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RESEARCH ARTICLE

Influence of *Staphylococcus Aureus* on the Oral *Candida Albicans*

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Abstract

Candida albicans is that the most current isolated from the human body and it consider as a natural part of the commensal microorganisms. The mouth is colonized by many of bacterial species, and it also the relationship between these oral bacteria and *C. albicans* has been a subject of interest in understanding the event of candidiasis. Many studies have tried to explain the bacteria-fungal relationship within the oral environment; however, few have targeted on the single bacterial species inhibiting growth of *C. albicans*. *Staphylococcus aureus* and *Candida albicans* were isolated and purified from the samples of 50 patients with the fixed orthodontic appliances and PCR was performed to identify this isolated. The microscopic examination and qPCR were carried out to determine the inhibitory impact of *S. aureus* on *C. albicans*. This study demonstrated *Staphylococcus aureus* displayed the strongest inhibitory effect on the growth of *Candida albicans*. By qPCR assay, all isolated *C. albicans* were inhibited on the growth by *S. aureus* in the various periods. Mixed culture experiments also demonstrated inhibitory of *C. albicans* by *S. aureus*.

Keywords: *PCR, Staphyloccous, Candida, Oral cavity.*

Introduction

The human oral cavity may be a diverse microorganism system comprised of bacteria, fungi, protozoa, and viruses [1]. Over 700 species of the microorganism are known to reside in the human mouth, and more than a hundred species are found within the oral cavity of a healthy individual at one time [2].Normally, the various species maintain an ecological homeostasis; but, because of constant competition for limited areas and nutrients; however, any disturbance of the biofilm via environmental factors or interspecies interactions will favor the growth of certain species and it may be caused by diseases [3].

Many studies have reported the mix culture of *S. aureus* and *C. albicans* from various biofilm associated diseases like periodontitis, denture stomatitis, cystic fibrosis, keratitis, and ventilator associated pneumonia, urinary tract and burn wound infections [4, 10]. Though these studies only reported associations and it didn't prove causing; however, the frequency with that *S. aureus* and *C. albicans* are co-isolated merits more study and additional directly relevant to bacteremia. It also is studied by Klotz *et al* [11].

Investigated the incidence of Candida blood infections in the hospitalized patients reported that *C. albicans* and *S. aureus* with 20 % of the cases. Additionally, animal studies via Carlson *et al* [12, 13].Demonstrated a major increase in the mortality of mice co-infected intraperitoneally with the sub lethal levels of *C. albicans* and *S. aureus*. This lethal synergism was found in the recent study via Peters & Noverr [14] wherever co-infection led to a 40 % mortality percentage and increased microorganism burden within the spleen and kidney.

The interaction between *S. aureus* and *C. albicans* doesn't seem to be strain specific, and it is also significantly higher than the interaction between C. albicans and other bacterial species [15, 16]. The aim of this study was to investigate the effect of *S. aureus* for the growth of *C. albicans* isolated from the oral cavity and it is also determining the relationship for these.

The effect of inhibition by *Staphylococcus aureus* was observed in the growth of *C. albicans* and this is evidence of the environmental imbalance within the oral cavity which causes diseases, especially when using the fixed orthodontic.

Materials and Methods

Sample Collection

Patients with the fixed orthodontic appliances were 50 randomly selected for the isolation. The participants carefully brushed their teeth after breakfast and samples were taken 2 hours after food consumption. Microbiological samples were obtained via the swabs method 6hours, 12hours, 24hours, 36hours, and 48hours in addition to 1 month, 2 months, 3 months, and 6 months prior to and after installation of the fixed orthodontic appliances.

Identification and Culture

Bacterial species were isolated on brain heart infusion (BHI) agar (1.5% agar) plates under both aerobic and anaerobic conditions. Purified colonies were grown in BHI medium and visualized microscopically (Olympus Microscope 60X/1.40 Oil Ph3). *Candida albicans* was cultured in CHRO Magar Candida identification petridishes (CHRO Magar, Paris, France) at 37C for (36-48) hours. Species were identified by PCR method (the primers in table 1), and stored in BHI containing 15% glycerol at -80°C [17].

Table 1: Primers used in this study

Primers	Sequence (5' to 3')
S. aureus -F	AGAGTTTGATCCTGGCTCAG
S. aureus -R	TACGGTTACCTTGTTACGACTT
C.albicans -F	GACTCAACACGGGGAAACT
C.albicans -R	ATTCCTCGTTGAAGAGCA

Results and Discussion

C. albicans inhibition by *Staphylococcus aureus* and it was used in mixed cultures. After and before treatment with the fixed orthodontic appliances, *C.albicans* and *S. aureus* were (39.4%) after treatment than before treatment was (50%) (Table2). *Candida* considers the organism's ability to change the morphology between yeast cells and hyphae forms so as to adhere to surfaces, form biofilms, and penetrate tissues [18, 19]. Hypha is believed to be invaded and the pathogenic form of *Candida* species, whereas yeasts are the commensal nonpathogenic form [20]. Some molecules generated by the Gram-negative bacteria were, according to inhibit the formation of hyphae in *C. albicans*, e.g. 3-Oxo-C12 homoserine lactone or cis-2-dodecenoic acid (BDSF) created by *Pseudomonas aeruginosa* or *Burkholderia cenocepacia*, respectively [21, 22]. Gram positive bacteria *Streptococcus mutans* also produces Competence Stimulating Peptide (CSP), trans-2-decenoic acid and the secondary metabolites to suppress filamentation in *C. albicans* [23, 24].

Table 2: Isolations at different treatment periods

Isolations	Before treatment	%	After treatment	%	Total	%
Candida albicans	57	46.7	39	26.5	96	35.6
Staphylococcus aureus	4	3.2	50	34	54	20
C. albicans + *S. aureus*	61	50	58	39.4	119	44.2
Total	122	100	147	100	269	100

Mono and dual species biofilms were quantified at early (6 h), intermediate (12h), mature (24 h), (36h), and (48h) stages of biofilm growth using the biomass assay. It was shown that dual-species biofilms (50%, 60%, 22.2%, 20%, and 25%) respectively (Table 3). Initial objectives of these series of studies were to make a reliable, functional assay to quantify and characterize the interaction between *C. albicans*, and *S. aureus*. It had been our hypothesis based on the previous studies that *C. albicans* inhibition via *S. aureus* biofilm formation [25].

We used the biofilm *S. aureus* to determine whether the presence of *C. albicans* may inhibit its ability to colonize and the form biofilms. Indeed, our hypothesis were confirmed with the observation that single *S. aureus* cells and the small clusters adhere to *C. albicans* germ tubes after only (6h) of the growth, whereas synchronously inhibiting overall biomass, significantly increasing throughout biofilm maturation. This is often in line with previous reports predicting the initiation of poly microbial biofilm occurs upon initial *C. albicans* germ tube formation [26, 27].

Table 3: Isolations after treatment during periods in hours (hr)

Isolations	After treatment										
	6 hr.	%	12 hr.	%	24 hr.	%	36 hr.	%	48 hr.	%	Total
C. albicans	0	0	1	20	4	44.4	3	30	5	41.6	13
S. aureus	2	50	1	20	3	33.3	5	50	4	33.3	15
C. albicans + *S. aureus*	2	50	3	60	2	22.2	2	20	3	25	12
Total	4	100	5	100	9	100	10	100	12	100	40

The installation of the fixed orthodontic appliances within 3 months, the rate of *C. albicans* and *S. aureus* significantly increased compared with those prior to treatment, particularly at 4 months after the fixed orthodontic appliances installation; these values then gradually decreased over time (Table 4). These findings may be because the fixed orthodontic appliances leading to a lowering of the local defense mechanism of the oral mucosal cells. Oral mucosal cells, that act as mechanical barriers, and metabolism play important roles in increasing the resistance of the mouth to infection. Therefore, *Candida* will easily adhere to any damage within the oral epithelia [28]. The interaction of *Candida* and the other oral bacteria, including adhesion between *C. albicans* and other microorganisms within the host cells and it is an important factor in maintaining the commensalism of bacteria in the human body. *Escherichia coli*, *Streptococcus*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* will restrain the pathogenicity of *Candida.* Obligate anaerobic bacteria will inhibit the proliferation and adhesion of *C. albicans* to mucosa. *S. aureus* and *Candida* have synergetic pathogenic characteristics [29].

Table 4: Isolations after treatment during periods (1 -6 months)

Period of treatment	*C. albicans*	%	*S. aureus*	%	*C. albicans* + *S. aureus*	%	Total	%
1 months	7	17.9	5	10	4	6.8	16	10.8
2 months	4	10.29	8	16	6	10.3	18	12.2
3 months	5	12.8	11	22	15	25.8	31	21
4 months	9	23	7	14	12	20.6	28	19
5 months	8	20.5	10	20	11	18.9	29	19.7
≥6 months	6	15.3	9	18	10	17.2	25	17
Total	39	100	50	100	58	100	147	100

After an 24 hours co-incubation at 37°C, all cultures were harvested, and total genomic DNA was isolated. PCR was used to measure the abundance of *C. albicans* by using specific primers .This primer pair didn't generate any PCR product when using *S. aureus*,and compared to the single culture control, *C. albicans* growth was reduced by presence of *S. aureus*.

This difference of *S. aureus* inhibitory effects can be because various characteristics of the clinically isolated strains of *C. albicans*, thus, more investigation is needed to determine the exact mechanism. Although most studies depicted both *C. albicans* and *S. aureus* exist in a cooperative relationship, quorum sensing molecule farnesol generated by *C. albicans* was found to interrupt *S. aureus* cell

membrane integrity and then inhibit its biofilm formation and viability [30,31]. Thus far, the inhibitory effect of *S. aureus* on *C. albicans* has not been reported. In this study, *S. aureus* was determined to be effective in inhibiting yeast growth. Using a PCR assay, 2 strains in 4 strains of *C. albicans* were identified as being the strongest inhibited on the growth by S. aureus, and the rest five *C. albicans* strains showed reduced growth to some extent in the presence of S. aureus (Figure 1). In a study exploring the antagonistic fungal, bacterial interaction between *C. albicans* and *P. aeruginosa*, Hogan and Kolter determined that *P. aeruginosa* was unable to bend or kill the yeast form of *C. albicans*, but it could form a dense biofilm on *C. albicans* hyphae, which killed the fungus .Surprisingly, in this study, *S. aureus* strain apparently suppressed the pseudohyphae/hyphae formation of all isolated *C. albicans* strains, but displayed the obviously inhibitory effects on their growth. Thus, further studies are required to figure out the mechanism(s) of repression of *S. aureus* on *C. albicans* [32].

Figure 1: Amplification of *C. albicans* from the oral cavity

Figure 2: Amplification of *S. aureus* from the oral cavity

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