Medical Mycology, 2018, 56, 972–978
doi: 10.1093/mmy/myx151
Advance Access Publication Date: 8 January 2018
Original Article

Original Article

DST659 genotype of *Candida albicans* showing positive association between biofilm formation and dominance in Taiwan

Jang-Jih Lu[1,2,3,†], Hsiu-Jung Lo[4,5,†], Yen-Mu Wu[6], Jia-Yuan Chang[7], Yin-Zhi Chen[4] and Shao-Hung Wang[7,*]

[1]Department of Laboratory Medicine, Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan, [2]Department of Medicine, College of Medicine, Chang Gung University, Taoyuan, Taiwan, [3]Department of Medical Biotechnology and Laboratory Science, Chang Gung University, Taoyuan, Taiwan, [4]National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Miaoli County, Taiwan, [5]School of Dentistry, China Medical University, Taichung, Taiwan, [6]Division of Infectious Diseases, Department of Internal Medicine, Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan and [7]Department of Microbiology, Immunology and Biopharmaceuticals, National Chiayi University, Chiayi City, Taiwan

*To whom correspondence should be addressed. S.-H. Wang, Department of Microbiology, Immunology and Biopharmaceuticals, National Chiayi University, 300 Syuefu Rd., Chiayi City, 60004, Taiwan (R.O.C.). Tel: 886-5-271-7225; Fax: 886-5-2717831; E-mail: shwang@mail.ncyu.edu.tw

†The first two authors contributed equally to this article, and both should be considered first authors.

Received 12 September 2017; Revised 18 October 2017; Accepted 22 November 2017; Editorial Decision 1 November 2017

Abstract

Based on multiple locus sequence typing, we previously found that DST659 and DST693 were dominant genotypes of *Candida albicans* among the bloodstream isolates at Chang-Gung Memorial Hospital at Linkou. Biofilm-forming activity, which is critical for *C. albicans* virulence, probably contributed to the dominance of antifungal sensitive isolates in hospital. Both *in vitro* membrane weighting and *in vivo* zebrafish egg infection assays were used to evaluate the biofilm-forming activity of DST659 and DST693 genotypes. Medical records of the patients infected by these two genotypes were retrospectively reviewed. High biofilm-forming activity of DST659 isolates was demonstrated *in vitro* and further proved with the zebrafish egg infection model, which showed a positive correlation between the biofilm-forming extent on chorion and the *in vitro* biofilm activity. Moreover, significantly less embryos survived when infected with DST659 isolates than those with DST693 (1.25% vs. 11.43%), and the high-biofilm subset of DST659 showed a greater reduction in survival of embryos at 48 h post-infection than the low-biofilm subset (0 vs. 1.92%). Patients infected with DST659 seemed to survive slightly worse than those infected with DST693, although the difference was insignificant. It is noteworthy that DST659-infected patients were associated with a higher incidence in renal insufficiency as compared to those with DST693, the low biofilm genotype. We suggest that a strong biofilm activity of DST659 contributed to a high mortality rate in zebrafish hosts

© The Author(s) 2018. Published by Oxford University Press on behalf of The International Society for Human and Animal Mycology. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com

Downloaded from https://academic.oup.com/mmy/article-abstract/56/8/972/4792815 by guest on 13 November 2018

and poor renal function in patients, as well as gaining the dominance in the northern Taiwan.

Key words: *Candida albicans*, diploid sequence type (DST), biofilm formation, zebrafish egg infection model, renal disease.

Introduction

Candida albicans, a human commensal microorganism, is a major cause of nosocomial bloodstream infections and caused up to 30–60% mortality worldwide.[1–4] Dissemination of *Candida* to the circulation system and deep organs could be lethal. The current risk factors of candidemia are numerous and include invasive surgeries, dialysis, central venous access, diabetes, burns, suppressed immunity, use of steroid drugs, and broad-spectrum antibiotics.[5–7]

Previously, DST693 and DST659 genotypes are found to be the most abundant multiple locus sequence typing (MLST) genotypes in Chang-Gung Memorial Hospital at Linkou (CGMHL) by the MLST method.[8] The decrease in fluconazole sensitivity may benefit DST693 isolates to continue to exist during prophylactic fluconazole treatment.[8] Among the collection between 2003 and 2011 in CGMHL, DST659 was the second dominant genotype, which, however, showed no detectable change in susceptibility against common antifungals. Peculiarly, DST659 ranked to clonal complex (CC) 11 (i.e., the 11th largest eBURST complex) in global collection before year 2000[9] and moved up to CC4 (*i.e.* the 4th largest complex) in year 2014.[10] This observation may imply that DST659 genotype seems to expand during 2000–2014.

In the current study, we took advantage of the optical transparency of zebrafish embryos that allows noninvasive, high-resolution, time-course and real-time experiments to monitor the infection processes with imaging techniques. And many similarities and counterparts of immune systems do exist between zebrafish and mammals. Since biofilm forming activity has been demonstrated as an important factor for the pathogenesis of *C. albicans*, as well as the survival advantage under stress, an *in vitro* biofilm weighting analysis[11] and the zebrafish egg infection model[12] were then used to evaluate the biofilm activities and the infectivity of *C. albicans* isolates. By doing so, we reported the relationship between biofilm formation and dominance of DST659 genotype in CGMHL. Meanwhile, a retrospective study was conducted to reveal the clinical importance of high biofilm forming activity of DST659 genotype.

Methods

Candida albicans isolates

C. albicans isolates were identified by conventional culture and germ tube formation methods, and any uncertainty was further clarified by MALDI-TOF mass spectrometry or CHROMagar Candida (BD). Therefore, a total of 20 *C. albicans* isolates (16 from bloodstreams and 4 from urine) of DST659 genotype and 22 DST693 isolates (19 from bloodstreams and 3 from urine), all defined by MLST typing method,[8] were included in this study. In addition, SC5314, a filamentous positive strain and HLC54, *cph1/cph1 efg1/efg1*, a nonfilamentous strain[13] were used as controls.

In vitro biofilm weighting analysis

The biofilm weighting analysis was adapted from the biofilm assay previously done with silicone elastomers.[11,14] In brief, sterile filter membrane (0.8-μm pore size) (MF-Millipore, Millipore) in a fixed size was placed into 12-well culture wells and incubated in 1-ml fetal bovine serum for 16 h. The membranes were washed in phosphate-buffered saline (PBS) and wet with Spider medium (1% nutrient broth, 1% mannitol, 0.2% K_2HPO_4), followed by inoculation with *C. albicans* for 90 min at 37°C. Unbound *C. albicans* yeasts were removed by PBS washing, and then the membranes were incubated with sterile Spider medium for 60 h. After PBS washing, the filter membranes were air dried and finally weighted. Blank was performed with non-inoculation membrane in the same procedure. A biofilm defective strain Δ*tec1* (*tec1/tec1*)[11] was used for negative control, and its congenic parental wild-type *C. albicans* SC5314 was used for positive control. To normalize the biofilm forming activity, the biofilm formation index was calculated by dividing the weights of sample membranes by that formed with the Δ*tec1* strain.

Candida albicans infection model with zebrafish eggs

Zebrafish egg infection model was conducted according to the previously reported studies.[12] In brief, approximately 20 zebrafish eggs at 1 day post-fertilization were cultured in egg water (0.03% sea salt) at 28°C overnight and then placed in a 24-well plate containing 0.7 ml MOPS-buffered RPMI-1640 medium each well. The embryos in wells were co-incubated with *C. albicans* (at 1 × 10^6 yeast form cell/ml) and shaken at 80 rpm, 30°C, for 4 h. The *C. albicans* SC5314 was used as a positive control for infection whereas HLC54 (*cph1/cph1 efg1/efg1*) mutant was used as a negative

Downloaded from https://academic.oup.com/mmy/article-abstract/56/8/972/4792815 by guest on 13 November 2018

Table 1. Levels of *Candida* biofilms on zebrafish eggs infected by DST693 and DST659 isolates.

Genotypes			Level* (Percentage criteria matched/analyzed images)				
			IV	III	II	I	Not detected
SC5314#		24 h	0	100.0 (5/5)	0	0	0
		48 h	100.0 (5/5)	0	0	0	0
HLC54#		24 h	0	0	0	0	100.0 (5/5)
		48 h	0	0	0	0	100.0 (5/5)
DST693$		24 h	0	0	13.6 (3 × 3)/(22 × 3)	36.4 (8 × 3)/(22 × 3)	50.0 (11 × 3)/(22 × 3)
		48 h	0	18.2 (4 × 3)/(22 × 3)	0	45.5 (10 × 3)/(22 × 3)	36.4 (8 × 3)/(22 × 3)
DST659$	Total	24 h	0	45.0 (9/20)	30.0 (6/20)	20.0 (4/20)	5.0 (1/20)
		48 h	25.0 (5/20)	65.0 (13/20)	5.0 (1/20)	5.0 (1/20)	0
	Gp. H	24 h	0	57.1 (4 × 3)/(7 × 3)	42.9 (3 × 3)/(7 × 3)	0	0
		48 h	42.9 (3 × 3)/(7 × 3)	57.1 (4 × 3)/(7 × 3)	0	0	0
	Gp. L	24 h	0	38.5 (5 × 3)/(13 × 3)	23.1 (3 × 3)/(13 × 3)	30.8 (4 × 3)/(13 × 3)	7.7 (1 × 3)/(13 × 3)
		48 h	15.4 (2 × 3)/(13 × 3)	69.2 (9 × 3)/(13 × 3)	15.4 (2 × 3)/(13 × 3)	0	0

*: A representative image of an isolate was semi-quantified with the following criteria: level IV, biofilm coverage ≧70% and hyphal length ≧250 μm; level III, biofilm coverage ≧70% and hyphal length ≧150 μm and <250 μm; level II, biofilm coverage ≧50% and hyphal length <150 μm; level I, biofilm coverage <50%.

#: SC5314 and HLC54 represented the biofilm positive and negative strains, respectively. Each experiment was repeated for five times.

$: DST693 and DST659 groups consisted of 22 and 20 isolates, respectively. DST659-H group (Gp. H): 7 isolates; DST659-L (Gp. L): 13 isolates. Each infection experiment was repeated for three times.

control. After washing, embryos were transferred into egg water supplemented with 0.5% YPD (Difco, Detroit, MI, USA) and incubated at 30°C for 48 h.

Candida biofilms on zebrafish chorions were semi-quantified by analyzing the photo images taken under microscope at 24 and 48 h post-infection (hpi). To quantify the biofilm on a chorion, representative images (100 ×) selected from 10 eggs in each infection were measured for the surface coverage and hyphal length of the *Candida*. Biofilm formation activity of individual isolate was categorized decreasingly into levels: IV, III, II, I and nondetected, of which details are described in Table 1.

To evaluate the survival rate after infection, approximately 20 eggs were used in a well and infected similarly as described above, and the infected eggs were monitored for 2 days. The survival analysis by using Kaplan–Meier curves was performed by Prism 7 (GraphPad, La Jolla, CA, USA). The calculated survival rate was the percentage of embryos with active heartbeat or young fishes hatched. The zebrafish protocol (NHRI-IACUC-101071-A) was reviewed and approved by the Institutional Animal Care and Use Committee of the National Health Research Institutes.

Medical records analysis

Retrospective data collected from the enrolled patients included demographics, comorbidities, risk factors, and outcomes. Comorbidities included solid tumors, diabetes mellitus (DM), chronic lung diseases, heart failure, hepatic dysfunction (defined as the serum total bilirubin level ≥2.0 mg/dl or liver cirrhosis), renal insufficiency (defined as a serum creatinine level ≥2.0 mg/dl or a requirement of hemodialysis), and hematological malignancies. Risk factors were scrutinized within 30 days prior to the onset of candidemia, including central venous access and total parenteral nutrition. Coinfection was defined as patients infected simultaneously with different *Candida* or bacterial species, which were isolated from the same blood sample.

Statistical analysis

K-means cluster analysis (SPSS) was performed to divide high biofilm-forming group from the low biofilm-forming one. For comparison of biofilm forming activity, statistical significance was determined by the Student *t* test. And χ^2 analysis was used to compare the difference of clinical presentations between the patients infected with DST659 and DST693. The Mantel-Cox (log-rank) test was used for survival comparisons in both zebrafish egg infection and in patients with candidemia. A *P* value <.05 was considered significant.

Results

Biofilm forming activity in CGMHL *C. albicans* isolates

To investigate the biofilm forming activity, an *in vitro* biofilm weighting analysis was applied to all DST659 and DST693 isolates. All of the tested isolates of DST659 and DST693 appeared to form biofilms on filter membranes and showed significantly higher biofilm formation indices than that of Δ*tec1*, a negative control strain for biofilm formation.[11] Moreover, several DST659 isolates formed biofilms better than the positive control, SC5314. The average biofilm formation indices of DST659 isolates (3.37 ± 0.82 indices) showed significantly higher than that of

Downloaded from https://academic.oup.com/mmy/article-abstract/56/8/972/4792815 by guest on 13 November 2018

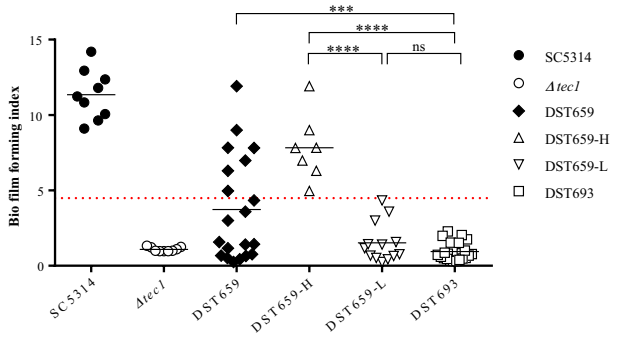

Figure 1. *Candida albicans* isolates formed *in vitro* biofilms on filter membranes. The biofilm formation indices of DST659 and DST693 isolates were illustrated by scatter dot plots with group means. A dotted line representing the K-means clustering cutoff at 4.49 was used to differentiate high and low biofilm activities. Seven of 20 of DST659 (DST659-H) possessed high biofilm-forming activity, while the others (DST659-L) had low forming activity. Δ*tec1* is a mutant defective in biofilm formation. a/α represents the SC5314 strain. The difference in the biofilm formation indices were calculated with Student *t* test. Three asterisks represent the *P*-values less than .001; four asterisks mean $P < .0001$.

DST693 (0.94 ± 0.28 indices) ($P < .001$) (Fig. 1). When the biofilm formation index at 4.49 was used as a cutoff value calculated by K-means clustering with both data sets of DST659 and DST693 genotypes, 7 in 20 DST659 isolates were classified as high biofilm-forming isolates but none in DST693 (Fig. 1). Among the isolates of DST659 genotype, the biofilm-forming activities varied and apparently could be clustered into two groups: high (DST659-H) and low (DST659-L) *in vitro* biofilm groups. The biofilm formation indices of DST659-H isolates were averaged at 7.83 ± 1.23 whereas those of DST659-L averaged at 1.52 ± 0.61, and they differed significantly ($P < .0001$) (Fig. 1).

Mutual validation between the *in vitro* and *in vivo* biofilm assays

To validate the *in vitro* biofilm-forming activity of *C. albicans* obtained above, zebrafish eggs were used as *in vivo* biotic surfaces for biofilm formation experiment.[12] All of the images of biofilm on eggs at 24 and 48 hpi were recorded and analyzed. And all biofilms on eggs infected by *Candida* isolates was semiquantified into different levels (Supplementary Table). It was noted that co-incubation of eggs with individual *C. albicans* isolates for 4 h showed apparent adhesion of yeasts on egg surface, but no difference was found between genotypes DST659 and DST693 (Fig. 2A). However, at 24 hpi, there were thick mycelia extended from the chorion surface of eggs infected by SC5314 and by 45%

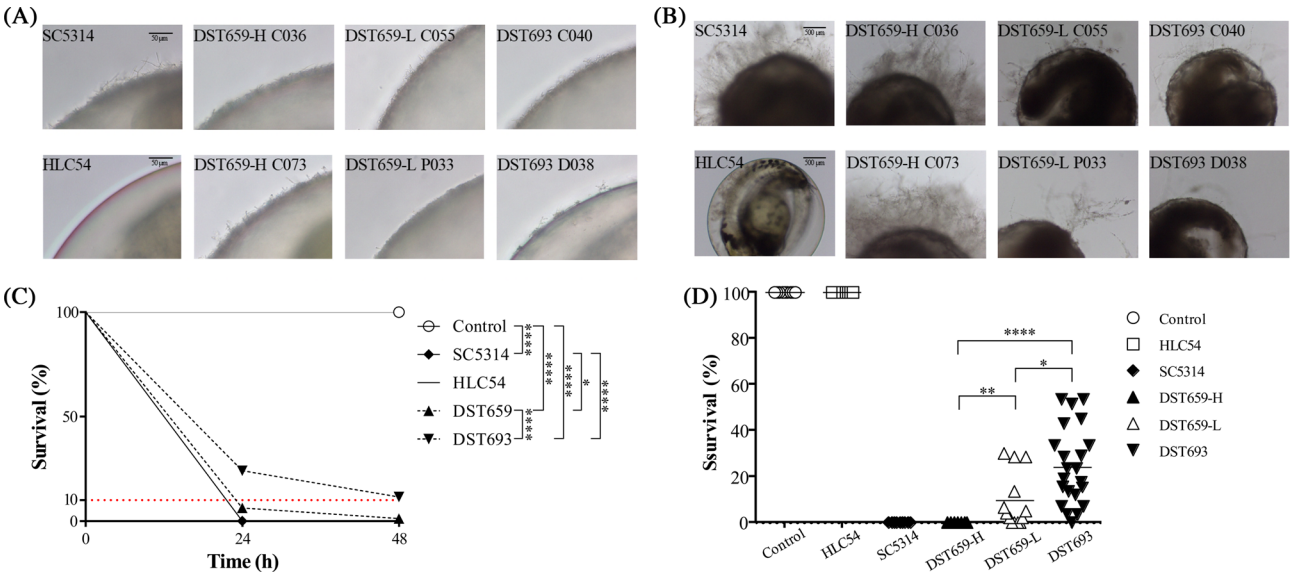

Figure 2. Survivals of zebrafish eggs infected with *C. albicans* isolates. Representative images (A and B) displayed those zebrafish eggs infected by *Candida* yeasts under phase contrast microscope. The corresponding survival analyses of zebrafish eggs (C and D) were performed. (A) The adhered *Candida* cells were observed on the surfaces of eggs co-incubated with *Candida* isolates for 4 h. The upper panels of photographs taken in the same condition showed the enlarged surface parts of eggs, and the lowers displayed whole embryos. (B) Biofilms on eggs infected by *Candida* for 48 h post-infection were observed under microscope. The upper panels showed the whole eggs with *Candida* biofilms, and the lowers displayed the structure of biofilms on eggs. Representative embryos were co-incubated with DST659-H isolates, C036 and C073, DST659-L isolates, C055 and P033, DST693 isolates, C040 and D038. *Candida albicans* SC5314 is a virulent control strain, while HLC54 is a non-filamentous negative control strain. (C) In sum, 22 DST693 and 20 DST659 isolates were used to infect zebrafish eggs, and the survival of eggs were monitored within 48 h. A dotted line notes the 10% survival rate. Kaplan–Meier curves were generated and their statistic differences were calculated by Prism 5.0 software. (D) The 24-h post-infection survivals of eggs infected by DST659-H, DST659-L and DST693 isolates were compared, and the statistic difference in-between was calculated by Student *t* test. For an infection test in a well, 20 zebrafish eggs were used. Three times of repeat gave similar results. An asterisk denoted $P < .05$; two asterisks mean $P < .01$; four asterisks mean $P < .0001$.

Downloaded from https://academic.oup.com/mmy/article-abstract/56/8/972/4792815 by guest on 13 November 2018

Table 2. Survival rates of zebrafish eggs after co-incubated with *Candida albicans*.

Genotypes		Survival rate (%, mean ± SEM) 24 hpi	48 hpi	Genotypes		Survival rate (%, mean ± SEM) 24 hpi	48 hpi
SC5314		0 ± 0	0 ± 0	**HLC54**		100 ± 0	100 ± 0
DST659		5.93 ± 1.77	1.25 ± 0.50	**DST693**		24.02 ± 2.94	11.43 ± 2.31
DST659-H	**Gp. H**	0 ± 0	0 ± 0	DST693	C049	0 ± 0	0 ± 0
	C036	0 ± 0	0 ± 0		C105	3.3 ± 3.33	0 ± 0
	C052	0 ± 0	0 ± 0		C040	3.3 ± 1.68	0 ± 0
	C053	0 ± 0	0 ± 0		C006	6.7 ± 4.41	6.7 ± 4.41
	C073	0 ± 0	0 ± 0		C163	6.7 ± 6.67	0 ± 0
	C131	0 ± 0	0 ± 0		P059	11.7 ± 7.26	0 ± 0
	C140	0 ± 0	0 ± 0		P043	13.3 ± 7.26	8.3 ± 4.41
	U008	0 ± 0	0 ± 0		C070	15.1 ± 3.04	5.0 ± 0.14
	Gp. L	9.12 ± 2.59	1.92 ± 0.75		C122	15.3 ± 2.90	6.7 ± 3.33
	C106	0 ± 0	0 ± 0		C072	17.2 ± 9.22	0 ± 0
	C160	0 ± 0	0 ± 0		U021	18.8 ± 4.75	1.8 ± 1.75
	D003	0 ± 0	0 ± 0		D038	23.3 ± 10.14	10.0 ± 10.00
	U031	0 ± 0	0 ± 0		D027	23.4 ± 10.06	3.3 ± 1.67
DST659-L	C055	1.7 ± 1.67	0 ± 0		P061	28.3 ± 14.81	20.0 ± 10.00
	U020	1.7 ± 1.67	0 ± 0		D048	29.0 ± 4.89	11.8 ± 4.34
	U032	3.7 ± 1.85	0 ± 0		P068	33.3 ± 16.91	6.7 ± 3.33
	P018	5.0 ± 2.89	3.3 ± 1.67		P067	33.5 ± 14.64	8.5 ± 5.96
	C078	6.4 ± 6.35	0 ± 0		U017	43.0 ± 18.89	29.5 ± 20.31
	C079	13.4 ± 6.71	1.5 ± 1.52		U009	45.0 ± 15.28	40.0 ± 17.56
	P033	28.3 ± 20.88	3.3 ± 1.67		P020	51.7 ± 10.93	30.0 ± 12.58
	C083	28.4 ± 12.94	10.1 ± 7.61		C109	53.3 ± 27.28	23.3 ± 18.56
	P053	30.0 ± 10.41	6.7 ± 3.33		P004	53.3 ± 21.67	40.0 ± 21.79

DST659 isolates (e.g., C036 or C073) but not by DST693 isolates (Fig. 2B and Table 1). DST659 isolates showed significantly heavier biofilms on eggs than DST693 at either 24 or 48 hpi (e.g., 25.0% vs. 0 at level IV; Table 1 and Supplementary Table). Moreover, DST659-H isolates also displayed much heavier biofilm formation on infected eggs than isolates of DST659-L and DST693 isolates at 48 hpi (e.g., 42.9%, 15.4%, and 0 at level IV, respectively; Table 1). The positive association between the two results of *in vitro* membrane weighting and *in vivo* zebrafish egg biofilm-forming activity was clearly seen with our clinical CGMHL isolates.

Low survival rates when zebrafish eggs infected with high biofilm-forming *C. albicans* isolates

Zebrafish eggs hatch around 48–72 h, and the survivals of *C. albicans* infected eggs were scored at 24 and 48 hpi. The survival rates of embryos infected with SC5314 and biofilm-defective HLC54[13] strains were 0 and 100%, respectively, at 48 hpi (Fig. 2C and Table 1). Those eggs infected with DST659 isolates displayed significantly low survival rates at 5.93% ± 2.34% at 24 hpi and 1.25% ± 0.61% at 48 hpi. And the latter was close to that seen with SC5314. Relatively, the egg survival rates with DST693 (24.02% ± 3.61% and 11.43% ± 2.79% at 24 and 48 hpi, respectively) were better than DST659-infected eggs (Table 2). The decrease in the survival rates of DST659-infected eggs than the DST693 infected was statistically significant when Kaplan-Meier curve was used for analysis ($P < .0001$) (Fig. 2C). In any case, the vast majority of DST659-infected eggs died with heavy biofilms on their surfaces at 48 hpi (Fig. 2B). Peculiarly, the survivals of DST693 infected eggs were surprisingly low at 11.43% at 48 hpi, but only mild biofilms formed on the eggs were observed (Table 2 and Fig. 2B). It is noteworthy that DST659-H isolates clearly damped the embryo viability more severely than DST659-L isolates did, a fact shown by the survival rates at 24 hpi (survival rate: 0 ± 0% vs. 9.12% ± 3.29% at $P < .05$, Fig. 2D). At 48 h, most embryos infected with DST659-H isolates formed heavy and dense biofilms on eggs. In contrast, isolates of DST659-L showed mild or moderate biofilms on egg surface with clear developed organs inside (Fig. 2B).

Differences in clinical outcomes between the candidemia patients infected with DST659 and DST693 genotypes

A retrospective study was conducted to evaluate the importance of high biofilm-forming activity in our CGMHL

Downloaded from https://academic.oup.com/mmy/article-abstract/56/8/972/4792815 by guest on 13 November 2018

Table 3. Demographic and clinical presentations of candidemia patients infected with DST659 and DST693 genotypes.

Characteristics	DST659	DST693	*P*
Sex (male)	4 (25%)	10 (52.6%)	.096
Coinfection	1 (6.3%)	4 (21.1%)	.347
DM	6 (37.5%)	3 (15.8%)	.245
Liver disease	5 (31.3%)	3 (15.8%)	.424
Renal disease	8 (50.0%)	2 (10.5%)	.022*
Lung disease	5 (31.3%)	4 (21.1%)	.700
Heart disease	2 (12.5%)	4 (21.1%)	.666
Solid tumor	4 (25.0%)	5 (26.3%)	1.000
Central venous access	14 (87.5%)	19 (100%)	.202
Parenteral nutrition	10 (62.5%)	9 (47.4%)	.371
Mortality	10 (62.5%)	10 (52.6%)	.557
3-day mortality	4 (25.0%)	2 (10.5%)	.628
7-day mortality	5 (31.3%)	3 (15.8%)	.650

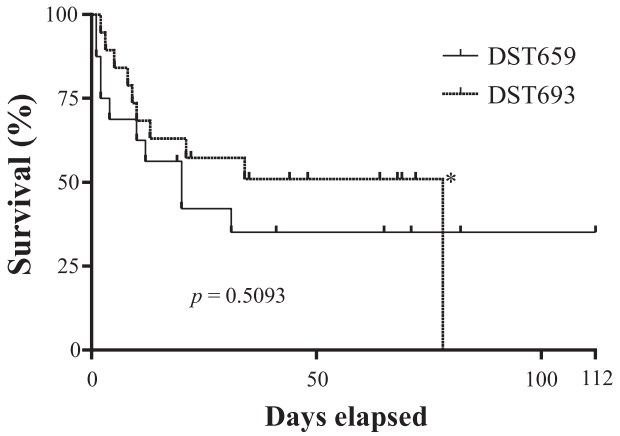

Figure 3. The survival curves of candidemia patients infected with DST659 and DST693 isolates in CGMHL. The medical records of candidemia patients infected with DST659 or DST693 genotypes were reviewed and their cumulative mortality in-hospital within 120 days were plotted by Kaplan–Meier's method, and their statistic differences were calculated by Prism 5.0 software. The last follow-up of DST693-infected patients was censored at day 112, and no data thereafter were available. The *P* value calculated by log-rank testing was .5093.

isolates. The medical records of the candidemia patients infected by DST659 and DST693 genotypes were reviewed; some clinical presentations were analyzed, and they included demographics, co-morbidities, risk factors, and outcomes. Notably, the candidemia patients with DST659 showed a discernible lower survival rate than DST693 infected (3-day and 7-day mortality, Table 3), although the difference was not statistically significant (log-rank testing, $P = .509$; Fig. 3). Comparing other clinical presentations, such as coinfection with other microbes, DM, diseases in major organs, solid tumor, central venous access, total parenteral nutrition, and mortality, gave no differences between the patients with DST659 and DST693 except for a significant difference ($P = .022$) in renal disease (Table 3). Apparently, infection by the higher biofilm-forming activity genotype DST659 showed a much higher incidence in renal failure than DST693 (50.0% vs. 10.5%).

Discussion

Previously we have reported that DST693 and DST659 were the most prevalent two genotypes in CGMHL during 2003–2011.[8] In the present study, we demonstrated that DST659 isolates showed higher biofilm formation than DST693 ones by both *in vitro* and *in vivo* assays. The high biofilm-forming activity of DST659 genotype is very likely to be the reason for being dominant in CGMHL *C. albicans* isolates. The allele ID numbers of DST659 are 11/26/6/4/34/60/119 (*AAT1a/ACC1/ADP1/MPIb/SYA1/VPS13/ZWF1b*), and those of DST693 are 1/7/15/6/61/105/112. Most of the sequence variations between DST659 and DST693 are silent mutations. Nonetheless, there are four missense variations found in *VPS13* located on chromosome 4, but only two in *ADP1* and *MPIb*, and one in *AAT1a*, *SYA1* and *ZWF1b*. The profiles of CAI microsatellite, located also on chromosome 4, indeed differentiated DST659 isolates from DST693 (unpublished data), a fact suggesting that there may be enormous variations in chromosome 4 between DST659 and DST693 genotypes. Interestingly, several important biofilm-associated genes were found located on chromosome 4, such as *BGL2*, *CZF1*, *ECE1*, *HWP1*, *HWP2*, *MED20*, *PGA7*, *PGA10*, *PHR1*, *RBT1*, *RLM1*, and *RBT5*.[15–21] The significance of chromosome 4 on biofilm formation remains to be explored.

According to the results of infection model with zebrafish eggs, *C. albicans* with high *in vitro* biofilm-forming activity was able to adhere on chorion surface, an event that may cause death of embryos, and examples are SC5314 and C036 (Fig. 2B and Table 2). The survival of embryos in the infection model is likely affected by the amount of mycelium. Although the zebrafish infection model has been used in the pathogenesis of *C. albicans*,[22] we found that the egg infection assay may provide additional information about the influence of adhesion or biofilm formation on hosts. In this study, the biofilm-forming activities of clinical *C. albicans* isolates were found closely associated with the zebrafish egg survival after infection with *Candida* yeasts. A scant of invading hyphae was found within the chorions during the infection. However, it is not possible at this time to exclude or include the hyphal invasion played a role toward the pathogenesis of *C. albicans*.

Downloaded from https://academic.oup.com/mmy/article-abstract/56/8/972/4792815 by guest on 13 November 2018

Biofilm has usually been considered as a risk factor for invasive candidiasis, especially for those patients with intravenous lines and bioprosthetic devices. The high biofilm-forming activity of clinical *C. albicans* was recently associated with increased mortality in candidemia patients in a nationwide study in Scotland, 2012–2013.[23] Our current study first demonstrated that a dominant genotype of clinical *C. albicans*, DST659, possesses a high biofilm-forming activity. It may cause a poor prognosis as DST693 does, although currently patient number was not sufficient to clarify the issue. Worthy of attention is the observation that a high incidence rate of renal dysfunction is associated with the DST659-infected patients. Except for underlying diseases, the major cause for mortality of *C. albicans*-disseminated candidiasis is believed to be kidney damage caused by growing fungi.[24] A study using a candidemia mouse model clearly demonstrated that systemic candidiasis can lead to renal failure.[25] The renal dysfunction in DST659-infected patients probably could be explained by an extraordinary amount of initial adherence and the fungal mass in patients' kidneys. In conclusion, high biofilm-forming activity was found to be an important factor for the dominance of DST659 genotype in north Taiwan, and that genotype tends to damp the renal function in patients.

Supplementary material

Supplementary data are available at *MMYCOL* online.

Acknowledgments

We greatly appreciate the help of Dr. Wan-Jr Syu in editing the manuscript. This study was reviewed and approved by the Institutional Review Board of Chang Gung Memorial Hospital (approval no. 201701182B0) and was supported by grants from Chang Gung Memorial Hospital (CMRPG3B1242, CMRPG3D1243, and CMRPG3D1612) and the Ministry of Science and Technology, Taiwan (MOST-104-2320-B-182A-005-MY3 and MOST 105-2320-B-415-006).

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References

1. De Rosa FG, Trecarichi EM, Montrucchio C et al. Mortality in patients with early- or late-onset candidaemia. *J Antimicrob Chemother*. 2013; 68: 927–935.
2. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev*. 2007; 20: 133–163.
3. Ortega M, Marco F, Soriano A et al. *Candida* species bloodstream infection: epidemiology and outcome in a single institution from 1991 to 2008. *J Hosp Infect*. 2011; 77: 157–161.
4. Ruan SY, Hsueh PR. Invasive candidiasis: an overview from Taiwan. *J Formos Med Assoc*. 2009; 108: 443–451.
5. Lortholary O, Dupont B. Antifungal prophylaxis during neutropenia and immunodeficiency. *Clin Microbiol Rev*. 1997; 10: 477–504.
6. Cheng MF, Yang YL, Yao TJ et al. Risk factors for fatal candidemia caused by *Candida albicans* and non-*albicans Candida* species. *BMC Infect Dis*. 2005; 5: 22.
7. Sardi JC, Scorzoni L, Bernardi T, Fusco-Almeida AM, Mendes Giannini MJ. *Candida* species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. *J Med Microbiol*. 2013; 62: 10–24.
8. Wang SH, Shen M, Lin HC et al. Molecular epidemiology of invasive *Candida albicans* at a tertiary hospital in northern Taiwan from 2003 to 2011. *Med Mycol*. 2015; 53: 828–836.
9. Odds FC, Bougnoux ME, Shaw DJ et al. Molecular phylogenetics of *Candida albicans*. *Eukaryot Cell*. 2007; 6: 1041–1052.
10. McManus BA, Coleman DC. Molecular epidemiology, phylogeny and evolution of *Candida albicans*. *Infect Genet Evol*. 2014; 21: 166–178.
11. Lin CH, Kabrawala S, Fox EP et al. Genetic control of conventional and pheromone-stimulated biofilm formation in *Candida albicans*. *PLoS Pathog*. 2013; 9: e1003305.
12. Chen YZ, Yang YL, Chu WL, You MS, Lo HJ. Zebrafish egg infection model for studying *Candida albicans* adhesion factors. *PLoS One*. 2015; 10: e0143048.
13. Lo HJ, Kohler JR, DiDomenico B et al. Nonfilamentous *C. albicans* mutants are avirulent. *Cell*. 1997; 90: 939–949.
14. Chandra J, Kuhn DM, Mukherjee PK et al. Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. *J Bacteriol*. 2001; 183: 5385–5394.
15. Nett JE, Sanchez H, Cain MT, Ross KM, Andes DR. Interface of *Candida albicans* biofilm matrix-associated drug resistance and cell wall integrity regulation. *Eukaryot Cell*. 2011; 10: 1660–1669.
16. Ding C, Vidanes GM, Maguire SL et al. Conserved and divergent roles of Bcr1 and CFEM proteins in *Candida parapsilosis* and *Candida albicans*. *PLoS One*. 2011; 6: e28151.
17. Uwamahoro N, Qu Y, Jelicic B et al. The functions of mediator in *Candida albicans* support a role in shaping species-specific gene expression. *PLoS Genet*. 2012; 8: e1002613.
18. Langford ML, Hargarten JC, Patefield KD et al. *Candida albicans* Czf1 and Efg1 coordinate the response to farnesol during quorum sensing, white-opaque thermal dimorphism, and cell death. *Eukaryot Cell*. 2013; 12: 1281–1292.
19. Taff HT, Nett JE, Zarnowski R et al. A *Candida biofilm*-induced pathway for matrix glucan delivery: implications for drug resistance. *PLoS Pathog*. 2012; 8: e1002848.
20. Kurakado S, Takatori K, Sugita T. Minocycline inhibits the *Candida albicans* budded-to-hyphal-form transition and biofilm formation. *Jpn J Infect Dis*. 2017; 70: 490–494.
21. Ene IV, Bennett RJ. Hwp1 and related adhesins contribute to both mating and biofilm formation in *Candida albicans*. *Eukaryot Cell*. 2009; 8: 1909–1913.
22. Chao CC, Hsu PC, Jen CF et al. Zebrafish as a model host for *Candida albicans* infection. *Infect Immun*. 2010; 78: 2512–2521.
23. Rajendran R, Sherry L, Nile CJ et al. Biofilm formation is a risk factor for mortality in patients with *Candida albicans* bloodstream infection-Scotland, 2012–2013. *Clin Microbiol Infect*. 2016; 22: 87–93.
24. Parker JC, Jr., McCloskey JJ, Knauer KA. Pathobiologic features of human candidiasis: a common deep mycosis of the brain, heart and kidney in the altered host. *Am J Clin Pathol*. 1976; 65: 991–1000.
25. Jae-Chen S, Young-Joo J, Seon-Min P et al. Mechanism underlying renal failure caused by pathogenic *Candida albicans* infection. *Biomed Rep*. 2015; 3: 179–182.

Downloaded from https://academic.oup.com/mmy/article-abstract/56/8/972/4792815 by guest on 13 November 2018