

# Identification of *candida albicans* and *nonalbicans candida* resistant species in tobacco users and oral squamous cell carcinoma patients: Comparison of HiCrome agar and automated VITEK 2 system

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## Abstract

**Introduction:** *Candida* is most common fungal pathogen in the immunocompromised and medically ill patients. Higher prevalence of *Candida albicans* has been reported in tobacco users and oral squamous cell carcinoma (OSCC) patients which may be due to immunosuppression. Recently, emergence of *nonalbicans candida* (NAC) species resistant to conventional antifungal treatment has been observed that requires accurate identification of organisms at species level for reduction of progression of suspicious oral lesions toward malignancy.

**Aims and Objectives:** To detect and compare the prevalence of *C. albicans* and NAC species smokeless tobacco chewers, histopathologically confirmed oral squamous cell carcinoma patients and the normal individuals. Effectiveness of automated Vitek 2 system in comparison to HiCrome agar color media in the identification of the candida species was also evaluated.

**Methodology:** One hundred and fifty patients (90 males, 60 females) aged between 20 and 76 years were divided into three groups: Group I individuals with habit of chewing Gutka, and betel quid/pan masala with or without tobacco, Group II individuals with clinically and histopathologically confirmed oral squamous cell carcinoma and Group III comprised of controls. Salivary samples were cultured on HiCrome agar color media and results were compared with those of Vitek 2 system in the accurate identification of candida species. Data were statistically analyzed and Chi-square test was used to estimate the effectiveness of color and Vitek method in the identification of candida species in all the three groups.  $P < 0.05$  was considered to be statistically significant.

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**Results:** HiCrome agar color method identified six candida isolates *C. albicans*, *Candida tropicalis*, *Candida krusei* and *Candida glabrata* isolates in all the three groups, with 0.00 unidentified organisms ( $P = 0.00001$ ) whereas VITEK 2 system identified five isolates of candida; *C. albicans*, *Candida famat*, *Candida ciferri*, *Candida gulleri*, *C. tropicalis*, unidentified organisms were observed in 26% of subjects. Further confirmation by supplemental tests indicated the presence of two or three organisms of different species/or subspecies with low reactivity biopattern. Higher incidence of opportunistic infections was seen in Group II OSCC patients ( $P = 0.00001$ ).

**Conclusion:** The results suggested that there is shift toward NAC species, with higher species diversity in OSCC patients followed by gutka, betel quid/pan masala with or without tobacco users. Conventional agar media culture methods of species identification should be used in conjunction with automated Vitek 2 method for better control of candida-associated oral cancer.

**Keywords:** Candida, infections, nonalbicans candida, Vitek 2

## INTRODUCTION

Candida infections are most prevalent opportunistic fungal infections encountered by oral physicians chiefly in immunocompromised and severely ill patients. Candida is fourth most common organism causing bloodstream infections, it constitutes 8% of nasocomial infections and usually shows good clinical response after antifungal therapy.<sup>[1]</sup> Recently, epidemiological data revealed that the use of topical and systemic antifungal agents (azole, polyenes) has resulted in remarkable increase of resistant nonalbicans candida (NAC) species such as *Candida tropicalis*, *Candida glabrata*, *Candida krusei*. In addition, organization of *Candida spp.* in biofilms, a protective shell, has enables them to survive under unfavorable conditions and also makes them resistant to antifungal therapy. Therefore, accurate identification of candida to the species level is essential for the establishment of etiological diagnosis and effective antifungal treatment.<sup>[2]</sup>

In India, tobacco usage has become a major public health problem and nicotine has been found to enhance candidal proliferation in oral potentially malignant lesions.<sup>[3]</sup> The possible association between oral cancer and *candida spp.* was first reported in 1969 by Cawson and Williamson.<sup>[4,5]</sup> Candida may promote carcinogenesis by producing carcinogenic compounds chiefly nitrosamines that bind with deoxyribonucleic acid (DNA) to form adducts with bases, phosphate residues or hydrogen binding sites leading to irregularities in DNA replication. These DNA mutations activate specific oncogenes that initiate the development of carcinogenesis. It was also proposed that ability of *Candida spp.* to catalyze the formation of potent carcinogen, N-nitrosobenzylmethylamine (NMBA) is strain dependent, i.e., strains with highest potential to form NMBA were isolated from advanced potentially malignant disorders such as speckled or nonhomogeneous, thick homogeneous

leukoplakia in comparison to early thin homogeneous leukoplakia.<sup>[6]</sup> *Candida albicans* is the predominant organism, but recently increase in incidence and diversity of candida species and emergence of fluconazole-resistant NAC species has challenged the clinical laboratories to develop more reliable and rapid candida identification methods based on various commercial systems utilizing mainly biochemical, morphological and physiological characteristics.<sup>[2]</sup> Isolation and culture of candida on the agar medium, Sabouraud Dextrose Agar or on differential culture media, chromogenic media, does not usually allow species identification, so to overcome the limitation of conventional culture media identification procedures, several automated approaches have been introduced that identifies and differentiates candida species accurately and reduces the frequency of low or misidentification of microorganisms. In this context, molecular biological techniques such as polymerase chain reaction, random amplified polymorphic DNA, DNA sequence analysis and mitochondrial large subunit ribosomal RNA gene sequencing have shown high specificity and sensitivity in species identification but these techniques are expensive and time consuming.<sup>[7]</sup>

Automated Vitek 2 YST (colorimetric) system has been introduced for the identification and susceptibility testing of the candida organisms. Meurmann O *et al.*<sup>[8]</sup> and Graf B *et al.*<sup>[9]</sup> have suggested that Vitek 2 system identified all isolates of *C. albicans*, i.e., *C. glabrata*, *C. krusei*, *C. dubliniensis* correctly. It comprises yeast identification cards (YST cards) that are incubated and interpreted automatically.<sup>[8,9]</sup> Thus, Agar culture media in conjunction with Vitek 2 system has proven to be a valuable, easy to perform and accurate method for candida species identification in clinical laboratories. The aim of the present study was to detect and compare the prevalence of *C. albicans* and nonalbicans candida species in individuals with habit of chewing gutka, betel quid/pan masala with or without

tobacco and clinically and histopathologically confirmed oral squamous cell carcinoma (OSCC) in relation to normal individuals with the absence of any adverse habits. Effectiveness of automated Vitek 2 system in the candida species identification in comparison to HiCrome agar color media was also evaluated.

## METHODOLOGY

The case-control study was conducted on 150 patients (86 males, 64 females) aged between 20 and 76 years attending the outpatient Department of Oral Medicine and Radiology in M. S. Ramaiah Dental College and Hospital, Bangalore, in the period from June 2013 to August 2014. Ethical clearance was obtained from the ethical review committee of the institute (MSRDC/EC/2013-14). Sample size was estimated using power analysis PASS software version 11.0, Kaysville, Utah, United States with the power of 90% and confidence interval of 95% sample size obtained was 50 in each of three groups of subjects. The study included individuals with adverse habit of smokeless tobacco usage, with clinically diagnosed and histopathologically confirmed OSCC as cases and individuals reported to have no deleterious habit were kept as controls. Individuals diagnosed with acquired immunodeficiency syndrome, type II diabetes mellitus with history of tobacco use, and on drugs such as antibiotics (for at least 5 days), antipsychotics, immunosuppressant's, prolonged corticosteroids, anticholinergics were excluded. Written informed consent was obtained from all 150 subjects and was subjected to thorough clinical examination. Based on habit history and clinical examination subjects were divided into three groups, Group I individuals with habit of chewing gutka and pan masala/betel quid with or without tobacco, Group II individuals with clinically and histopathologically confirmed OSCC and Group III comprised of controls with absence of any deleterious habit.

### Collection of saliva samples

After history and thorough clinical examination, all the patients were asked to swish the oral cavity with 10 ml of 7.2 pH buffer (alkaline phosphate) for 1 min and then spit it out in sterile uricol container. Unstimulated whole saliva samples were collected from patients in Group I and Group III and after histopathological confirmation of OSCC in Group II patients. The collected salivary samples were processed within 3 h.

### Preparation and isolation of candida colonies on a HiCrome agar media

Dehydrated HiCrome agar was mixed with distilled

water (42.72 g in 1000 ml of distilled water) and was heated to dissolve the agar media (Peptone, Yeast extract, Dipotassium hydrogen phosphate, Chromogenic mixture, Chloramphenicol, Agar). After this, it was allowed to cool up to 50°C and was poured into sterilized petridishes. The collected unstimulated saliva was admixed with 7.2 pH alkaline phosphate buffer, transferred into the labeled disposable test tube and was centrifuged at 3000 rpm for 10 min. The supernatant formed following centrifugation was discarded and the pellet left behind was streaked onto the petridishes. These petridishes were kept in the incubator at the temperature of 37°C for 48 h, allowing the *Candida* to grow on the petridishes. All the petridishes positive for the growth of *Candida* species were taken to The Elbit Laboratory, Bangalore, for the further identification of subspecies of *Candida* [Figure 1a-c].

### Identification of *Candida* species by VITEK 2 compact test

A sterile swab or applicator stick was used to transfer a sufficient number of colonies of a pure culture and to suspend them in 3.0 mL of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5-7.0) in a 12 mm × 75 mm clear plastic (polystyrene) test tube. The turbidity was adjusted to 1.8-2.2 McFarland turbidity range and measured using a turbidity meter called the DensiChek.<sup>TM</sup>

Identification cards were inoculated with microorganism suspensions using an integrated vacuum apparatus. A test tube containing the microorganism suspension was placed into a special rack (cassette), and the identification card was placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube. The filled



**Figure 1:** (a) HiCrome differential Color Agar Media (b) Isolated *Candida* Colonies on Petridish (c) Centrifugation machine

cassette was placed manually into a vacuum chamber station. After the vacuum was applied, the microorganism suspension was forced through the transfer tube into microchannels that filled all the test wells. Inoculated cards were sealed and incubated online at  $35.5^{\circ}\text{C} \pm 1.0^{\circ}\text{C}$ . Each card was removed from the carousel incubator once every 15 min, transported to the optical system for reaction readings and then returned to the incubator until the next read time. Data were collected at 15-min intervals during the entire incubation period [Figure 2].<sup>[8]</sup>

### INTERPRETATION OF TEST REACTIONS AND ANALYSIS OF RESULTS

A transmittance optical system analyzed test reactions using different wavelengths in the visible spectrum. During incubation, each test reaction was read every 15 min to measure either turbidity or colored products of substrate metabolism. In addition, a special algorithm was used to eliminate false readings due to small bubbles that may be present. The final test reaction results, i.e., biopattern of an unknown organism was compared with the Vitek database of reactions for each taxon and numerical probability calculation was performed. Four result categories were obtained as follows: (i) correct identification (to the species level), (ii) low level of discrimination (either identification to the genus level or low level of discrimination between two or more species, including the correct species), (iii) un identification and (iv) misidentification (the species identified was different from that identified by the reference method).<sup>[8,9]</sup> Various qualitative levels of identification were assigned based on the numerical probability calculation.

Identification results may also be interpreted as “Nonreactive bio-pattern” for an unknown organism. If one encounters a nonreactive bio-pattern, a note will appear that states: “Organism with low reactivity bio-pattern – please check viability.”<sup>[9]</sup> Mixed taxon identification occurs when the biopattern is representative of a collective taxon and generates a genus-level, group-level or slash line

identification. In rare cases, species-level identification can be a mixed taxon comprising two subspecies. In such situations, two or three organisms listed as viable choices in order of their probability calculations are subjected to additional tests such as fermentation, assimilation of trehalose, cellobiose, nitrate, and lactate, phenol oxidase production, Wickerham method and microscopic morphology may be used to delineate representative species or subspecies of these collective taxon.<sup>[8,9]</sup>

### Statistical analysis

Data obtained was analyzed with the Statistical Package for the Social Service version 21 software (SPSS Inc., Chicago, IL, USA). Chi square test was used to estimate the effectiveness of color and VITEK method in the identification of candida species prevalent in the three groups. Intergroup comparison for the presence of different species was also done by Chi Square test. Results were measured as mean  $\pm$  standard deviation.  $P < 0.05$  was considered to be statistically significant.

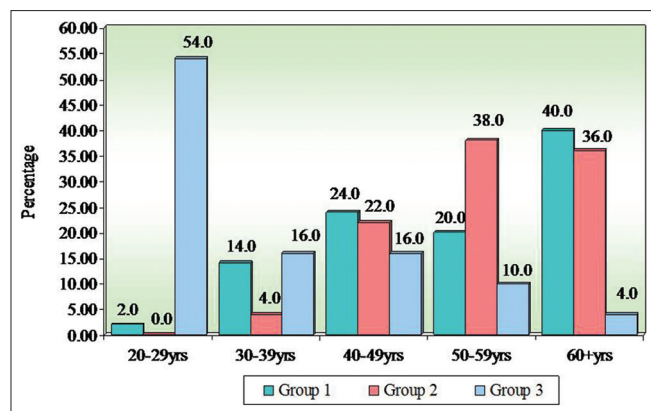
### RESULTS

Total 150 subjects (90 males and 60 females) with mean age  $47.25 \pm 15.07$  years divided into three groups (30 males and 20 females in each group) after history and clinical examination based on inclusion criteria. Mean age of Group I patients was  $53.90 \pm 12.91$  years, for Group II, it was  $54.60 \pm 7.82$  and it was  $33.20 \pm 12.65$  years for Group III, Maximum individuals were betel quid chewers (51.33%), 42 subjects in Group I and 35 in Group II used to chew betel quid in comparison to other adverse agents, i.e., gutka (4.67%), panmasala (2.67%), betel quid/panmasala with tobacco (8.00%) [Figures 3 and 4]. When frequency and duration of chewing were assessed, oral SCC was seen more in patients (Group II) who chewed 6–10 times/day for more than 16 years. In our study, it was observed that the incidence of OSCC was less in subjects who chewed  $>11$  times/day which suggested that duration of chewing has profound impact on progression to malignancy.

Six types of candida species; *C. albicans*, *Candida famata*, *Candida ciferri*, *C. tropicalis*, *Candida gulleri*, *C. krusei*, *Candida glabrata* were identified. The distribution of candida species; *C. albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata* was 2 (4%), 7 (14%), 4 (8%), 1 (2%) in Group I, 20.8 (40%), 7 (14%), 34 (68%), 14 (28%) in Group II, and 6 (12%), 2 (4%), 1 (2%), 2 (4%) in Group III subjects ( $P = 0.00001$ ), that was highly significant and none of the subjects showed isolates of *C. famata*, *C. ciferri*, *C. gulleri*. Thus, Color Media identified all the species correctly with “good



**Figure 2:** Automated Vitek 2 Compact System and YST 2 identification cards

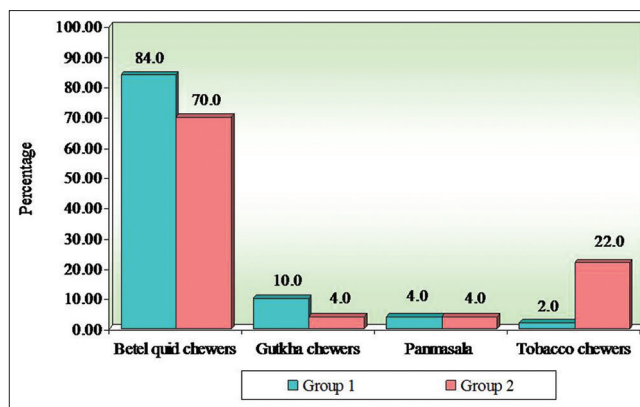


**Figure 3:** Distribution of samples according to age groups in three groups

identification,” and final number of unidentified organisms was almost 0.00. On intergroup comparison, statistically significant results were obtained for species identification; *C. albicans*; Group 1 versus Group 2 ( $P = 0.0019$ ), Group 2 versus Group 3 ( $P = 0.0158$ ), *C. krusei*; Group 1 versus Group 2 ( $P = 0.00001$ ), Group 2 versus Group 3 ( $P = 0.00001$ ) and *C. glabrata* Group 1 versus Group 2 ( $P = 0.0251$ ). HiCrome agar color method showed higher incidence of opportunistic *C. albicans*, *C. tropicalis*, *C. krusei* and *C. glabrata* isolates in Group II OSCC patients in comparison to Group I and Group III [Tables 1 and 2].

Vitek 2 system identified five isolates of candida; *C. albicans* 1 (2%) subjects in Group II, *C. famata* 4 (8%), 9 (18%), 2 (4%) in Groups I, II and III, respectively, *C. zifferri* 1 (2%), 5 (10%) in Group II and GROUP III, respectively, *C. galleri*, *C. tropicalis* 1 (2%) in Group III but number of unidentified organisms were seen in 39 (26%) subjects, i.e., 10 in Group I, 23 in Group II and 6 in Group III that indicates they did not belong to single species [Table 3]. On intergroup comparison also, Vitek system showed low discrimination results which may be due to technical errors or missing reaction, this necessitates supplemental or repeated tests for final identification [Table 4]. Similar results were seen on repeated Vitek testing that excluded the possibility of technical error or missing reaction. Final differentiation of strains done by supplemental tests indicated the presence of two or three strains of different species/or subspecies of low reactivity biopattern that resulted in false-negative biochemical reaction.

These findings highly suggested that low discrimination is the highest level of identification that can be achieved by Vitek 2 system, if required, supplemental tests could be used for delineation of closely related Candida species in a mixed taxon in comparison to conventional culture media. When Vitek and color method was compared for three groups,



**Figure 4:** Distribution of samples according to habits in Group I and Group II

statistically significant results for species identification were seen for Group II patients ( $P < 0.05$ ) [Table 5].

## DISCUSSION

Incidence of Oral Candida infections has been reported to increase remarkably, especially in immunocompromised patients. Candida species are common oral commensals both in normal and medically compromised individuals, and their transition to opportunistic infection depends upon virulence of organism and the host defense.<sup>[2]</sup> Candida can invade and penetrate the epithelium by two different mechanisms. It can cause degradation of the epithelial surfaces by secretion of aspartic proteases to allow physical movement of fungal hyphae into, or between, host cells. Second, it can induce epithelial cell endocytosis by stimulating keratinocytes to produce pseudopod like structures that surround the fungus and draws it into the cell. Recently, it has been proposed that keratinocytes type and stage of differentiation also influence the susceptibility of epithelial cell surfaces to the fungus.<sup>[6]</sup> Studies have reported that oral Candida colonization is more common in tobacco users and opportunistic infections occur frequently in cancer patients due to inherent immunosuppression and use of cytotoxic drugs.<sup>[10,11]</sup>

In the literature, several hypotheses have been proposed for increased candida colonization in tobacco users. It has been stated that tobacco constituents (nicotine, nitrosoproline, nitrosodiethelamine, polycyclic aromatic hydrocarbons and polonium) lead to decrease in salivary immunoglobulin A and suppress the function of polymorphonuclear leukocytes, thus providing nutritious media for candida proliferation. Nicotine has been found to trigger liver to release glycogen into the bloodstream thereby increasing blood glucose levels that provide favorable media for growth of candida species. It also has been hypothesized that smoke form of tobacco promotes adhesion, growth

**Table 1: Comparison of three groups (I, II, III) with the presence of different species by color method**

Species	Present (%)				$\chi^2$	P
	Group I	Group II	Group III	Total		
<i>Candida albicans</i>	2 (4.00)	20 (40.00)	6 (12.00)	28 (18.67)	23.3790	0.00001*
<i>Candida famata</i>	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0.0000	1.0000
<i>Candida ciferrii</i>	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0.0000	1.0000
<i>Candida tropicalis</i>	7 (14.00)	7 (14.00)	2 (4.00)	16 (10.67)	3.4750	0.1760
<i>Candida gulleri</i>	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0.0000	1.0000
<i>Candida krusei</i>	4 (8.00)	34 (68.00)	1 (2.00)	39 (26.00)	68.7690	0.00001*
<i>Candida glabrata</i>	1 (2.00)	14 (28.00)	2 (4.00)	17 (11.33)	20.6930	0.00001*
Un identified	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	-	-

\*Statistically significant

**Table 2: Pairwise comparison of three groups with presence of different species by color method**

Species	Group I versus Group II (P)	Group I versus Group III (P)	Group II versus Group III (P)
<i>Candida albicans</i>	0.0019*	0.4906	0.0158*
<i>Candida famata</i>	1.0000	1.0000	1.0000
<i>Candida ciferrii</i>	1.0000	1.0000	1.0000
<i>Candida tropicalis</i>	1.0000	0.3888	0.3888
<i>Candida gulleri</i>	1.0000	1.0000	1.0000
<i>Candida krusei</i>	0.00001*	0.6051	0.00001*
<i>Candida glabrata</i>	0.0251*	0.8632	0.0386
Un identified	1.0000	1.0000	1.0000

\*Statistically significant

and biofilm formation of *C. albicans*.<sup>5,6]</sup> Similarly, in this study, higher candidal proliferation and prevalence of NCA species resistant to conventional antifungal treatment were observed in Group II OSCC patients ( $P = 0.0001$ ). Tao *et al.* explained the phenomenon of “white-opaque” transition denoted as bistable phenotypic switching system in *C. albicans*. White and opaque cells differ in morphology that is white cells form small, round and shiny colonies on solid media whereas opaque cells form flatter and rougher colonies. Moreover, white and opaque phenotypes differ in gene expression profiles, mating competency and virulence characteristics. According to hypothesis, *C. tropicalis* and *C. dubliniensis* are two fungal pathogens closely related to *C. albicans* which can undergo phenotypic switching and intermediate phenotypes are observed during high temperature transitions. They discovered novel morphological “gray” phenotype of *C. albicans* which along with white and opaque phenotype forms a tristable heritable switching system in *C. albicans*. Therefore, this phenomenon may be a pathogenic mechanism for transition between commensal and pathogenic variants of *C. albicans* and emergence of NCA species in tobacco users.<sup>12]</sup>

*C. albicans* genotype A strain is most commonly isolated from oral cancer patients but simultaneous presence of NCA strains is significant risk factors for progression of malignancy due to resistance to antifungal therapies.<sup>11]</sup> Therefore, for effective definitive treatment, identification of candida at species level has become important. Conventional

identification methods are still considered as a gold standard, but due to emergence of NCA resistant strains, there is a need for rapid and more accurate methods for yeast identification.<sup>[13,14]</sup> To the above consideration, in this study, automated Vitek 2 system was compared with conventional HiCrome agar media in the identification of candida species. Color media identified six *Candida* isolates and number of unidentified organisms was almost 0.00. Group II patients showed that higher prevalence of *C. albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata* was 20.8 (40%), 7 (14%), 34 (68%), 14 (28%) than Group I and Group III individuals. In contrast to above, Vitek 2 methods identified five candida isolates and the number of unidentified organisms was seen in 39 (26%) of subjects. Further identification by supplementary tests suggested the presence of two or three organisms of different species with low reactive biopattern.

When Vitek and color method was compared for three groups, more species variation and transition were observed in Group II patients, followed by Group I and Group III normal individuals ( $P = 0.00001$ ). Such species diversity may be due to altered host immune responses in oral cancer patients.<sup>[8]</sup> Vitek 2 system was able to differentiate between the closely related *Candida* species and offers rapid and accurate identification within 15 h in comparison to color media. Previous literature studies have well documented the reliability of molecular and automated systems in identification and differentiation of close taxon of the genus candida.<sup>[8,9,15]</sup> Esmat *et al.* found that sensitivity of Vitek 2 with regard to correct identification of *Candida* species was 96%; and specificity was 100%, which was slightly more than CHROM agar that showed sensitivity and specificity of 89% and 100%, respectively.<sup>[7]</sup> They suggested that when both a HiCrome agar and Vitek 2 were used, sensitivity of strain detection increased from 93% to 97% for *C. albicans* and up to 100% for some of *Candida* species. Thus, Vitek 2 could be an additional help in those situations where CHROM agar could not give correct identification.<sup>[3]</sup> In another study by Meurman *et al.*, it was concluded that Vitek 2 system in comparison with conventional assimilation, fermentation and morphological methods has ability to identify yeast

**Table 3: Comparison of three groups (I, II, III) with the presence of different species by Vitek 2 method**

Species	Present (%)				$\chi^2$	P
	Group I	Group II	Group III	Total		
<i>Candida albicans</i>	0 (0.00)	1 (2.00)	0 (0.00)	1 (0.67)	2.0000	0.3680
<i>Candida famata</i>	4 (8.00)	9 (18.00)	2 (4.00)	15 (10.00)	5.7390	0.0570
<i>Candida ciferrii</i>	1 (2.00)	5 (10.00)	0 (0.00)	6 (4.00)	7.2430	0.0270*
<i>Candida tropicalis</i>	0 (0.00)	0 (0.00)	1 (2.00)	1 (0.67)	2.0000	0.3680
<i>Candida gulleri</i>	0 (0.00)	0 (0.00)	1 (2.00)	1 (0.67)	2.0000	0.3680
<i>Candida krusei</i>	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0.0000	1.0000
<i>Candida glabrata</i>	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0.0000	1.0000
Un identified	10 (20.00)	23 (46.00)	6 (12.00)	39 (26.00)	16.4240	0.0002*

\*Statistically significant

**Table 4: Pairwise comparison of three groups with presence of different species by Vitek 2 method**

Species	Group I versus		
	Group II (P)	Group III (P)	Group II versus Group III (P)
<i>Candida albicans</i>	0.8632	1.0000	0.8632
<i>Candida famata</i>	0.3888	0.7303	0.2277
<i>Candida ciferrii</i>	0.4906	0.8632	0.3888
<i>Candida tropicalis</i>	1.0000	0.8632	0.8632
<i>Candida gulleri</i>	1.0000	0.8632	0.8632
<i>Candida krusei</i>	1.0000	1.0000	1.0000
<i>Candida glabrata</i>	1.0000	1.0000	1.0000
Un identified	0.0250*	0.4905	0.0034*

\*Statistically significant

**Table 5: Comparison of color and Vitek 2 method in the assessment of species in three groups**

Species	Color and Vitek method					
	Group I		Group II		Group III	
	Z	P	Z	P	Z	P
<i>Candida albicans</i>	0.0000	1.0000	3.6322	0.0003*	2.2014	0.0277*
<i>Candida famata</i>	1.8257	0.0679	2.6656	0.0077*	0.0000	1.0000
<i>Candida ciferrii</i>	0.0000	1.0000	2.0226	0.0431*	0.0000	1.0000
<i>Candida tropicalis</i>	2.3664	0.0180*	2.3664	0.0180*	0.5345	0.5930
<i>Candida gulleri</i>	0.0000	1.0000	0.0000	1.0000	0.0000	1.0000
<i>Candida krusei</i>	1.8257	0.0679	5.0862	0.0001*	0.0000	1.0000
<i>Candida glabrata</i>	0.0000	1.0000	3.2958	0.0010*	0.0000	1.0000

\*Statistically significant

isolates from among 151 clinical specimens and 16 known type culture or quality control strains. All isolates of *C. albicans*, *C. glabrata* and *C. krusei* were identified correctly.<sup>[8]</sup>

In the present study, *C. albicans* was most common species identified in all the three groups by both methods and most common NAC species as identified by color media was *C. tropicalis* 7 (14%) in Group I and Group III 2 (4%), *C. krusei* 34 (68%) showed higher prevalence in Group II patients. Vitek 2 system found higher percentage of *C. famata* 9 (18%) in Group II and *C. ciferrii* 5 (10%) in Group III subjects. Our results are in accordance with Belazi *et al.*,<sup>[16]</sup> and Redding *et al.*<sup>[17]</sup> who found that the prevalence of NCA species increased by 12%–30% in oral cancer patients and *C. tropicalis* (42.85%) was the most common NAC species isolated.

## CONCLUSION

The results of our analysis suggested that pattern of candida species infection is changing, with higher prevalence of NAC strains in tobacco users and oral cancer patients. Conventional methods of species identification should be used in conjunction with automated Vitek 2 method for better prevention and control of candida-associated oral cancer. Future demands more clinical trials to confirm the efficacy of new automated Vitek 2 compact system in Candida identification to species level.

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## Conflicts of interest

There are no conflicts of interest.

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