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# Oral cavity infection: an adverse effect after the treatment of oral cancer in aged individuals

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## ABSTRACT

Objective: The immune compromised patients after treatment of oral cancer may have a chance of infection by drug-resistant opportunistic microbes. We investigated the occurrence of opportunistic microorganisms in aged individuals receiving follow-up examinations after treatment of oral cancer in China. Material and Methods: These patients were used as test group and the respective age grouped healthy individuals as control group. In this study, the oral cavity microorganisms such as bacteria and yeast were taken for the analysis. After the screening of representative microorganisms, their aptitude of pervasiveness against drugs was studied. Here, we used antimicrobial agents which are common in clinical practice. We also performed studies to investigate the presence of toxin genes in methicillin-resistant *S. aureus* (MRSA). Results: The results indicate that the prevalence of drug-resistant microbes was more pronounced in oral cancer patients after initial treatment above 70 years old. The oxacillin resistance of *S. aureus* isolate confirms that the prevalence of MRSA is increasing in accordance to age-factor and immune compromise in elderly patients. Conclusions: This study reveals the occurrence of drug-resistant opportunistic microorganisms in oral cavity after treatment for oral cancer in aged individuals. Special attention should be directed to MRSA during the treatment of oral cancer, and to realize the fact of immune compromise in elderly patients.

**Keywords:** Oral cancer. Dental infection control. Drug resistance. Opportunistic infections. Prevention and control.

## INTRODUCTION

The term oral cavity refers to lips, buccal mucosa, alveolar ridges, retro molar trigone, hard palate, floor of the mouth and anterior two-thirds of the tongue. Oral cancer or oral cavity cancer, a subtype of head and neck cancer, is any cancerous tissue growth located in the oral cavity[24]. The most common oral cancer is squamous cell carcinoma (SCC) that affects the tissue lining of the oral cavity[5]. Oral cancer is the eleventh most common cancer in the world with an estimated 267,000 cases and 128,000 deaths in around 2000, two-third of which occur in developing countries. The incidence of oral cancer is increasing in several parts of the world, particularly in Australia, Japan and parts of Europe. Oro-pharyngeal cancer is a significant part of the global burden of cancer. Oral cancer occurrence is particularly high in males. Incidence rates for oral cancer vary in men from 1 to 10 cases *per* 100,000 populations in many countries. Tobacco and alcohol are regarded as the major causes of oral cancer[10].

The immune compromised patients after treatment of oral cancer may have a chance of infection by drug-resistant opportunistic microbes. Such microbes that originate in the oral cavity, representative microorganisms include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*[1,14]. *Pseudomonas aeruginosa* is increasingly recognized as an emerging opportunistic pathogen of clinical relevance. One of the most worrying characteristics of *P. aeruginosa* is its low antibiotic susceptibility[23]. This low susceptibility is attributable to a concerted action of multidrug efflux pumps with chromosomally

encoded antibiotic resistance genes[15]. Most of these organisms have become drug-resistant, which has resulted in difficulties in curing the related infectious diseases. The *Candida* species educe the infectious disease candidiasis, which causes infections such as oral thrush and vaginitis as well as life-threatening diseases, known as candidemia[16]. *C. albicans* are yeast that normally inhabits the human mouth and skin, where it generally uneventfully coexists with a variety of other microorganisms. An infection occurs when the balance of bacteria in the body is disrupted, especially in immunocompromised situations, allowing drug-resistant *Candida* species to proliferate and overcome other healthy microorganisms[11]. Immunocompromised situations are frequently seen in older individuals, infants, people infected with HIV, and individuals with cancer; oral cancer can reduce immunity in the maxillofacial region[1,14]. The principal treatments for oral cancer are surgical excision, radiotherapy, and chemotherapy, employed alone or in combination[13].

Currently, the systemic applications of antibacterial drugs have shown better results on curing diseases than local application, which could induce drug-resistant bacteria in the particular area[2,3]. In this study, we have investigated the reason behind the frequent cause of oral infection after the treatment of oral cancer in elderly Chinese.

## MATERIAL AND METHODS

### Patient study

Out of several elderly patients (60–95 years old), 128 patients who had undergone treatment for oral-cavity related problems participated in the study at Ninth People's Hospital, Shanghai Jiao Tong University. We have excluded patients with other systemic diseases like autoimmune disease or diabetes to avoid misleading of our parameter. The participants were divided into two groups: Group I - the patients undergone oral cancer treatment (n=93; 41 men, 52 women; average age 68.1±8.3 years) ranging from 1 month to 7 years after the initial treatment, 43 members were involved in species identification, remaining 50 members involved in drug-resistant test. These patients have been treated with surgery, chemo and/or radiotherapy; Group II - the control (n=35; 15 men, 20 women; average age 70.2±10.1 years), who had received treatment for oral cavities or with no history of any cancer treatment. The Institution Review Board of Affiliated Ninth People's Hospital of Shanghai Jiao Tong University, according to Helsinki Declaration II, approved the study. Written informed consent was obtained from each participant.

### Sample collection

Microbes were collected from the areas of surgery (Group I), tongue, gingiva, and palate by using sterilized dry cotton swabs. After wetting, the cotton swab was immediately put into an airtight sterilized test tube. To collect anaerobic microbes samples, syringe was used to collect fluid under the deep area of the incision. Collected samples were immediately put into an anaerobic sample collection flask for cultivations. About 1 mL of saliva samples was collected from each subject for the analysis.

### Microbial cultivation

The microorganisms were cultured on nalidixic acid/cetrimide agar (Sigma-Aldrich Chemie GmbH, Switzerland) for the first screening of *Pseudomonas* species, while Mannital Salt agar (Acumedia Manufacturers, USA) was used for the first screening of *Staphylococcus* species and Brilliance *Candida* Agar (Thermo Fisher Scientific Inc.) was used for the first screening of *Candida* species. The nalidixic acid/cetrimide agar and Mannital Salt agar plates were incubated at 37°C under aerobic conditions for 2 days, while *Candida*-GS agar plates were incubated at 30°C for 3 days.

### Microbial species detection and antimicrobial testing

The morphological assessment was carried out from the colonies on the plates, and those with different shapes were collected, gram stained and observed under a light microscope

| S.No. | Antibiotics | Sensitivity dose | Resistance dose |
|---|---|---|---|
| | | (µg/mL) | (µg/mL) |
| 1 | Oxacillin | <0.25 | >2 |
| 2 | Arbekacin | <4 | >8 |
| 3 | Vancomycin | <2 | >8 |
| 4 | Teicoplanin | <8 | >16 |
| 5 | Linezolid | <2 | >4 |
| 6 | Fluconazole | <8 | >64 |
| 7 | 5-fluorocytosine | <4 | >32 |
| 8 | Itraconazole | <0.125 | >1 |
| 9 | Miconazole | <0.5 | >1 |
| 10 | Amphotericin B | <1 | >2 |
| 11 | Voriconazole | <1 | >4 |
| 12 | Micafungin | <1 | >2 |
| 13 | Imipenem/ cilastatin | <16 | >16 |
| 14 | Amikacin | <32 | >32 |
| 15 | Ciprofloxacin | <4 | >4 |

**Figure 1-** Antibiotics used to test the antimicrobial activity

for species detection. Genomic DNA from each colony was obtained using a Wizard genomic extraction kit (Promega, Madison, WI, USA). For *Staphylococcus* species, cells were treated with 1 mg/mL of lysostaphin in Tris-EDTA buffer (TE; 10 mM Tris-Cl, 1 mM EDTA, pH 8.0) at 37°C for 1 h before using the kit. Final identification was done by polymerase chain reaction (PCR) amplification with bacterial universal primers for 16S rDNA and 26S rDNA for fungus, followed by DNA sequencing. DNA sequencing was performed using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Tokyo, Japan) with a Big Dye Cycle Sequencing reaction kit (AB Applied Biosystems). Identification of experimentally determined nucleotide sequences using sequence databases was performed by Basic Local Alignment Search Tool (BLAST). The antimicrobial activity was tested with respective antibiotics used commonly in clinical practice (Figure 1).

### Statistical analysis

Statistical analyses were conducted using SPSS 15.0 (SPSS Inc., U.S.A.) and any differences at $p<0.05$ level were considered as statistically significant. The samples of respective parameters were compared using independent student t-test.

## RESULTS

### Identification of microorganisms

The samples were collected from surgical scar, saliva, gingiva, and palate of 78 elderly Chinese (43 cases of group I and 35 cases of group II). The majority of identified microorganisms were *Candida*, *Staphylococcus* and *Pseudomonas* species. The graphical representation for the percentage of species identification from one species in one isolates or two species in same isolates were presented in two sets of group I & II in each species (Figure 2). Statistical analysis revealed no significant difference between groups in the number of isolated microorganisms or the

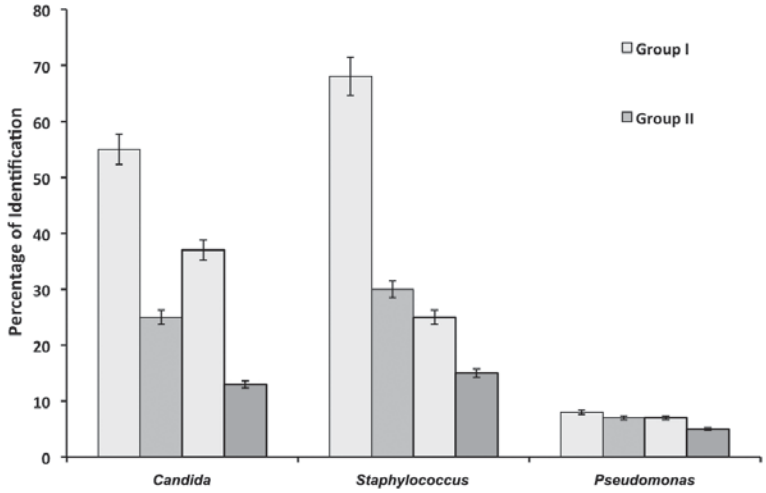


**Figure 2-** Graphical representation of the percentage of species identification, identified as one species in one isolates or two species in same isolates were presented in two sets of group I & II in each species

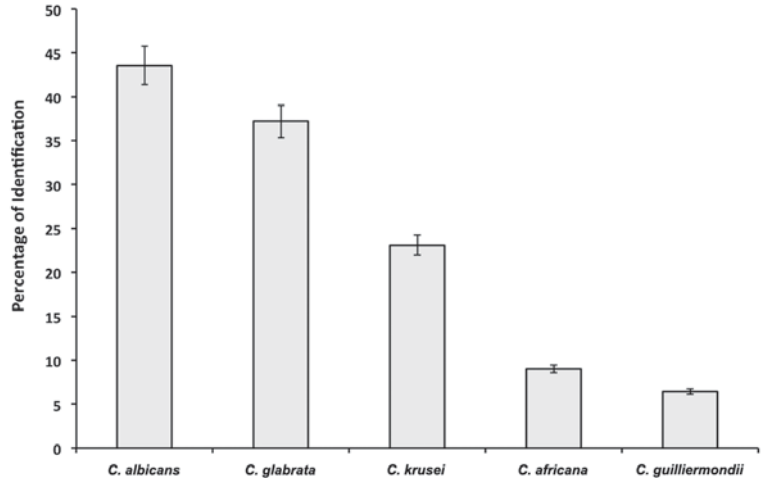


**Figure 3-** Graphical representation of the overall percentage of identified Candida species

number of participants with these isolates.

### *Candida* species

The universal primers for fungi, 26S rDNA sequencing was used to identify the *Candida* species (Figure 3). The *Candida* species isolated from 78 participants (both groups) are identified as *C. albicans* (43.6%), *C. glabrata* (37.2%), *C. krusei* (23.1%), *C. africana* (9%) *and C. guilliermondii* (6.4%). The number of *Candida* species isolated from group I were greater than that of group II. On comparison between the groups, the *C. albicans* were found to be the dominant species in both group I (55.8%) and group II (28.6%). However, there is a significant difference ($p<0.05$) found between groups. Similarly, *C. glabrata* were found to be the next dominant species with significant difference between group I (48.8%) and group II (22.8%) participants (Table 1). However, no significant difference was found for *C. africana and C. guilliermondii* species, rather very less percentage of the species were identified in both groups (Table 1).

### Bacterial species

The universal primers for bacteria, 16S rDNA sequencing was used for species identification (Figure 4). The bacterial species isolated from the 78 participants (both groups) are identified as *Staphylococcus* (59%), *Pseudomonas* (53.8%), *Streptococcus* (44%), *Neisseria* (37.2%), *Actinomyces* (29.5%) and *Veillonella* (25.6), which are mostly found in the surgical scar and saliva. On comparison between two groups of isolates, the *Streptococcus*, *Staphylococcus*, *Pseudomonas* and *Neisseria* showed significant differences ($p<0.05$) in percentage of these species between groups I and II (Table 2). In Group I, the numbers of positive isolates of bacterial species were greater than that of the control group using traditional oral care methods. For *Veillonella* and *Actinomyces*, no significant differences were shown between the two groups (Table 2).

### Occurrence of microorganism against respective antimicrobial agents

The species identification reveals that there are higher percentages of dominant microorganisms found in group I than those of group II. To investigate

**Table 1-** Identification of *Candida* species and their percentage of occurrence

| ***Candida* Species Identified** | **Group I (n=43)** | | **Group II (n=35)** | |
|---|---|---|---|---|
| | **No. of positive samples** | **Percentage (%)** | **No. of positive samples** | **Percentage (%)** |
| *C. albicans* | 24 | 55.8 | 10 | 28.6* |
| *C. glabrata* | 21 | 48.8 | 8 | 22.8* |
| *C. krusei* | 12 | 27.9 | 6 | 17.1 |
| *C. guilliermondii* | 4 | 9.3 | 1 | 2.8 |
| *C. africana* | 5 | 11.6 | 2 | 5.7 |

*There were significant differences between the two groups ($p<0.05$)

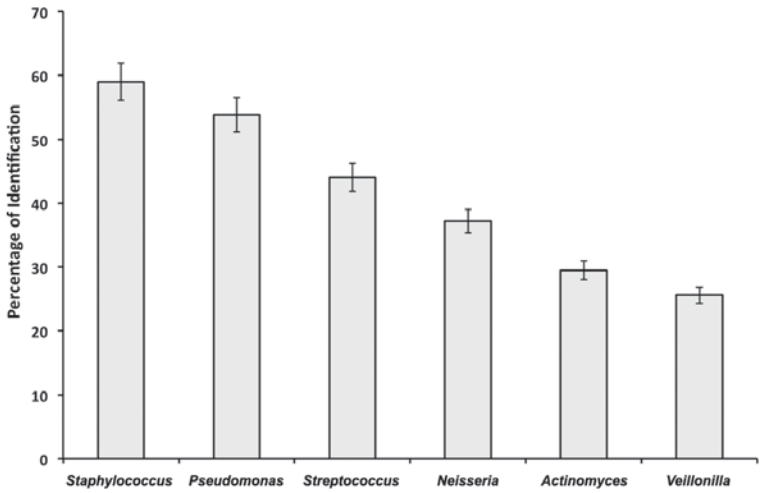


**Figure 4-** Graphical representation of the overall percentage of identified bacterial species

the reason behind this dominant character, we studied the occurrence of microorganisms against the antimicrobial agents by categorizing the remaining group I participants (50 cases) with their history of treatment with commonly practiced antimicrobial agents (Table 1) after the treatment for oral cancer. These 50 participants were divided as 25 cases for the study against antifungal agents and remaining 25 against antibacterial agents.

The participants after the treatment of oral cancer were taking commonly practiced antifungal agents such as Itraconazole, Miconazole, Fluconazole, 5-fluorocytosine, Amphotericin B, Voriconazole and Micafungin. The isolates were tested for the presence of *Candida* species. Except for the *C. glabrata* and *C. krusei* species, all other species were susceptible to the antifungal agents. Interestingly, Itraconazole had 100% resistance by *C. glabrata* and 60% resistance by *C. krusei* species. The *C. glabrata* showed resistance to Miconazole (20%), Fluconazole (20%), 5 fluorocytosine (8%). However, *C. krusei* showed 44%, 32% and 28% of resistance respectively. The other drugs were susceptible to all *Candida* species (Table 3).

The antibacterial agents were tested for the drug-resistant activity of staphylococci subspecies, particularly on *methicillin-resistant Staphylococcus aureus* (MRSA), *S. epidermidis* and *S. haemolyticus*. The tested agents are commonly practiced antibiotics such as Oxacillin, Arbekacin, Vancomycin, Teicoplanin, Linezolid, Imipenem/cilastatin, Amikacin and Ciprofloxacin. Surprisingly, MRSA showed drug-resistance to almost all antibiotics with variation in percentage of resistance. The MRSA showed the highest percentage of resistance to Oxacillin (96%) and Arbekacin. However, the lowest percentage (4%) of resistance was shown in relation to Amikacin and Ciproflaxacin (Table 4). The other species, *S. epidermidis* and *S. haemolyticus,* showed resistance to Oxacillin with 60 and 40%, respectively. However, *S. haemolyticus* showed the lowest percentage (4%) of resistance in relation to Arbekacin. All other antibiotics were found susceptible to these species.

**Table 2-** Identification of bacterial species and their percentage of occurrence

| Bacterial Species Identified | Group I (n=43) | | Group II (n=35) | |
|---|---|---|---|---|
| | No. of positive samples | Percentage (%) | No. of positive samples | Percentage (%) |
| *Streptococcus* | 24 | 55.8 | 10 | 28.6* |
| *Staphylococcus* | 32 | 74.4 | 14 | 40* |
| *Pseudomonas* | 30 | 69.8 | 12 | 34.3* |
| *Veillonella* | 12 | 27.9 | 8 | 22.8 |
| *Neisseria* | 20 | 46.5 | 9 | 25.7* |
| *Actinomyces* | 15 | 34.9 | 8 | 22.8 |

*There were significant differences between the two groups ($p<0.05$)

**Table 3-** Prevalence of Candida species against respective antibiotics

| Antibiotics | Percentage of drug-resistance in 25 cases | | | |
|---|---|---|---|---|
| | *C. glabrata* | | *C. krusei* | |
| | Sample # | % | Sample # | % |
| Itraconazole | 25 | 100 | 16 | 60 |
| Miconazole | 5 | 20 | 11 | 44 |
| Fluconazole | 5 | 20 | 8 | 32 |
| 5-fluorocytosine | 2 | 8 | 7 | 28 |
| Amphotericin B | S | - | S | - |
| Voriconazole | S | - | S | - |
| Micafungin | S | - | S | - |

S=Susceptible

**Table 4-** Prevalence of bacterial species against respective antibiotics

| Antibiotics | Percentage of drug-resistance in 25 cases | | | | | |
|---|---|---|---|---|---|---|
| | MRSA | | *S. epidermidis* | | *S. haemolyticus* | |
| | Sample # | % | Sample # | % | Sample # | % |
| Oxacillin | 24 | 96 | 15 | 60 | 10 | 40 |
| Arbekacin | 12 | 48 | S | - | 1 | 4 |
| Vancomycin | 8 | 32 | S | - | S | - |
| Teicoplanin | 5 | 20 | S | - | S | - |
| Linezolid | 3 | 12 | S | - | S | - |
| Imipenem/ cilastatin | 3 | 12 | S | - | S | - |
| Amikacin | 1 | 4 | S | - | S | - |
| Ciprofloxacin | 1 | 4 | S | - | S | - |

S=Susceptible

## DISCUSSION

In this study, a prevalence of drug-resistant microorganisms in the oral cavity after treatment of oral cancer was performed in aged Chinese. The study was carried out in Ninth People's Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai. The participants were the patients from this hospital who had undergone oral cancer treatment and others without any history of cancer but that could be patients who had undergone treatment for other oral diseases. This study was aimed to investigate the reason behind the frequent cause of oral infection after the treatment of oral cancer in elderly individuals.

Several lines of evidence support our views that there is a possibility of drug-resistant microbes present in immunocompromised patients, particularly after the treatment of oral cancer. In general, infections are commonly found in oral cancer patients after surgical excision of the tumor[17–20]. This might be due to wound exposure during and after the operation, even if sutured, when microorganisms may infect oral regions, oropharynx, nasal cavity, and paranasal sinuses areas. Patients after oral cavity surgery often appear to have complications after bacterial infections, as well. Colonization of pathogenic bacteria in oral cavity is thought to increase the risk of infections such as pneumonia and bacteraemia[4,7]. It is therefore of high importance the prevention from or cure for the infections. During the development of tumor, the tumor cells or soluble products produced by tumor cells inactivate lymphocytes and provoke immunosuppression in the body[9,12].

The high percentages of microbial species identified from the isolates of participants were *Candida*, *Staphylococcus, Streptococcus* and *Pseudomonas* species (Figure 2). The highest numbers of these species were found in patients after the treatment of oral cancer in comparison with the non-cancer patients (Tables 1 and 2). Similar results were obtained in a phase 1 clinical trial with the application of an antibacterial dressing spray in the prevention of post-operative infection in oral cancer patients[25]. Antimicrobial drugs have been very helpful to prevent infections after surgery. People, however, sometimes misuse them and have antimicrobial drugs abuse. The abuse of antimicrobial drugs brings severe adverse effects to people, for example, allergy, toxic reaction, and opportunistic infections[18]. As a result, a new and ideal preventive method is needed for people who have surgery to reduce the chances of bacterial infections.

In this study, after revealing the high percentage of microorganisms found particularly in post-treatment of oral cancer, we started to focus on screening or identification of drug-resistant microbes within the species of already identified microbes. The antimicrobial agents (Figure 1), which are common in clinical practice, were taken for this study. The isolates were tested for the presence of *Candida* species and subspecies of staphylococci, particularly on MRSA, *S. epidermidis* and *S. haemolyticus*. Except for the *C. glabrata* and *C. krusei* species, all other *Candida* species were susceptible to the antifungal agents (Table 3). The MRSA showed the highest percentage of resistance to Oxacillin (96%) and Arbekacin. The other species, *S. epidermidis* and *S. haemolyticus,* showed resistance to Oxacillin with 60 and 40%, respectively. All other antibiotics were found susceptible to these species (Table 4). According to a study performed in the United Kingdom, 13 (41.9%) of 31 *S. aureus* isolates from the oral mucosa and pockets of patients with gingivitis/

periodontitis were mecA-positive (antimicrobial resistance)[6]. Another study, in the United States, reported that the prevalence of MRSA organisms in the nasal and oral cavities of nursing home residents was 20–35%[8]. Furthermore, a Japanese group investigated MRSA colonization in neonatal intensive care units and found that 207 (49.9%) of 415 newborns had MRSA organisms[22]. When compared with these results, the drug-resistance (96%) of MRSA against oxacillin was extremely high, possibly due in part to the tendency to prescribe long-term, high-dose antibiotic treatment in Japan[21].

## CONCLUSION

Our study revealed a group of opportunistic-microorganisms such as *C. glabrata, C. krusei* and subspecies of staphylococci, particularly *Methicillin-resistant S. aureus* (MRSA), *S. epidermidis* and *S. haemolyticus*. Nevertheless, anti-tumor drugs used in tumor treatment will also lead to immune and bone marrow suppression. Therefore, post-treatment infections occurred[13,14]. Continuous monitoring and a basic infection control strategy, including standard precautions, are important for older individuals, especially those receiving follow-up care for oral cancer. There is always a risk that they may become immunocompromised hosts easily susceptible to oral infections. Clinicians must also pay careful attention to opportunistic-microorganisms during the treatment of oral cancer, and to realize the fact of immune compromise in elderly patients.

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