple, grape, and papaya, respectively, were resistant to fluconazole. The fluconazole-resistant rate of *C. tropicalis* from the grape orchards in the 2018 survey was significantly higher than that in the 2012 survey (62.1% vs. 23.5%, $P = .02$).

We detected four and one instances of *C. tropicalis* from farmers' hands and oral rinse, respectively, in each survey (Table 1 and Supplementary Table 1). It is worth noting that none of the isolates from farmers in the 2012 survey were resistant to fluconazole, whereas 2/5 (40%) from the 2018 survey were resistant to fluconazole.

Susceptibility of *Candida tropicalis* to three azole fungicides

The MICs of difenoconazole, tebuconazole, and triadimenol of the 101 isolates are summarized in Figure 2 and Table 2. The MIC_{50} of difenoconazole and tebuconazole for the isolates from the 2012 survey and the 2018 survey were the same, 1 mg/l, whereas those for triadimenol in the 2012 survey and the 2018 survey were different (2 vs. 8 mg/l). The MIC_{90} of triadimenol for the isolates from both surveys was the same, >64 mg/l, whereas those of difenoconazole (2 mg/l in the 2012 survey vs. 4 mg/l in the 2018 survey) and tebuconazole (2 mg/l in the 2012 survey vs. 8 mg/l in the 2018 survey) were increased from the 2012 survey to the 2018 survey. There were significantly more isolates from the 2018 survey than those from the 2012 survey with ≥4 mg/l MICs of tebuconazole (34.5% vs. 4.3%, $P = .0002$). *Candida tropicalis* was cross-resistant to fluconazole and the other three fungicides (Fig. 2 and Table 2). The strength of correlations between susceptibilities to fluconazole and to difenoconazole (0.688–0.832), tebuconazole (0.789–0.912), and triadimenol (0.856–0.946) became stronger from the 2012 survey to the 2018 survey.

Genetic relatedness among *Candida tropicalis* isolates

The distribution of genotypes of *C. tropicalis* is shown in Figure 3. In the 2012 survey, 77.8% (7/9, including three out of four from fruits and four out of five from soil) of fluconazole-resistant isolates belonged to the clade 4 genotype. The remaining two belonged to the clade 1 genotype. In the 2018 survey, 92.6% (25/27, including all eight from fruits and two from farmers' hands, and 15 out of 17 from soils) of fluconazole-resistant isolates belonged to the clade 4 genotype. The remaining two belonged to the clade 3 genotype.

There were two and eight *C. tropicalis* isolates from oral rinse and hand, respectively. Two isolates from the 2018 survey, YFA180932 (DST506) and YFA181013 (DST225), from farmers hands of CGRF17 and CGRF05 orchards, respectively, were azole-resistant, and both belonged to the clade 4 genotype (Supplementary Table 1). There were clade 4 azole-resistant *C. tropicalis* detected from the soil samples from both orchards as well.

The distribution of genotypes among fluconazole-nonresistant *C. tropicalis* was more diverse (Supplementary Table 1). The 12 fluconazole-SDD isolates were distributed across six genotypes, including seven belonging to clade 4

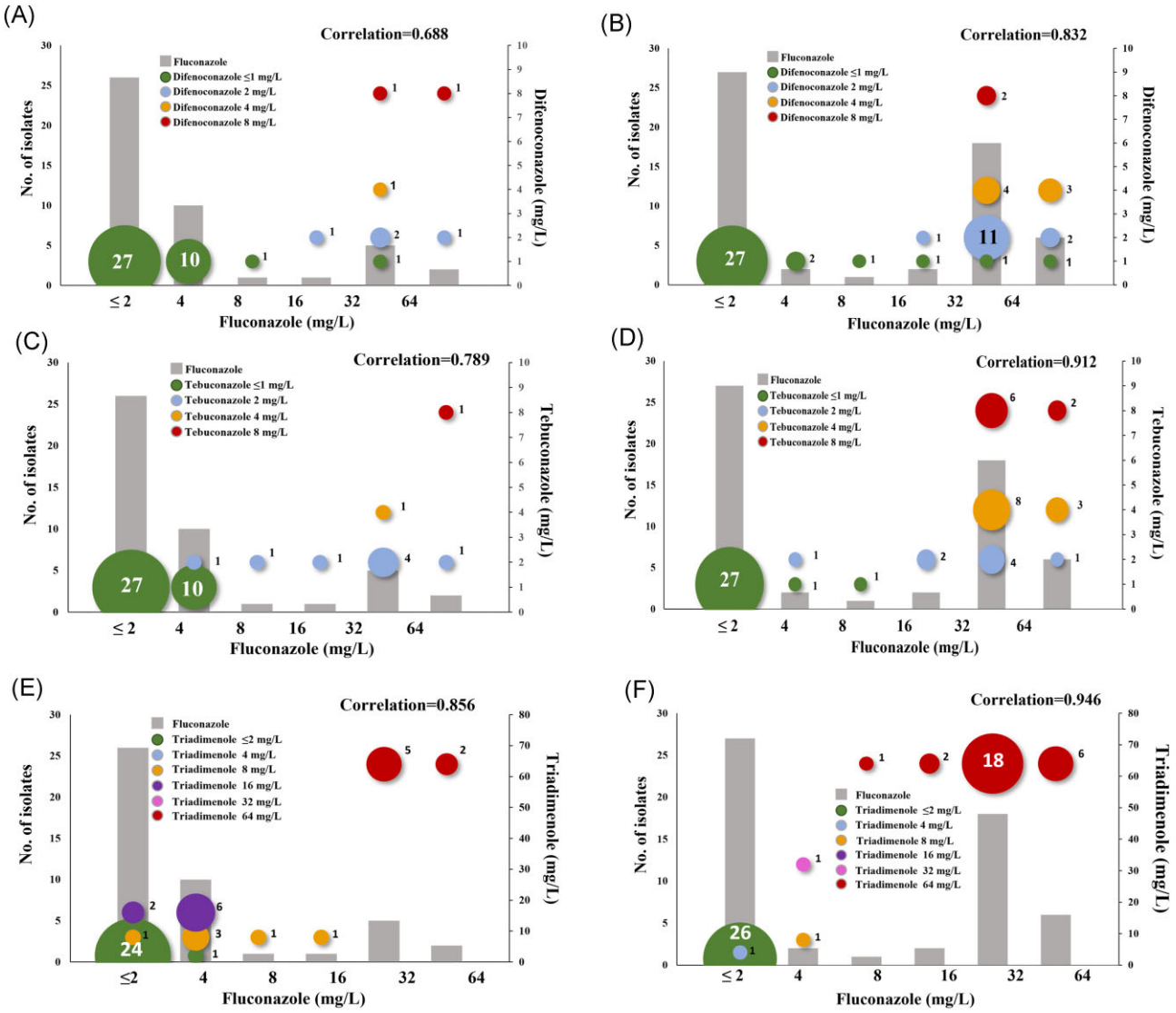

Figure 2. *Candida tropicalis* cross-resistant to fluconazole and three other azole fungicides. The distributions of MICs of fluconazole and difenoconazole of isolates from the 2012 survey (A) and the 2018 survey (B). Those of fluconazole and tebuconazole of isolates from the 2012 survey (C) and the 2018 survey (C). Those of fluconazole and triadimenole of isolates from the 2012 survey (E) and the 2018 survey (F). The left vertical axis represents the number of *C. tropicalis* isolates. The horizontal axis represents the concentration of fluconazole, and the right vertical axis represents the concentration of the azole fungicide. The gray bars represent the fluconazole susceptibilities, and the circles refer to the fungicide susceptibilities of isolates. The different color circles indicate different MICs of the azole fungicide, with the number of isolates indicated and also shown by the size of circle.

and one each from clades 1, 5, 7, 8, and 18. Similarly, the 53 fluconazole-susceptible isolates were classified into 11 genotypes, comprising 19 from clade 1, 12 from clade 3, 10 from clade 4, 5 from clade 5, and one isolate from each of seven different genotypes.

Discussion

The fact that azole-resistant *C. tropicalis* isolated from fruits, soils, water, and farmers' hands were genetically closely related and belonged to clade 4 demonstrates that this drug-resistant genotype is persistent in all three different orchard types. Furthermore, there was a significantly higher azole-resistant rate in the 2018 survey than in the 2012 survey, especially for samples from grape orchards. In fact, there was significantly increased use of azole fungicides in grape orchards from the 2012 survey to the 2018 survey (35.5% vs. 82.4%, $P = .007$), which was significantly associated with the increased rate of detection of azole-resistant of *C. tropicalis* (23.5% in the 2012 survey and 62.1% in the 2018 survey).

Among the three types of orchards, wax apple orchards had the lowest detection rate of *C. tropicalis* (30.4% and 47.8% of the 23 orchards in the 2012 survey and the 2018 survey, respectively). The wax apples were grown on the southwest coast of Taiwan. Every year during the typhoon season, seawater floods back, and it is common to see wax apple trees soaked in salty water. The observation that salt resulted in a successful 'black-pearl' wax apple variety in Taiwan led farmers to develop many kinds of farming techniques to improve the flavor and appearance of wax apple, including adding salts to the soil. Consequently, the soil of wax apple orchards has become even more salty than from the effect of typhoons alone.[28] Kim et al. demonstrated that *C. tropicalis* could not grow on agar medium containing 3.0 M NaCl.[29] Therefore,

Table 2. Cross-resistance between fluconazole and other three azole fungicides.

	Fluconazole MICs (mg/l)							Spearman's correlation coefficient	
	≦2	4	8	16	32	64	Total	Correlation*	*P*-value
				The 2012 survey§					
	26	10	1	1	5	2	45		
Difenoconazole								0.688	<.001
≦1	26	10	1	0	1	0	38		
2	0	0	0	1	2	1	4		
4	0	0	0	0	1	0	1		
8	0	0	0	0	1	1	2		
Tebuconazole								0.789	<.001
≦1	26	9	0	0	0	0	35		
2	0	1	1	1	4	1	8		
4	0	0	0	0	1	0	1		
8	0	0	0	0	0	1	1		
Triadimenol								0.856	<.001
≦2	23	1	0	0	0	0	24		
4	0	0	0	0	0	0	0		
8	1	3	1	1	0	0	6		
16	2	6	0	0	0	0	8		
32	0	0	0	0	0	0	0		
≧64	0	0	0	0	5	2	7		
				The 2018 survey					
	Fluconazole MICs (mg/l)							Spearman's correlation coefficient	
	≦2	4	8	16	32	64	Total	Correlation	*P*-value
	27	2	1	2	18	6	56		
Difenoconazole								0.832	<.001
≦1	27	2	1	1	1	1	33		
2	0	0	0	1	11	2	14		
4	0	0	0	0	4	3	7		
8	0	0	0	0	2	0	2		
Tebuconazole								0.912	<.001
≦1	27	1	1	0	0	0	29		
2	0	1	0	2	4	1	8		
4	0	0	0	0	8	3	11		
8	0	0	0	0	6	2	8		
Triadimenol								0.946	<.001
≦2	26	0	0	0	0	0	26		
4	1	0	0	0	0	0	1		
8	0	1	0	0	0	0	1		
16	0	0	0	0	0	0	0		
32	0	1	0	0	0	0	1		
≧64	0	0	1	2	18	6	27		

*0.00–0.19 as very weak; 0.20–0.39 as weak; 0.40–0.59 as moderate; 0.60–0.79 as strong; 0.80–1.0 as very strong.
§The 2012 survey indicates data selected from the 53 orchards of the previously published in Tseng et al. *Emerg Infect Dis*. 2024.

the high salt concentration in the wax apple environment may exert a certain level of stress on *C. tropicalis*, potentially impacting its distribution although *C. tropicalis* is a relative salt-tolerant yeast, salt concentration still represents an environmental stress that may affect its growth rate or competitiveness. It is worth noting that despite the low detection rate of *C. tropicalis*, the rates of fluconazole-resistant *C. tropicalis* were 37.5% and 50% in 2012 and the 2018 survey, respectively.

It is interesting that even though the detection rates of *C. tropicalis* on papaya orchards (84.6% and 81.8% of the 13 orchards in 2012 and the 2018 survey, respectively) were higher than those of wax apple, the rates of fluconazole-resistant *C. tropicalis* were the lowest among three types of orchards (9.5% in 2012 and 14.3% in the 2018 survey). For conserving soil moisture, improving fertility and health of the soil, and reducing weed growth, a layer of material is applied to the surface of the soil in papaya orchards. In Taiwan, either straw mats, plastic sheets, leaves, or grass clippings are used. It would be interesting and important to determine whether regularly clearing the mulch on the surface of the soil can prevent azole fungicides from staying on the soil, resulting in decreased the selection stress of the azole-resistant *C. tropicalis* in the soil. Thus, it would be interesting to investigate whether the amount of azole fungicides used on the papaya orchards is lower than on the other two types of orchards.

There are limitations of the present study. Variations in cultivation cycles among the studied crops may influence the timing and frequency of fungicide applications. In addition, geographical factors, such as the distance between two orchards, means of irrigation water, and wind, could affect exposure to azole fungicides. Restricting the recording of azole fungicide use to the 30 days prior to sampling may not sufficiently capture information on azole fungicide exposure for each orchard. Additionally, given the relatively long half-life of azole fungicides in the environment, which can persist for months,[30] it would be more informative to collect data on azole fungicide residues in soil to determine the impact of azole fungicide on the detection of azole-resistant *C. tropicalis* in the environment.

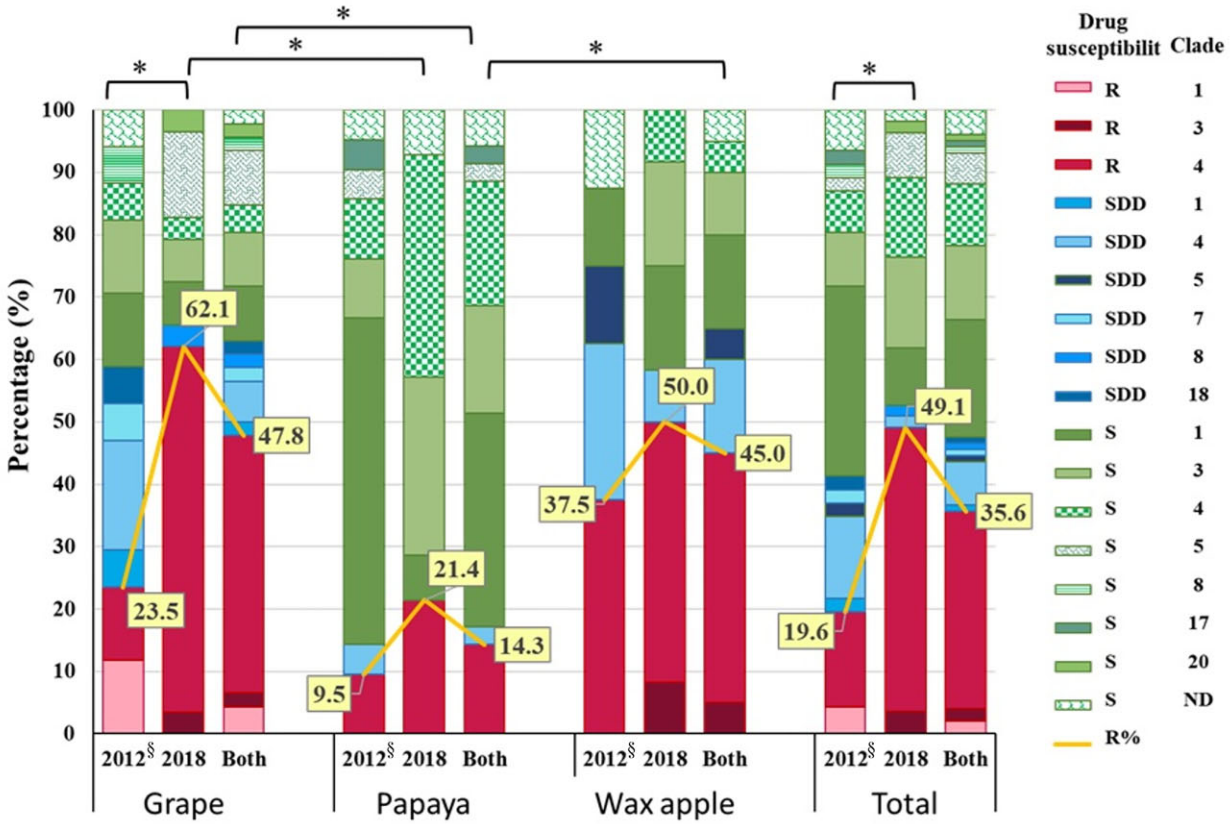

Figure 3. Characteristics of fluconazole-resistant *Candida tropicalis* isolates. The percentages of fluconazole-resistant (R, red bars), -susceptible-dose dependent (SDD, blue bars), and -susceptible (S, green bars) isolates of each orchard in the 2012 survey and the 2018 survey are shown, along with the averages of the two surveys. Organ lines and numbers represent the resistant trend and resistant rates to fluconazole, respectively. ∗ Statistically significant ($P \leq .05$) between two different surveys or orchards. § 2012 survey indicates data previously published in Tseng et al. *Emerg Infect Dis.* 2024.[21]

Of the 53 orchards, there were more orchards that used azole fungicides within 30 days before collection in the 2018 survey than in the 2012 survey (14 vs. 7) (Supplementary Table 3). Of the 21 orchards that used azole fungicides, only one was wax apple in the 2012 survey, and the 20 remaining were grape. Despite the limitations of the present study, the observation that of the 17 grape orchards, there were more grape orchards that used azole fungicides in the 2018 survey than in the 2012 survey (14 vs. 6, $P = .007$) suggests that azole fungicide use is associated with the increased detection rate of azole-resistant *C. tropicalis*.

The amounts of azole-type compounds used in agriculture in Taiwan are estimated according to 'Domestic Manufacturers Production & Sale of Pesticides,' an annual publication by the Taiwan Crop Protection Industry Association.[31] There are 29 different types of azole fungicides used in Taiwan. The annual use of azole fungicides in agriculture in Taiwan increased from 130.6 tons in 2012 to 160.6 tons in 2018. The use of difenoconazole increased from 27.2 tons in 2012 to 49 tons in 2018, while tebuconazole was similar, 11.2 tons in 2012 to 10 tons in 2018, and triadimenol decreased from 0.7 tons in 2012 to 0 tons in 2018. Even though the amount of triadimenol used has been reduced recently, isolates resistant to fluconazole showed cross-resistance to triadimenol since other azole fungicides use has been increased.

Notably, azole-resistant clade 4 genotype *C. tropicalis* could be traced to a few farmers in the 2018 survey. In the future, systematic studies should be conducted in evaluating the horizontal transmission of this predominant genotype in the agricultural setting and its implications in the clinical setting. Moreover, an active surveillance to detect emergence and dissemination of azole-resistant *C. tropicalis* in clinical settings should be considered beyond Taiwan, especially in tropical Asia and Latin America.

Developing novel antifungals, implementing antifungal stewardship, improving disease management and diagnosis, and surveillance are strategies to tackle the issues of antifungal drug resistance.[32–35] Importantly, we also demonstrated that the use of azole fungicides was significantly associated with the rate of azole-resistant *C. tropicalis* detection in the environment. Moreover, we also identified grape orchard among the three different types has a priority to conduct intervention for the cultivation habits of farmers, especially on azole fungicide use, in Taiwan. Hence, antimicrobial stewardship efforts in hospitals and discontinuing in agriculture the use of antimicrobial classes applied in human medicine are equally important for preserving the few medical treatment options for fungal infections.

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Author contributions

Yin-Zhi Chen (Conceptualization, Investigation, Methodology, Writing – original draft), Kuo-Yun Tseng (Conceptualization, Investigation, Methodology, Writing – original draft), Min-Nan Tseng (Conceptualization, Investigation), Jyh-Nong Tsai (Conceptualization, Investigation), Ching-Ching Hsu (Conceptualization, Investigation), Yu-Chieh Liao (Formal analysis), Chih-Chao Lin (Methodology), De-Jiun Tsai (Methodology), Feng-Jui Chen (Formal analysis), Li-Yun Hsieh (Formal analysis), Chiao-Mei Lin (Methodology), Chi-Jung Wu (Conceptualization, Investigation), Huey-Kang Sytwu (Formal analysis), Hsiu-Jung Lo (Conceptualization, Formal analysis, Writing – original draft)

Supplementary material

Supplementary material are available at *Medical Mycology* online.

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