Evaluation of Exoenzyme Activities, Biofilm Formation, and Co-hemolytic Effect in Clinical Isolates of *Candida parapsilosis* Species Complex

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Abstract

Candida parapsilosis species complex is considered as important emerging pathogens and little is known about their pathogenicity factors and co-hemolytic activity with different bacteria species. The aim of this study was to determine *in vitro* exoenzyme activities, biofilm formation, and co-hemolytic effect of different bacteria species on clinical *C. parapsilosis* complex isolates. In total, 67 *C. parapsilosis* complex isolates consist of *C. parapsilosis sensu stricto* 63/67 and *Candida orthopsilosis* 4/67 were used in this study. To determine the hemolytic activity of these species, Sabouraud dextrose sheep blood agar was used. Evaluation of the CAMP-like phenomenon carried out in the presence of *Staphylococcus aureus, Staphylococcus saprophyticus, Staphylococcus epidermidis,* and *Streptococcus agalactiae*. Tube test method with ethylenediaminetetraacetic acid-rabbit plasma was used to determine coagulase activity, and biofilm formation was assessed by the tube method in assist of Sabouraud glucose broth (8%) medium. Fisher's exact tests were used for data statistical analysis. Sixty-six of 67 (98.5%) and 3/67 (4.5%) of the species showed hemolysin and coagulase activity, respectively. Fifty-five of 67 (82.1%) of species had ability for biofilm formation, and none of the samples exhibited co-hemolytic effect in the presence of four mentioned bacteria. No significant difference was found between the level of enzyme production and biofilm formation among the isolates.

Keywords: Biofilm, CAMP-like, *Candida orthopsilosis*, *Candida parapsilosis*, coagulase

Introduction

Candida species have been recognized as normal flora of human skin. Although *Candida albicans* is the most frequently isolated fungal species from human infections, *Candida parapsilosis* has become the second or third most common cause of fungal infections in human.[1] Nowadays, these species have been reclassified as a *C. parapsilosis* species complex comprising three distinct species: *C. parapsilosis*, *Candida orthopsilosis,* and *Candida metapsilosis*, using different molecular methods.[2] Several factors, such as adherence, persistence, germ tube formation, phenotypic switching, interference with host defense systems, synergism with bacteria, and the production of hydrolases, have been proposed to be *Candida* spp. virulence factors.[3] The ability of *C. albicans* to form biofilms and adhere to host tissues is important pathogenesis factor. Biofilm formation can act as a reservoir of agents, allow coinfection with other pathogens, promote the persistence of infection, and increase mortality.[4] The role of exoenzymes as a virulence factor of *C. albicans* has been intensively investigated, but its contribution to the virulence of *C. parapsilosis* species complex remains uncertain.[5,6] The co-hemolytic effect which was named the "CAMP reaction," first described by Christie, Atkins, and Munch-Peterson in 1944. The cooperative (CAMP-like) lytic processes are the result of the interaction of at least two membrane-active agents of bacteria, with biological membranes. *Streptococcus agalactiae* (B group streptococci) produces a thermostable, extracellular, diffusible protein that

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DOI: 10.4103/jgid.jgid_93_17

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How to cite this article: Pakshir K, Ravandeh M, Khodadadi H, Motamedifar M, Zomorodian K, Alipour S. Evaluation of exoenzyme activities, biofilm formation, and co-hemolytic effect in clinical isolates of *Candida parapsilosis* species complex. J Global Infect Dis 2018;10:163-5.

acts synergistically with the *Staphylococcus aureus* b-lysine to produce a zone of enhanced lysis in ovine and bovine erythrocyte cultures.[7]

According to our knowledge, there are no data about co-hemolytic effect of bacteria species which are commonly part of normal skin flora or any association in mucocutaneous area with infected lesions caused by *Candida* infections. The aim of this study was to evaluate hemolysin and coagulase activities, biofilm formation, and determination of co-hemolytic effect (CAMP-like factor) of *C. parapsilosis* species complex with standard strain of *S. aureus, Staphylococcus saprophyticus, Staphylococcus epidermidis,* and *S. agalactiae*.

Microbiology Report

A subset of 67 clinical isolates including 63 *C. parapsilosis sensu stricto* and four *C. orthopsilosis* were included in this study. Hemolytic was evaluated with a sheep blood (7% v/v and 3% w/v glucose) plate assay[8] The presence of a distinctive translucent halo around the inoculum site indicated positive hemolytic activity. Coagulase activity was evaluated with standard tube test method using ethylenediaminetetraacetic acid-rabbit plasma.[9] The presence of a clot formation in tube tests expresses as positive coagulase test. Biofilm formation was evaluated by the tube method, visual detection (Sabouraud dextrose broth 8%).[10] The tubes were examined for the presence of an adherent visible film at the bottom of the tubes and the results were expressed as negative–, weak+, moderate++, and strong+++. Standard strains of bacteria including *S. aureus* (ATCC25923), *S. saprophyticus* (PTCC1440), *S. epidermidis* (PTCC1435), and *S. agalactiae* (PTCC1768) were used for the evaluation of co-hemolytic effects. After 2 days of incubation for assessing the hemolytic activity, a loop was used to streak each bacterium in straight lines across the plate at a distance of 10 mm from the edge of the border of yeast colony. A distinct arrowhead of hemolysis at the intersection of the tester strain and the *Candida* colony streaks considered as a positive result for CAMP-like reactions.[7] Chi-square and Fisher's exact tests were used for data statistical analysis. Out of 67clinical isolates, 66/67 (98.5%) presented hemolysin activity. Beta-hemolysin activity was detected in 52/67 (78.78%) isolates. Coagulase activity was detected in 3/4 (4.5%) isolates which all belongs to *C. parapsilosis sensu stricto* [Table 1]. There was no co-hemolytic effect between positive hemolytic isolates in association with bacteria species [Table 1 and Figure 1]. A total of 55 (82.1%) of the 67 *Candida* strains were positive for biofilm production [Figure 2].

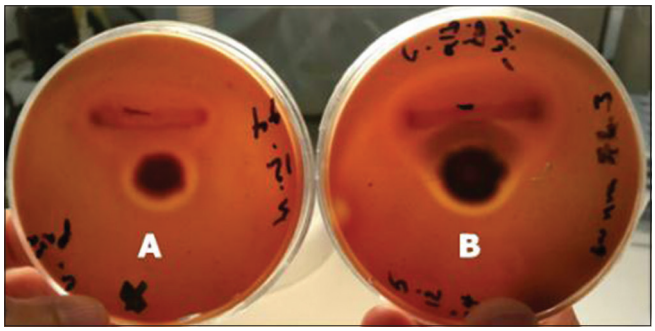

Figure 1: Hemolysin and co-hemolytic (CAMP-like) effect. (A), *C. parapsilosis* complex with *Staphylococcus aureus* (CAMP negative). (B -), *C. albicans* as control with *Staphylococcus aureus* (CAMP positive , a zone of half-moon shape hemolysis)

Conclusion

In general, our study reveals that secretion of hemolysin and biofilm-forming ability are two major factors that play role in pathogenesis of *C. parapsilosis* species complex and none of the bacteria species did used in this study had any role in cooperative (CAMP-like) hemolytic activities with these strains.

Acknowledgments

This study was extracted from an MS thesis by Mostafa Ravandeh and was funded by Deputy of Research and Technology

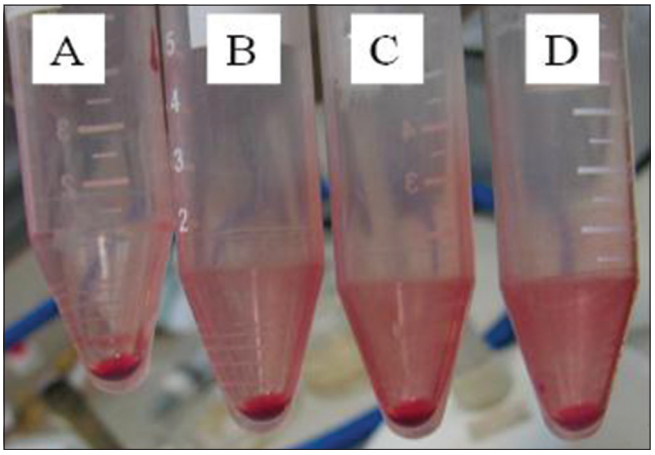

Figure 2: Biofilm formation: (a) Negative (control), (b) Positive (weak+), (c) Positive (mediate++), and (d) positive (strong+++)

Table 1: Distribution profile of enzymatic activity, biofilm production and CAMP-like effect on clinical species of *C. parapsilosis* complex

Tests species	Coagulase		Hemolysin			CAMP-like effect				Biofilm			
	+	-	Alpha	Beta	Gama	sap.	aure.	epi.	aga.	-	1+	2+	3+
C. parapsilosis sensu stricto (63)	3	60	14	48	1	-	-	-	-	12	25	8	18
C. orthopsilosis (4)	0	4	0	4	0	-	-	-	-	0	3	0	1
Total (67)	3	64	14	52	1					12	28	8	19

S. aureus(*aure*), *S. saprophyticus*(*sap*), *S. epidermidis*(*epi*) and *S. agalactiae*(*aga*)

of Shiraz University of Medical Sciences, Shiraz, Iran (Grant No. 11975).

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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