The Rate of Nasal and Oral Colonization and Expression Levels of Hyphal Adhesin Als3p and mecA Genes in Methicillin-Resistant Staphylococcus aureus and Candida spp. Isolated From Patients With Lung Cancer

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Abstract

Background: Patients with cancer is considered highly susceptible group to both nosocomial and community-acquired infections.

Objectives: In the present research, we aimed to determine the rate of nasal and oral colonization and expression level of Als3p and mecA genes among Candida spp. and methicillin-resistant Staphylococcus aureus (MRSA) in co-colonization and single colonization conditions.

Materials and Methods: In total, 110 oral swab samples and 110 nasal swab samples were gathered from patients with lung cancer. The frequency of MRSA isolates (oxacillin-resistant Staphylococcus aureus) was determined using the disk diffusion method. In addition, the frequency and expression levels of Als3p and mecA genes among MRSA and Candida spp. isolates were determined and compared using PCR and qRT-PCR methods, respectively.

Results: Candida spp. and S. aureus were found in 42.7% (n = 47/110), and 9.1% (n = 10/110) of oral samples, respectively, while Candida spp. and S. aureus were found in 5.5% (n = 6/110) and 16.4% (n = 18/110) of nasal samples, respectively. Additionally, 55.3% (n = 10/18) of S. aureus isolates obtained from nasal samples were MRSA. Candida albicans (n = 23/110; 20.9%) had the highest frequency among Candida species. In all MRSA and Candida spp. isolates, the Als3p and mecA gene expression increased two and three times in co-colonization condition compared to single colonization condition, respectively.

Conclusion: The present study revealed that co-colonization has a synergistic effect on the expression level of mecA and Als3p genes. Our finding suggested that co-colonization can facilitate the invasion of S. aureus and leads to systemic and severe infections in co-colonized patients.

Keywords: Candida spp., Als3p, Staphylococcus aureus, MRSA, mecA

Background

In general, patients with cancer due to several reasons such as cytotoxic chemotherapy, long-time hospitalization, use of broad-spectrum antibiotics, use of immunosuppressive drugs, and invasive procedures have a weakened immune system.1 Therefore, this group of patients is considered highly susceptible to both nosocomial and community-acquired infections.2 Staphylococcus aureus is an opportunistic organism that can cause different infections such as oral and nasal infections in humans.3 This pathogen contributes to high morbidity and mortality rates among cancer patients, worldwide.4 Methicillin-resistant S. aureus (MRSA) is considered a public health threat, worldwide. Health workers’ contaminated hands are the main route of MRSA transmission. However, MRSA can be transmitted from patient to patient or from environment to patient.5 MRSA strains are resistant to different antibiotics including β-lactam agents, β-lactamase inhibitor combinations, monobactams, and cepheks. The production of beta-lactamase enzyme and an altered penicillin-binding protein (PBP2a) are the main mechanisms of methicillin resistance in MRSA strains. The mecA gene encodes PBP2a, which is located on the staphylococcal cassette chromosome mec (SCCmec) (a chromosomal mobile genetic element).6,7 The synergistic relationship between S. aureus and Candida spp. has been revealed in several studies.8,9 These microorganisms possess numerous virulence factors and can transition from commensals to pathogens, particularly in individuals under conditions of immune dysfunction.10 The ability to switch between yeast and hyphal forms is
a major virulence factor of *Candida*. The dissemination of *Candida* species to target organs is facilitated in yeast form.

*Candida albicans* have several virulence factors including invasins such as agglutinin-like sequence 3 adhesin (Als3p) which allows the *Candida* to cause systemic diseases. A breach in mucosal barriers of the host endothelial and epithelial cells is required for *S. aureus* infection. It is revealed that *S. aureus* attaches to the Als3p in the *C. albicans* cell wall and this adherence leads to penetration of tissue. Studies suggested that co-colonization facilitates the invasion of *S. aureus* and leads to systemic and severe infections in co-colonized patients. Furthermore, it is suggested that co-colonization can increase the resistance to antibiotics in *S. aureus*.

In the current research, we aimed to determine the nasal and oral colonization rates and expression levels of Als3p and mecA genes in *Candida* spp. and MRSA isolates in co-colonization and single colonization conditions.

### Materials and Methods

#### Inclusion and Exclusion Criteria

Patients with lung cancer and those who signed the written assent/consent form were included in the current study. On the other hand, patients with other malignancies and cancers including acute lymphocytic leukemia, blood cancers, and dialysis, patients who died in the study period, patients who refused to sign the written consent form or failed to answer the questions, and persons whose parents did not agree to participate were excluded from the present study.

#### Study Setting, Sampling, and Population

The current study is cross-sectional research performed in Masih Daneshvari hospital, Tehran, Iran. Briefly, from January and December 2021, all patients with lung cancer referred to Masih Daneshvari hospital were included in the present study. A total of 220 clinical samples including oral swabs (n = 110) and nasal swabs (n = 110) were gathered, which were analyzed by standard microbiological methods. For *S. aureus* identification, standard microbiological tests including culture on microbiological media (sheep blood agar and mannitol salt agar), catalase, oxidase, growth on 6% NaCl, DNase, and coagulase tests were applied. The frequency of MRSA isolates (oxacillin-resistant *S. aureus*) was determined using the disk diffusion method. We used *S. aureus* ATCC 33591 and ATCC 29213 as methicillin-resistant and sensitive control strains, respectively.

The frequency of *Candida* spp. was identified using various standard assays including direct microscopic observation, culture on a different medium (cornmeal agar with Tween-80, Sabouraud Dextrose agar containing chloramphenicol, and CHROM agar *Candida* medium), and germ tube test.

### Polymerase Chain Reaction

DNA extraction was performed using the specific DNA extraction kit (GeNet Biotech Company, Daejeon, Korea; Cat. No, K-3000), and the presence and frequency of the *mecA* gene among phenotypically confirmed MRSA strains were determined using polymerase chain reaction (PCR) assay with specific primers (Table 1). The volume of materials used in the PCR assay and PCR conditions were determined according to the previously published study by Shariati et al. We used the *S. aureus* ATCC 25923 and ATCC 33591 as the negative and positive controls, respectively.

On the other hand, we used the Exgene cell SV-mini 10×10³ DNA tissue kit (GeneAll, South Korea) for total DNA extraction of *Candida* spp. The frequency of the Als3p gene among *Candida* spp. was identified by the PCR method. PCR condition and the volume of materials for the Als3p gene were determined according to Ardehali et al. DNA safe stain (SinaClon Co., Iran) was used for staining the PCR products, and all products were electrophoresed on 1.5% agarose gel under UV light.

### RNA Extraction and cDNA Synthesis

Total RNA extraction was done using the RNeasy Mini Kit (SinaClon) from grown *S. aureus* and *Candida* species (OD600, 1.5–2.0).

DNase I (Fermentas, Waltham, MA, USA) was used for removing the possible DNA contamination and all RNA samples were suspended in 50 µL of diethylpyrocarbonate-treated water (0.1% v/v). The total RNA concentration was determined using a Nanodrop DS-11 spectrophotometer (DeNovix, USA), and the cDNA synthesis was done using the Takara kit (Shiga, Japan).

### Semiquantitative Real-time PCR

The comparative expression levels of *mecA* and Als3p genes were evaluated in triplicate by qRT-PCR. The qRT-PCR assay was done on a Rotor-Gene RT-PCR machine (Corbett Research, Sydney, Australia; model RG3000, software version 6).

The primer sequences used in the qRT-PCR assay are shown in Table 1. The volume of materials used in qRT-PCR assay and reaction conditions are described as follows: 11 µL 2 × iQ™ SYBR® Green Supermix (Takapouzist Co., Tehran, Iran), 0.5 µM of forward primer (10 mM), 0.5 µM

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<th>Primers Name</th>
<th>Sequence (5′→3′)</th>
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| mecA         | F: TCCGATTTACAACTTACCCAGG  
R: CACTTCATTATCTTGTAAGG |
| 16s rRNA     | F: GACGGTCCTGTCGATGAGATGT  
R: GTAAGGCACCATGATGACCTT |
| Als3p        | F: CAATGCGGTATTAGAAGAAAACA  
R: AGAAACAGAAAAACCAAGACACCT |
of reverse primer (10 mM), 1.5 μL of the cDNA reaction mixture, and sterile distilled water up to 20 μL. The qRT-PCR protocol consisted of a denaturation step at 95°C for 15 minutes, followed by 45 cycles of 94°C for 15 seconds, 60°C for 45 seconds (mecA gene), 58°C for 30 seconds (Als3p gene), and a melting-curve step (60–95°C). The 16S ribosomal RNA housekeeping gene (16s rRNA) was used to normalize the data and the relative expression levels were calculated using the 2−ΔΔCt formula.13

Results

Study Population
A total of 110 oral samples and 110 nasal samples were gathered from 110 patients with lung cancer. Male patients (63.6%; 70/110) were more in number than the female (36.4%; 40/110) ones in our study. In this study, 36.4% (40/110) of the patients were from the age group 56-65 years, followed by the age groups 45-55 (27.3%) years and 35-44 (17.3%) years. Our analyses revealed that 70.9% (n = 78/110) and 6.4% (n = 7/110) of patients had a history of surgery and organ transplantation, respectively. Furthermore, 34.5% (n = 38/110) of patients had a history of hospitalization in the intensive care units (ICUs). The length of hospitalization in 62.7% (n = 69/110) patients was ≤ 3 days. In general, 9.1% (n = 10/110) and 4.5% (n = 5/110) of the included patients had a record of antibacterial and antifungal therapy.

Distribution and Frequency of Pathogens
Of the 110 patients included in the current study, 9.1% (n = 10/110) were positive for S. aureus in oral samples, while S. aureus was detected in 16.4% (n = 18/110) of the nasal samples. Results showed that 70% (n = 7/110) of S. aureus isolates obtained from oral samples were MRSA, while the prevalence of MRSA isolates in nasal samples was 55.5% (n = 10/18). S. aureus was found in 4.5% (n = 5/110) of both oral and nasal samples. Candida spp. was found in 42.7% (n = 47/110) and 5.5% (n = 6/110) of oral and nasal samples, respectively. Based on the conventional microbiological methods, C. albicans (n = 23/110; 20.9%) had the highest frequency among Candida species. The frequency of other Candida species was as follows: C. glabrata (n = 17/110; 15.5%) and C. tropicalis (n = 3; 2.7%). C. albicans and C. glabrata were isolated simultaneously from 8 patients. Furthermore, two patients were positive simultaneously for C. glabrata and C. tropicalis. S. aureus and Candida spp. were isolated simultaneously from 6.4% (n = 7/110) and 3.6% (n = 4/110) of both oral and nasal samples, respectively. Moreover, Candida spp. and MRSA were simultaneously found in 1.8% (n = 2/110) and 4.5% (n = 5/110) of nasal and oral samples, respectively.

Relative Gene Expression
The comparative expression levels of mecA and Als3p genes were evaluated among 20 MRSA and Candida spp. in single and co-colonization conditions. Results obtained from the quantitative analysis indicated that in Candida species, the Als3p gene expression increased more than three times in co-colonization conditions compared to single colonization conditions. Moreover, results indicated that mecA gene expression increased more than six times in co-colonization conditions compared to single colonization conditions. It was revealed that the Als3p and mecA gene expression increased two times and three times in the co-colonization condition compared to the single colonization condition, respectively.

Discussion
In general, the treatment and control of polymicrobial infections are time-consuming and very difficult. These infections are related to resistance to different groups of antibiotics.14 Immunocompromised persons are considered a susceptible group to polymicrobial infections.6 Among different microorganisms, MRSA and Candida spp. are the main opportunistic pathogens causing high mortality in immunocompromised patients.2 Several types of research have shown a synergistic relationship between S. aureus and Candida spp., which can lead to systemic infections.8,14-16

In the current study, we determined the frequency of oral and nasal colonization of S. aureus and Candida spp. in patients with lung cancer. Moreover, we evaluated the impact of co-colonization on the expression level of Als3p and mecA genes in MRSA and Candida spp. Results of our study showed that 16.4% and 9.1% of patients were positive for S. aureus in nasal and oral samples, respectively. Moreover, 70% and 55.5% of S. aureus isolates from oral and nasal samples were MRSA, respectively. According to a recently published study, 55% of human nasal commensals belong to the family Staphylococcaceae. Therefore, it is predictable that the load of S. aureus was higher in the nasal than in the oral cavity.17

Globally, different studies have surveyed the colonization rate of MRSA in persons with different malignancies and various results have been reported. Montazeri et al revealed that S. aureus was isolated from 48.1% of patients with cancer. Moreover, 76.3% of S. aureus isolates were MRSA.1 Emge et al showed that 34% of the 50 S. aureus isolates obtained from erythrodermic cutaneous T-cell lymphoma patients were MRSA.18 The prevalence rates of MRSA in studies performed by Ghanem et al in Saudi Arabia,19 Shiomori et al in Japan,20 and Djoudi et al in Algeria21 were 3%, 75.4%, and 1.5%, respectively.

In the current study, 42.7% and 5.5% of patients were positive for Candida spp. in oral and nasal samples, respectively. C. albicans with a 20.9% frequency had the highest rate among Candida species. Obtained results
are comparable with those of previous studies from Finland (two studies), South Africa, the USA, and Greece, which reported that the frequency of Candida spp. in oral samples of patients with malignancy was 75%, 74%, 84.2%, 27%, and 76.9%, respectively. Moreover, in all studies stated above, C. albicans had the highest rate among Candida species.

Staphylococcus aureus and Candida spp. were isolated simultaneously from 6.4% and 3.6% of both oral and nasal samples, respectively. Moreover, we surveyed the relative expression levels of mecA and Als3p genes in MRSA and Candida spp. in single and co-colonization conditions. It was revealed that the Als3p and mecA gene expression increased two and three times in co-colonization conditions compared to the single colonization condition, respectively. The interaction between S. aureus and Candida spp., especially C. albicans, has been shown in biofilm-associated diseases and bloodstream infections. However, in the oral and nasal mucosa, the synergic interaction between these two opportunistic pathogens is rarely stated in clinical studies. Studies revealed that both microorganisms were simultaneously isolated from polymicrobial infections.

In general, the ability to switch between yeast and hyphal forms is a major virulence factor of Candida spp. In contrast, breaks in host epithelial layers are required for S. aureus systemic infections. S. aureus has propensity to bind to the Candida (C. albicans) cell wall adhesin Als3p. In the co-colonization model, it is revealed that S. aureus uses Candida Als3p to enter and invade the host tissues. Candida can enable the transmission and invasion of S. aureus to host tissue and mucosal barriers. It is presumed that S. aureus secretes signaling chemical molecules (quorum sensing molecules) such as tyrosol and alters the Candida growth. However, further studies are required to understand these molecules and their mechanisms.

The present study has several limitations as well. There was no control group (healthy individuals) in this study. Therefore, we are unable to compare the frequency of S. aureus, MRSA, and Candida spp. between patients with lung cancer and healthy individuals. Additionally, we did not have access to data on the treatment process, treatment outcomes, and mortality rate among the included patients.

In conclusion, our results showed that the frequency of S. aureus was higher in the nasal cavity than in the oral cavity of patients. On the other hand, Candida spp. was more frequently isolated from the oral cavity of patients. These findings revealed that S. aureus and Candida spp. are common commensals of the nasal and oral cavities, respectively. Moreover, the present study revealed that co-colonization has a synergistic effect on the expression level of mecA and Als3p genes.

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Competing Interests
None.

Ethical Approval
The current study was approved by the Ethics Committee of Pediatric Infectious Research Center, Shahid Beheshti University of Medical Sciences (IR.SBMU.RICH.REC.1399.035).

References


