Clin Oral Invest (2016) 20:459–467
DOI 10.1007/s00784-015-1524-2

ORIGINAL ARTICLE

Occurrence of oral *Candida* colonization and its risk factors among patients with malignancies in China

Haiyan Sun[1] · Yong Chen[2] · Xuan Zou[1] · Huan Li[3] · Xiuyun Yin[4] · Haifeng Qin[5] · Rongrui Liu[6] · Changlin Yu[7] · Qihong Li[1] · Kaitao Yu[1] · Xuelin Han[2] · Jingcai Zou[1] · Cheng Ge[1] · Li Han[2]

Received: 18 August 2014 / Accepted: 2 July 2015 / Published online: 30 July 2015

Abstract
Objectives Oral colonization of *Candida* could lead to later development of oropharyngeal candidiasis or candidemia among the immunocompromised patients. This study aims to describe the occurrence and risk factors of oral *Candida* colonization in patients with malignancies.
Materials and methods From October 2012 to March 2013, 78 patients with pulmonary cancer (group I), 101 patients with gastrointestinal tract tumor (group II), 79 patients with hematopoietic system malignant tumor (group III), and 101 healthy controls were consecutively recruited in a hospital in Beijing, China. The oral rinse samples were taken and *Candida* species were identified; the enzymes activities were tested.
Results In total, 110 and 27 *Candida* strains were isolated from 91 patients and 26 controls, respectively. The oral colonization rate with *Candida albicans* in group III (12.7 %) was significant lower than that in group I (30.8 %), group II (33.7 %), and control group (25.7 %). The oral colonization rates with non-*albicans Candida* species in group I, group II, and group III were 15.4, 10.9, and 12.7 %, respectively, while only one non-*albicans Candida* strain was identified in control group. The non-*albicans Candida* species exhibited a lower virulence than *C. albicans*. Age was an independent risk factor for *Candida* colonization in patients with pulmonary cancer and digestive tract malignant tumor, "Teeth brush <1 time/day" was an independent risk factor for *Candida* colonization in patients with hematopoietic system tumor.
Conclusions The differences of risk factors for oral *Candida* colonization in patients with different cancers require different strategies for the prevention and control of *Candida* infection.
Clinical relevance Old aged patients with pulmonary cancer and digestive tract malignant tumor are high-risk population for *Candida* colonization. Increasing frequency of teeth brush might be helpful for preventing *Candida* colonization.

Keywords *Candida albicans* · Non-*albicans Candida* species · Colonization · Biological characteristics · Malignancies · Risk factor

Haiyan Sun, Yong Chen and Xuan Zou contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00784-015-1524-2) contains supplementary material, which is available to authorized users.

✉ Cheng Ge
drgechengde@163.com

✉ Li Han
hanlicdc@163.com

[1] Department of Stomatology, 307 Hospital, PLA, 8# Dongda Str., 100071 Beijing, China

[2] Center for Hospital Infection Control, Chinese PLA Institute for Disease Control and Prevention, Academy of Military Medical Sciences, 20# Dongda Str., 100071 Beijing, China

[3] Department of Oral Medicine, School of Stomatological, Jiamusi University, Jiamusi, China

[4] Department of Clinical Laboratory, 307 Hospital, PLA, Beijing, China

[5] Department of Digestive Tract Malignant Tumor, 307 Hospital, PLA, Beijing, China

[6] Department of Pulmonary Cancer, 307 Hospital, PLA, Beijing, China

[7] Department of Hematopoietic System Tumor, 307 Hospital, PLA, Beijing, China

Introduction

In healthy human, *Candida* species present as part of normal microbial population in the oral cavity and gastrointestinal tract [1]. It has been reported that *Candida* could be isolated in the oral cavity of 15.2 to 75 % of healthy individuals [2, 3]. The difference in population characteristics, sampling approach, and geographic area might contribute to the diversity of detection rates [4–6]. When appropriate conditions such as local or systemic deficiencies in the host defenses exist, they multiply, even causing multiple infections [7]. The *Candida* infection in oral cavity is common in patients with prolonged, severe diseases, including diabetes mellitus (DM), solid organ transplant, AIDS, or undergoing antineoplastic chemotherapy [8–10]. It also happened when the diverse local factors made the buccal tissues susceptible to *Candida* infection, such as poor oral hygiene [11], reduced saliva flow [12], and prosthetic dentures use [13].

Oral colonization (up to 93 %) and infection (up to 30 %) occurred frequently in the radiotherapy patients [14]. The oral carriage rate of *Candida* in patients with advanced cancer is much higher [15, 16]. The direct factors leading to oral candidiasis seemed to be oral mucosal histological changes [17], together with salivary quantitative and qualitative changes, which were superimposed on other immunological deficiency. However, the underlying risk factors causing the oral *Candida* colonization and infection remain controversial.

Most *Candida* infections originate from endogenous host reservoirs, and the oral cavity and digestive tract are thought to be the principle source for hematogenous invasion, especially in critically ill patients. Invasive *Candida* infections are significantly higher in patients with prior yeast colonization compared to individuals with no evidence of colonization [18, 19]. Therefore, the increased oral fungal burden in cancer patients might play a potentially important role in the subsequent development of invasive infection and correlate with severe clinical complications.

Non-*albicans Candida* species played an increasingly important role in fungal infection. Many studies have shown that the prevalence of non-*albicans Candida* species increased significantly in uncontrolled AIDS [20], cancer [15], and critically ill patients [21]. However, to date, the study on the characterization of different oral *Candida* species colonized in patients with malignant tumor in China and their risk factors was rare. This study aimed to investigate the occurrence of oral *Candida albicans* and non-*albicans Candida* species colonization in several groups of cancer patients undergoing cytostatic therapy and healthy controls in China, and to determine the biological characteristics of these *Candida* species and analyze the risk factors for *Candida* colonization.

Materials and methods

Study population

This prospective observational study was conducted from October 2012 to March 2013 in a large tertiary hospital (The 307th Hospital of PLA) in Beijing. Seventy-eight patients suffering from pulmonary cancer (group I), 101 patients suffering from gastrointestinal tract malignant tumor (group II), and 79 patients suffering from hematopoietic system malignant tumor (group III) were consecutively recruited from the in-patient department of the Tumor Therapy Center of the hospital. One hundred and one healthy controls were recruited randomly from the Medical Examination Center of the hospital. All the participants in each group were informed about the purpose of this study and all signed informed consent to participate in the study. The participants with certain affirmed risk factors for oral candidosis such as uncontrolled diabetes, maxillofacial radiotherapy, and intraoral prostheses were excluded from the study. The unconscious patients and those with significant physical defects were also excluded as they could not cooperate. All the participants were given oral hygiene instructions and were investigated for complete haemogram and blood sugar level. Oral sample was taken after 1 h fasting. To prevent circadian variations, the samples were collected between 9 a.m. and 12 a.m. The study was approved by the Institutional Ethics Committee of Academy of Military Medical Sciences, Beijing, China.

Data collection

The information on population demographics, medication variables, and oral local factors were collected by two trained dentists. The demographic data included age, gender, body mass index (BMI), and smoking habits. Medication variables included hospital length of stay, underlying diseases, stay in bed, neutropenia, cycles of chemotherapy, corticoid therapy, invasive treatment, present of central venous line, radiation therapy of non-oral maxillofacial region, and use of antibacterial and antifungal drug. Oral local risk factors included periodontal health status, oral mucositis, frequency of teeth brushing, use of drug mouthwash, and xerostomia.

Oral examination, specimen collection and *Candida* identification

Before collecting the oral rinse sample, regular oral examination was performed by two dentists. The participants were checked according to the following items: number of cavity, oral hygiene evaluation, periodontal health condition, symptom of oral mucosa, the unstimulated saliva flow. Then the oral rinse sampling was taken as previously described [22]. Each participant was supplied with 10 ml sterile phosphate-

buffered saline (0.1 M PBS, pH 7.2) in a universal container and to rinse the mouth for 60 s before expectorating the saliva-buffer mixture back into the container. The sample was sent immediately to the microbiology laboratory and was centrifuged at 1700×*g* for 10 min. The supernatant was discarded and the pellet was resuspended in 2 ml PBS on a vortex mixer for 30 s. A spiral plater (model DU; Spiral Systems) was used to dispense the precipitated sampling.

Fifty microliters of the suspension was separately inoculated onto CHROMagar-*Candida* (bioMérieux, Marcy L´Etoile, France) and Sabouraud-Dextrose Agar (bioMérieux, Marcy L´Etoile, France) for 72 h in 35 °C incubator. The growth of any *Candida* colonies was recorded and the subject was defined as a *Candida* carrier. The number of colonies on each plate was counted and the *Candida* colonization density was calculated based on the number of colony-forming units (CFU) per milliliter rinse sample. The "high load" *Candida* carrier referred to those with ≥500 CFU per ml of rinse sample, while the "low load" *Candida* carrier with <500 CFU per ml of rinse sample [23].

Initial identification of *Candida* species was based on colony color because the CHROMagar-*Candida* medium contains chromogenic substances that allow a presumptive identification of some species of *Candida*. Further identification of the yeast was done by API 20C AUX (bioMérieux, Marcy L´Etoile, France) according to the method of Williamson et al. [24] and the following polymerase chain reaction (PCR) assays.

Genotyping of *C. albicans*

The genotype of *C. albicans* was determined by PCR assays and distinguished from *Candida dubliniensis*. Cellular DNA was extracted according to previously described methods [25]. The fungus-specific, universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) were used to amplify a small conserved portion of the 18S rDNA region and a small portion of the 28S rDNA region, yielding products with variable sizes among the major species. *C. albicans* resulted in a 218- or 219-bp product.

Genotyping of *C. albicans* was conducted according to the previous study [26]. The expected PCR product spans the site of the transposable intron in the 25S rDNA. *C. albicans* resulted in products for *C. albicans* genotypes A (450 bp), B (840 bp), C (450 and 840 bp), D (1080 bp), and E (1400 bp). The genotype of D was proved to be *C. dubliniensis* [27].

Determination of virulence

The biological characteristics of the *Candida* were evaluated by detecting the virulence activities including phospholipase activity, proteinase activity, and hemolysin activity. The *C. albicans* were screened for the three extracellular enzymes' activity by measuring the ratio of the diameter of the colony to that of the precipitation or clear zone on the various culture agars. The protocol referred to the previous studies [28, 29]. Each assay was conducted in duplicate for each yeast isolate. Reference strains of *C. albicans* (ATCC 10231) served as positive controls and *C. parapsilosis* (ATCC 22019) served as a negative control.

Antifungal susceptibility test

The susceptibility of all the *Candida* to five antifungal drugs including 5-flucytosine, amphotericin B, fluconazole, itraconazol, and voriconazol was conducted using the commercial Candifast kit (International Micobio, France) according to the manufacturer's instructions. Minimum inhibitory concentrations (MICs) were determined and the following resistance breakpoints were used according to CLSI guidelines [30]: for fluconazole, resistant ≥64 μg/mL, susceptible-dose dependent (S-DD) 16–32 μg/mL, and susceptible ≤8 μg/mL; for 5-flucytosine, resistant ≥32 μg/mL, S-DD 8–16 μg/mL, susceptible ≤4 μg/mL; For itraconazol, resistant ≥1 μg/mL, S-DD 0.25–0.5 μg/mL, susceptible ≤0.0125 μg/mL; for voriconazol, resistant ≥4 μg/mL, S-DD 2–4 μg/mL, susceptible ≤1 μg/mL. There were no CLSI breakpoints defined for amphotericin B. For susceptibility to amphotericin B, the breakpoint established by Yang et al. [31] was adopted as follows: resistant ≥2 μg/mL, susceptible ≤1 μg/mL.

Statistical analysis

Categorical variables were evaluated using the chi-square and teo-tailed Fisher's exact tests as appropriate. For continuous variables, the *t* test or nonparametric test was used. Univariate and multivariate logistic regression analyses were performed for the risk factor analyses. Variables that were found as significant ($p<0.05$) in univariate analysis were considered as candidates for multivariate analysis. Odds ratio and 95 % confidence interval were calculated. All statistical analyses were performed with Statistical Package for the Social Sciences (SPSS Inc., Version 16.0, Chicago, IL, USA). A p value of <0.05 was considered statistically significant. All tests of significance were two-tailed.

Results

Population characteristics and the occurrence of oral *Candida* colonization

The demographic characteristics of the patients and randomly selected healthy controls were shown in Table 1. The age of patients in group I (pulmonary cancer) and group II (digestive

Table 1 Summary of population demographics

Variable	Patient groups				Health control group (n=101)	χ^2 value or t value[a]	p value
	Pulmonary cancer (n=78)	Digestive tract malignant tumor (n=101)	Hematopoietic system tumor (n=79)	Total (n=258)			
Age(years), means±SD	56.8±11.0	54.3±13.0	37.5±15.6	49.9±15.7	41.3±11.7	5.59	<0.001
Male sex	24 (30.8)	31 (30.7)	34 (43.0)	89 (34.5)	61 (61.0)	20.8	<0.001
BMI, means±SD	24.5±3.3	21.9±3.8	22.8±3.7	22.9±3.8	24.1±3.6	2.5	0.013
Smoking	8 (10.3)	12 (11.9)	5 (6.4)	25 (9.7)	21 (21.0)	8.15	0.004

[a] The results of comparison between the patient and healthy control group

tract malignant tumor) was higher than those in group III (hematopoietic system tumor) and healthy controls. There were more male participants and a higher prevalence of smoking in healthy controls, which might be due to the differences in their lifestyle and health status.

In total, 110 and 27 *Candida* isolates were isolated from 91 patients and 26 controls, respectively. The most common *Candida* species was *C. albicans*, which was isolated from 26.4 and 25.7 % of oral rinse samples of the patients and controls, respectively (Table 2). The oral colonization rate with *C. albicans* in group III (12.7 %) was significantly lower than that in group I (30.8 %), group II (33.7 %) and control group (25.7 %). The oral colonization rates with "high load" *C. albicans* in group I (9.0 %) and group II (7.9 %) were significantly higher than that in group III (1.3 %) and control (1 %). Only one patient from group I had combined clinical and microbiological evidence of oral candidosis. The oral colonization rates with non-*albicans Candida* species in groups I, II, and III were 15.4, 10.9, and 12.7 %, respectively, which were all significantly higher than that in controls (1 %). There was one case with "high load" non-*albicans Candida* species in group I and one case in group II.

Sixty-eight isolates (61.8 %) were identified as *C. albicans* among the 110 *Candida* isolates from patient groups. The four most common non-*albicans Candida* species isolated from patient groups were *C. parapsilosis* (n=17), *Candida tropicalis* (n=5), *Candida krusei* (n=5), and *Candida glabrata* (n=3), respectively, which accounted for 15.4, 4.5, 4.5, and 2.7 % of all the *Candida* isolates from patient groups. Twelve *Candida* isolates could not be classified into any subspecies based on current phenotypic assay. Only one *C. parapsilosis* strain was isolated from the health control group (Table 3). Among the *Candida*-positive participants, the proportion of co-colonization by more than two species of *Candida* was 21.9 % (group I), 15.0 % (group II), 21.1 % (group III), and 3.8 % (control group), respectively (Supplemental Table S1).

The genotypes and virulence of *C. albicans*

The predominant genotype of *C. albicans* from both the patient groups and control group was type A, for which the prevalence are respectively 47.8 % (group I), 61.8 % (group II), 77.8 % (group III), and 46.2 % (control group). No significant difference was found over the distribution of three

Table 2 The prevalence of oral *C. albicans* and non-*albicans Candida* species in patients and healthy controls

Groups	Numbers	*C. albicans*, *n* (%)			Non-*albicans Candida* species, *n* (%)		
		Low load	High load	Total	Low load	High load	Total
Patients							
Pulmonary cancer	78	17 (21.8)	7 (9.0)[a]	24 (30.8)	11 (14.1)[a]	1 (1.3)	12 (15.4)[a]
Digestive tract malignant tumor	101	26 (25.7)	8 (7.9)[a]	34 (33.7)	10 (9.9)[a]	1 (1.0)	11 (10.9)[a]
Hematopoietic system tumor	79	9 (11.4)[a]	1 (1.3)	10 (12.7)[a]	10 (12.7)[a]	0	10 (12.7)[a]
Total	258	52 (20.2)	16 (6.2)	68 (26.4)	31 (12)	2 (0.8)	33 (12.8)
Controls	101	25 (24.8)	1 (1.0)	26 (25.7)	1 (1.0)	0	1 (1.0)

[a] The differences of prevalence of oral *C. albicans* or non-*albicans Candida* species in patients and healthy controls were significant (p<0.05)

Table 3 Distribution of oral *Candida* species in patients and healthy controls

Candida species	Patient groups			Controls (n=27)
	Pulmonary cancer (n=39)	Digestive tract malignant tumor (n=48)	Hematopoietic system tumor (n=23)	
C. albicans	24 (61.5)	34 (70.8)	10 (43.5)	26 (96.3)
Non-*albicans Candida*				
C. parapsilosis	6 (15.4)	5 (10.4)	5 (21.7)	1 (3.7)
C. tropicalis	2 (5.1)	2 (4.2)	1 (4.3)	0
C. krusei	2 (5.1)	3 (6.3)	0	0
C. glabrata	0	2 (4.2)	1 (4.3)	0
C.guilliermondii	0	0	1 (4.3)	0
Others	5 (12.8)	2 (4.2)	5 (21.7)	0

genotypes in *C. albicans* among different population groups (p=0.572) (Fig. 1).

In general, *C. albicans* exhibited higher virulence than non-*albicans Candida* species (data not shown). The phospholipase activity, proteinase activity, and hemolytic activity of *C. albicans* isolated from controls were all higher than those from three patient groups (Table 4). There were no significant differences on the virulence activities of *C. albicans* of different genotypes (p>0.05).

Antifungal susceptibility

All the 66 *C. albicans* isolates from patients and healthy controls were sensitive to amphotericin B. Two *C. albicans* isolates (3.0 %) from group II were resistant to fluconazole, itraconazole, and voriconazole. Dose-dependent susceptibility to 5-flucytosine was seen in three *C. albicans* isolated from patient groups. None of the non-*albicans Candida* species from the patient groups and control group were resistant to the five antifungal drugs tested.

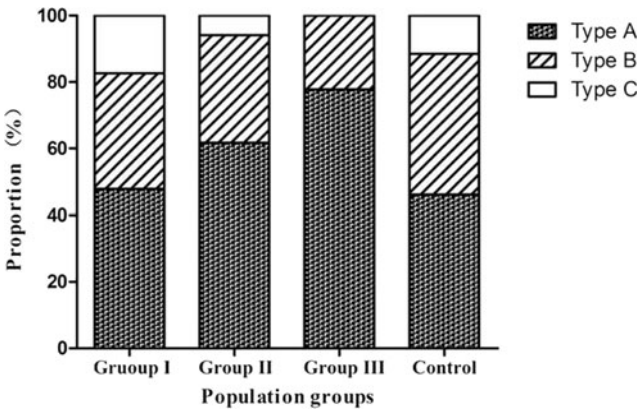

Fig. 1 The distribution of three genotypes in *C. albicans* among different population groups

Risk factors for oral *Candida* colonization

For patients of pulmonary cancer and digestive tract malignant tumor, univariate analysis showed that age and stay in bed were significantly associated with carriage of either all *Candida* species or *C. albicans* alone. Corticoid therapy also acted as a risk factor for carriage of *Candida*. Multivariate analysis showed that age was an independent risk factor for the carriage of either all *Candida* species or *C. albicans* alone. Corticoid therapy was another independent risk factor of oral *Candida* colonization (Supplemental Table S2). Due to the differences of patient characterizations, the risk factor analysis of oral *Candida* colonization in hematopoietic system tumor patients was conducted independently with other two patient groups. In hematopoietic system tumor patients, "Teeth brush <1 time/day" became an important risk factor for carriage of either all *Candida* species or *C. albicans* alone. However, no association was found between age of hematopoietic system tumor patients and carriage of either all *Candida* species or *C. albicans* alone (Supplemental Table S3).

The results of univariate and multivariate analyses showed that age and invasive treatment were significantly associated with carriage of "high load" *C. albicans* in pulmonary cancer and digestive tract malignant patients (Table 5). In hematopoietic system tumor patients, the cases of "high load" *C. albicans* carriage were too few to conduct risk factor

Table 4 The virulence of *C. albicans* from all participants (means±SD)

Virulence activities	Group I	Group II	Group III	Controls
Phospholipase (Pz)	0.73±0.09	0.72±0.07	0.71±0.16	0.62±0.07[a]
Proteinase (Prz)	0.37±0.07	0.36±0.07	0.40±0.09	0.27±0.02[a]
Hemolysin (Hz)	0.36±0.09	0.35±0.04	0.32±0.03	0.28±0.03[a]

[a] There were significant differences between groups I\II\III and controls over three virulence activities (p<0.001)

Table 5 Univariate and multivariate analyses of the risk factors for *C. albicans* high load in pulmonary cancer and digestive tract malignant patients, *n* (%)

Variables[a]	"High load" *C. albicans* patients(B) (*n*=15)	*Candida* negative patients (C) (*n*=107)	OR (CI 95 %)	*p* value
Univariate analysis				
Male sex	11 (73.3)	77 (72.0)	1.07 (0.32–3.63)	0.912
Age (years)	63.7±14.9	52.6±10.2	1.09 (1.03–1.15)	0.001
CVD (cardiovascular and cerebrovascular diseases)	6 (40.0)	20 (18.7)	2.90 (0.93–9.08)	0.068
Smoking	4 (26.7)	11 (10.3)	3.17 (0.86–11.68)	0.082
Hospital length of stay (days)	16.0±12.8	11.4±9.1	1.04 (0.99–1.09)	0.100
Stay in bed	8 (53.3)	33 (30.8)	2.56 (0.86–7.65)	0.092
Cycles of chemotherapy ≥3	6 (40.0)	56 (52.3)	0.61 (0.20–1.83)	0.374
Corticoid therapy	6 (40.0)	31 (29.0)	1.63 (0.54–4.98)	0.387
Invasive treatment	8 (53.3)	26 (24.3)	3.56 (1.18–10.77)	0.024
Central venous line present	5 (33.3)	13 (12.1)	3.62 (1.07–12.25)	0.039
Accepting radiation therapy ever	4 (26.7)	35 (32.7)	0.75 (0.22–2.52)	0.639
Active periodontal disease	0 (0.0)	7 (6.5)	–	0.595
Teeth brush <1 time/day	3 (20.0)	12 (11.2)	1.98 (0.49–8.03)	0.339
Dry mouth	11 (73.3)	49 (45.8)	3.26 (0.98–10.87)	0.055
Mucosal symptoms	1 (6.7)	10 (9.3)	0.69 (0.08–5.83)	0.736
Drug mouthwash	1 (6.7)	5 (4.7)	1.46 (0.16–13.40)	0.739
Antibacterial drug use ≥3 days	2 (13.3)	16 (15.0)	0.88 (0.18–4.25)	0.868
Antifungal drug use ≥3 days	0 (0.0)	1 (0.9)	–	1
Multivariate analysis				
Age (years)			1.11 (1.05–1.18)	0.001
Invasive treatment			8.70 (1.78–42.68)	0.008

OR odds ratio, *CI* confidence interval

[a] Continuous data are presented as mean (SD) and categorical data as number (%)

analysis. As to the carriage of non-*albicans Candida* species, no significant risk factor was found in all the patient groups.

Discussion

To investigate the occurrence of oral yeast carriage and oral candidiasis, the oral rinse sampling method was used in this study, as it was the most appropriate and sensitive technique for evaluating the overall yeast carriage comparing to imprint culture, swab, or saliva sampling [32]. The results showed that *C. albicans* was isolated from the oral cavity of 26.4 % of patients with malignant tumor and 25.7 % of healthy controls, which were similar to previous studies in healthy individuals and DM patients, but a little lower than those with uncontrolled AIDS and advanced cancer [33]. However, the prevalence of high load *C. albicans* in patients (6.2 %) was higher than that in controls (1 %). Unexpectedly, the oral colonization rate with *C. albicans* was significantly lower in hematopoietic system tumor patients. The possible reason could be that these patients were all provided with chlorhexidine and alkaline mouthwash during hospitalization. The previous study has proved the effect of chlorhexidine in decreasing colonization of *Candida* [33].

Although *C. albicans* was the most frequent yeast in all study groups, there was a significant higher proportion of non-*albicans Candida* species in patient groups than control group, only one healthy control was colonized with non-*albicans Candida* species. This result was in accordance with previous studies, which showed that non-*albicans Candida* species have been detected more frequently, especially in immunosuppressed individuals [34, 35]. This finding may be indicative of the possibility that several non-*albicans Candida* species, which are normally more vulnerable to host immunity, are allowed to thrive in the immunosuppressed host environment. The altered proportion of *C. albicans* and non-*albicans Candida* species suggested the changing epidemiology of *Candida* species. The main explanation for this phenomenon was supposed to be the selective pressure of long-term use of antifungal drugs [36]. It was reported that non-

albicans Candida species exhibited a higher resistance rate to certain antifungal drugs [37]. However, antifungal drug resistance was rare in our study, especially in non-*albicans Candida* species.

Extracellular enzymes such as phospholipases, aspartyl proteinases, and hemolysin are important virulence factors contributing to the pathogenesis of *Candida* [38, 39]. Our study showed that the three enzymes exhibited a higher activity in *C. albicans* than in non-*albicans Candida* species for all patient groups and control group, and the *C. albicans* from all patients group also exhibited a higher activity of these extracellular enzymes than those from control group. Previous studies also found that the activities of proteinases and phospholipases were high in *C. albicans*, while non-*albicans Candida* species usually presented low activities of these enzymes [40]. The results suggested that the level of extracellular enzymes might be less important for successful colonization and infection of *C. albicans* in cancer patients although the reasons were as yet unknown. We supposed that the factors associated with colonization and infection of the *Candida* in oral cavity might lie in local or system performance of individual. It could also be the reason for why patients with hematopoietic system tumor were colonized with a high prevalence of non-*albicans Candida* species, which were seldom found in healthy controls. This finding was confirmed by Krcmery et al., who found invasive *C. glabrata* infection presented a higher mortality and occurred predominantly in the most critically ill patients, although *C. glabrata* exhibited a low enzymatic activity and virulence in animal models of infection comparing to other non-*albicans Candida* species [35].

The risk factors for oral *Candida* colonization and infection in immunosuppressed patients were complex [41], including local oral factors, such as low salivary flow rates and pH, smoking, wearing dentures, and poor oral hygiene [42, 43], and other systemic factors such as patient age, disease types, treatment methods, and the physical performance of patients [44, 45]. We found the colonization of *Candida* in patients with pulmonary cancer and digestive tract malignant tumor was significantly associated with age, which was the same as previous report [46]. Although neutropenia is a common pre-disposing factor [28, 47], oral *Candida* carriage rate and density were not associated significantly with neutropenia in this study. The reason could be that few patients suffered from prolonged severe neutropenia in this study, as it had been demonstrated that fungal colonization and infection occurred more often in patients with prolonged neutropenia, especially in patients with prolonged, severe neutropenic episodes with extreme age [48, 49].

It was still controversary whether the use of antimicrobial drugs contribute to the oral colonization and infection of *Candida* in the previous studies. We also found no significant relationship between them; however, systematic use of corticosteroids contributed to some cases of oral *Candida* colonization. The possible mechanism might be that the systematic application of corticosteroids suppresses the immune system, which results in the proliferation of yeasts. Moreover, it had been reported that the routine prophylactic use of antifungal agents in transplant patients with high levels of oral *Candida* colonization was probably inappropriate, unless oral infection is present [50]. In our study, the prophylactic use of antifungal agents in oral cavity was rare, which might be the reason for the low prevalence of antifungal resistance. In addition, genotype A was prominent in *C. albicans* from all groups, followed by genotypes B and C, which is in accordance with other studies [51, 52]. No relationship was found between the genotypes and antifungal resistance.

In hematopoietic system tumor patients, teeth brush <1 time/day was associated with oral *Candida* colonization. For these patients with hematologic diseases, mouth washing was usually performed instead of brushing teeth. The bacteria plaque on the surface of teeth and mucosa would make *Candida* more prior to adhere. We have also found that invasive treatment might contribute to high load of *C. albicans* in patients with pulmonary and digestive tract malignant cancer. But the reason was not clear. To date, no similar results were reported for these groups of patients because there were only a small number of patients in each group. Future studies were needed for further investigation.

In conclusion, we demonstrated firstly the higher density of carriage and the virulence of oral *Candida* in patients with malignant tumor in Chinese hospitals. The diversity of occurrence and risk factors of oral *Candida* colonization required different strategies for the prevention and control of *Candida* infection in patients with different cancers.

Acknowledgments We thank the staff in Department of the Tumor Therapy Center and Medical Examination Center, The 307th Hospital of PLA, for their help and assistance in recruiting participants and collecting samples. The study was supported by a grant from Beijing Natural Science Foundation, China (No. 7122133), a grant from National "973" Program (No. 2013CB531606), and the National Natural Scientific foundation of China (No. 81102168).

Author contribution Li Han and Cheng Ge designed the study. Haiyan Sun, Huan Li, and Xuan Zou performed the study. Xiuyun Yin, Haifeng Qin, Rongrui Liu, Changlin Yu, Qihong Li, Kaitao Yu, and Xuelin Han participated in collecting the clinical samples and medical information. Yong Chen and Huan Li collected the data and carried out the statistical analyses. Haiyan Sun and Yong Chen wrote the paper; Jingcai Zou took in charge of reviewing and editing the research manuscript. All of the authors read and approved the final manuscript.

Declaration of interest The authors have no conflicts of interest to declare.

References

1. Cannon RD, Chaffin WL (1999) Oral colonization by *Candida albicans*. Crit Rev Oral Biol Med 10:359–383
2. Yang YL, Leaw SN, Wang AH, Chen HT, Cheng WT, Lo HJ (2011) Characterization of yeasts colonizing in healthy individuals. Med Mycol 49:103–106
3. Ghannoum MA, Jurevic RJ, Mukherjee PK, Cui F, Sikaroodi M, Naqvi A, Gillevet PM (2010) Characterization of the oral fungal microbiome (mycobiome) in healthy individuals. PLoS Pathog 6, e1000713
4. Sanchez-Vargas LO, Ortiz-Lopez NG, Villar M, Moragues MD, Aguirre JM, Cashat-Cruz M, Lopez-Ribot JL, Gaitan-Cepeda LA, Quindos G (2005) Oral *Candida* isolates colonizing or infecting human immunodeficiency virus-infected and healthy persons in Mexico. J Clin Microbiol 43:4159–4162
5. Wu CJ, Lee HC, Yang YL, Chang CM, Chen HT, Lin CC, Lee NY, Chu WL, Hsieh LY, Wang YL, Lauderale TL, Tseng FC, Ko NY, Ko WC, Lo HJ (2012) Oropharyngeal yeast colonization in HIV-infected outpatients in southern Taiwan: CD4 count, efavirenz therapy and intravenous drug use matter. Clin Microbiol Infect 18:485–490
6. Blignaut E (2007) Oral candidiasis and oral yeast carriage among institutionalised South African paediatric HIV/AIDS patients. Mycopathologia 163:67–73
7. Cannon RD, Holmes AR, Mason AB, Monk BC (1995) Oral *Candida*: clearance, colonization, or candidiasis? J Dent Res 74: 1152–1161
8. Dongari-Bagtzoglou A, Dwivedi P, Ioannidou E, Shaqman M, Hull D, Burleson J (2009) Oral *Candida* infection and colonization in solid organ transplant recipients. Oral Microbiol Immunol 24:249–254
9. Al-Attas SA, Amro SO (2010) Candidal colonization, strain diversity, and antifungal susceptibility among adult diabetic patients. Ann Saudi Med 30:101–108
10. Saiman L, Ludington E, Dawson JD, Patterson JE, Rangel-Frausto S, Wiblin RT, Blumberg HM, Pfaller M, Rinaldi M, Edwards JE, Wenzel RP, Jarvis W, National Epidemiology of Mycoses Study G (2001) Risk factors for *Candida* species colonization of neonatal intensive care unit patients. Pediatr Infect Dis J 20:1119–1124
11. Darwazeh AM, Hammad MM, Al-Jamaei AA (2010) The relationship between oral hygiene and oral colonization with *Candida* species in healthy adult subjects*. Int J Dent Hyg 8:128–133
12. Karbach J, Walter C, Al-Nawas B (2012) Evaluation of saliva flow rates, *Candida* colonization and susceptibility of *Candida* strains after head and neck radiation. Clin Oral Investig 16:1305–1312
13. Daniluk T, Tokajuk G, Stokowska W, Fiedoruk K, Sciepuk M, Zaremba ML, Rozkiewicz D, Cylwik-Rokicka D, Kedra BA, Anielska I, Gorska M, Kedra BR (2006) Occurrence rate of oral *Candida albicans* in denture wearer patients. Adv Med Sci 51(Suppl 1):77–80
14. Epstein JB, Freilich MM, Le ND (1993) Risk factors for oropharyngeal candidiasis in patients who receive radiation therapy for malignant conditions of the head and neck. Oral Surg Oral Med Oral Pathol 76:169–174
15. Lalla RV, Latortue MC, Hong CH, Ariyawardana A, D'Amato-Palumbo S, Fischer DJ, Martof A, Nicolatou-Galitis O, Patton LL, Elting LS, Spijkervet FK, Brennan MT, Fungal Infections Section OCSGMAoSCiCISoOO (2010) A systematic review of oral fungal infections in patients receiving cancer therapy. Support Care Cancer 18:985–992
16. Davies AN, Brailsford SR, Beighton D (2006) Oral candidosis in patients with advanced cancer. Oral Oncol 42:698–702
17. Cankovic M, Bokor-Bratic M (2010) *Candida albicans* infection in patients with oral squamous cell carcinoma. Vojnosanit Pregl 67: 766–770
18. Redding SW, Marr KA, Kirkpatrick WR, Coco BJ, Patterson TF (2004) *Candida glabrata* sepsis secondary to oral colonization in bone marrow transplantation. Med Mycol 42:479–481
19. Westbrook SD, Kirkpatrick WR, Freytes CO, Toro JJ, Bernardo S, Patterson TF, Redding SW, Lee SA (2007) *Candida krusei* sepsis secondary to oral colonization in a hemopoietic stem cell transplant recipient. Med Mycol 45:187–190
20. Bandar IN, Widodo D, Djauzi S, Muthalib A, Soegondo S, Wahyuningsih R (2006) Correlation between CD4 count and intensity of *Candida* colonization in the oropharynx of HIV-infected/AIDS patient. Acta Med Indones 38:119–125
21. Charles PE, Dalle F, Aube H, Doise JM, Quenot JP, Aho LS, Chavanet P, Blettery B (2005) *Candida* spp. colonization significance in critically ill medical patients: a prospective study. Intensive Care Med 31:393–400
22. Tsang CS, Samaranayake LP (2010) Immune reconstitution inflammatory syndrome after highly active antiretroviral therapy: a review. Oral Dis 16:248–256
23. Silverman S Jr, Gallo JW, McKnight ML, Mayer P, deSanz S, Tan MM (1996) Clinical characteristics and management responses in 85 HIV-infected patients with oral candidiasis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 82:402–407
24. Williams DW, Wilson MJ, Lewis MA, Potts AJ (1995) Identification of *Candida* species by PCR and restriction fragment length polymorphism analysis of intergenic spacer regions of ribosomal DNA. J Clin Microbiol 33:2476–2479
25. Sullivan DJ, Westerneng TJ, Haynes KA, Bennett DE, Coleman DC (1995) *Candida dubliniensis* sp. nov.: phenotypic and molecular characterization of a novel species associated with oral candidosis in HIV-infected individuals. Microbiology 141(Pt 7): 1507–1521
26. McCullough MJ, Clemons KV, Stevens DA (1999) Molecular and phenotypic characterization of genotypic *Candida albicans* subgroups and comparison with *Candida dubliniensis* and *Candida stellatoidea*. J Clin Microbiol 37:417–421
27. McCullough M, Ross B, Reade P (1995) Characterization of genetically distinct subgroup of *Candida albicans* strains isolated from oral cavities of patients infected with human immunodeficiency virus. J Clin Microbiol 33:696–700
28. Kaczmarczyk D, Morawiec-Sztandera A, Niedzwiecka I, Kurnatowski P (2011) Difficulties associated with the diagnosis of mycosis of the oral cavity and throat in chronic lymphocytic leukemia (CLL). Wiad Parazytol 57:155–158
29. Luo G, Samaranayake LP, Yau JY (2001) *Candida* species exhibit differential in vitro hemolytic activities. J Clin Microbiol 39:2971–2974
30. Clinical and Laboratory Standards Institute (CLSI) (2008) Reference method for broth dilution antifungal susceptibility testing of yeasts: 3rd ed. M27-A3. Clinical and Laboratory Standards Institute, Wayne
31. Yang YL, Wang AH, Wang CW, Cheng WT, Li SY, Lo HJ (2008) Susceptibilities to amphotericin B and fluconazole of *Candida* species in Taiwan Surveillance of Antimicrobial Resistance of Yeasts 2006. Diagn Microbiol Infect Dis 61:175–180
32. Byadarahally Raju S, Rajappa S (2011) Isolation and identification of *Candida* from the oral cavity. ISRN Dent 2011:487921
33. Torres SR, Peixoto CB, Caldas DM, Akiti T, Barreiros MG, de Uzeda M, Nucci M (2007) A prospective randomized trial to reduce oral *Candida* spp. colonization in patients with hyposalivation. Braz Oral Res 21:182–187
34. Bagg J, Sweeney MP, Lewis MA, Jackson MS, Coleman D, Al MA, Baxter W, McEndrick S, McHugh S (2003) High prevalence

of non-albicans yeasts and detection of anti-fungal resistance in the oral flora of patients with advanced cancer. Palliat Med 17:477–481
35. Krcmery V, Barnes AJ (2002) Non-*albicans Candida* spp. causing fungaemia: pathogenicity and antifungal resistance. J Hosp Infect 50:243–260
36. Lee I, Fishman NO, Zaoutis TE, Morales KH, Weiner MG, Synnestvedt M, Nachamkin I, Lautenbach E (2009) Risk factors for fluconazole-resistant *Candida glabrata* bloodstream infections. Arch Intern Med 169:379–383
37. Nadagir SD, Chunchanur SK, Halesh LH, Yasmeen K, Chandrasekhar MR, Patil BS (2008) Significance of isolation and drug susceptibility testing of non-*Candida albicans* species causing oropharyngeal candidiasis in HIV patients. Southeast Asian J Trop Med Public Health 39:492–495
38. Mane A, Gaikwad S, Bembalkar S, Risbud A (2012) Increased expression of virulence attributes in oral *Candida albicans* isolates from human immunodeficiency virus-positive individuals. J Med Microbiol 61:285–290
39. Ibrahim AS, Mirbod F, Filler SG, Banno Y, Cole GT, Kitajima Y, Edwards JE Jr, Nozawa Y, Ghannoum MA (1995) Evidence implicating phospholipase as a virulence factor of *Candida albicans*. Infect Immun 63:1993–1998
40. Kumar CP, Kumar SS, Menon T (2006) Phospholipase and proteinase activities of clinical isolates of *Candida* from immunocompromised patients. Mycopathologia 161:213–218
41. McNeil J, Kan V (1995) Oral yeast colonization of HIV-infected outpatients. Aids 9:301–302
42. Nishiyama Y, Inaba E, Uematsu H, Senpuku H (2010) Effects of mucosal care on oral pathogens in professional oral hygiene to the elderly. Arch Gerontol Geriatr 51:e139–e143
43. Redding SW, Zellars RC, Kirkpatrick WR, McAtee RK, Caceres MA, Fothergill AW, Lopez-Ribot JL, Bailey CW, Rinaldi MG, Patterson TF (1999) Epidemiology of oropharyngeal *Candida* colonization and infection in patients receiving radiation for head and neck cancer. J Clin Microbiol 37:3896–3900
44. Sahin I, Oksuz S, Sencan I, Gulcan A, Karabay O, Gulcan E, Yildiz O (2005) Prevalance and risk factors for yeast colonization in adult diabetic patients. Ethiop Med J 43:103–109
45. Peres-Bota D, Rodriguez-Villalobos H, Dimopoulos G, Melot C, Vincent JL (2004) Potential risk factors for infection with *Candida* spp. in critically ill patients. Clin Microbiol Infect 10:550–555
46. Zaremba ML, Daniluk T, Rozkiewicz D, Cylwik-Rokicka D, Kierklo A, Tokajuk G, Dabrowska E, Pawinska M, Klimiuk A, Stokowska W, Abdelrazek S (2006) Incidence rate of *Candida* species in the oral cavity of middle-aged and elderly subjects. Adv Med Sci 51(Suppl 1):233–236
47. Alberth M, Majoros L, Kovalecz G, Borbas E, Szegedi I, J Márton I, Kiss C (2006) Significance of oral *Candida* infections in children with cancer. Pathol Oncol Res 12:237–241
48. Lai YY, Bao L, Lu XJ, Lu J, Yu J, Li RY, Huang XJ (2009) *Candida* colonization and invasive fungal infection in hospitalized patients with hematological malignancies. Zhonghua Yi Xue Za Zhi 89: 239–242
49. Stinnett EA, Childers NK, Wright JT, Rodu BK, Bradley EL Jr (1992) The detection of oral *Candida* in pediatric leukemia patients. Pediatr Dent 14:236–239
50. Heald AE, Cox GM, Schell WA, Bartlett JA, Perfect JR (1996) Oropharyngeal yeast flora and fluconazole resistance in HIV-infected patients receiving long-term continuous versus intermittent fluconazole therapy. Aids 10:263–268
51. Qi QG, Hu T, Zhou XD (2005) Frequency, species and molecular characterization of oral *Candida* in hosts of different age in China. J Oral Pathol Med 34:352–356
52. Nawrot U, Pajaczkowska M, Wlodarczyk K, Mecler I (2010) rDNA-based genotyping of clinical isolates of *Candida albicans*. Pol J Microbiol 59:213–216