P-056	**Activity of zinc oxide emulsion against yeasts isolated from health worker hands.**	

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INTRODUCTION - OBJECTIVES

Nosocomial infections are important causes of morbidity and mortality in pediatric hospitals (Cavalcante 2006). Systemic fungal infections have increased steadily in last decades (Agarwal 2004) and have been highlighted by health institutions, universities and hospitals in various countries (Gudlaugsson 2003). Among the fungi, *Candida* spp has been reported as a common agent of hospital infections with increase in frequency, morbidity and mortality (Agarwal 2004).

Importance of *Candida* spp in nursery and intensive care units is increasing; candidemia is generally associated with high mortality (Agarwal 2004). More recently, non-albicans *Candida* have emerged as important opportunistic pathogens, notably *C. tropicalis*, *C. glabrata* and *C. parapsilosis*, this later one being most prevalently isolated in later years (Agarwal 2004).

Among other factors to justify the increase of candidemia and non-albicans species, especially in neonatal intensive care units (NICUs), the horizontal transmission has been noted, for example, from the hands of the hospital staff (Bonassoli 2005). However, the measures for control of this situation have not been effective yet.

Hand hygiene is considered to be the simplest and most effective measure to prevent against cross-transmission of microorganisms and health-care-associated infections (Pittet 2005).

The use of a barrier cream would supposedly have the function of covering the baby's skin with a layer of protection in order to treat possible infections horizontally transmitted. This study aims to evaluate the antifungal activity of a new product, an emulsion with zinc oxide, on yeasts isolated from the hands of hospital professionals in search of a new tool for treatment of fungal infections.

MATERIAL AND METHODS

Organisms: Ten yeasts isolated from hands of hospital staff were studied: *C. parapsilosis*, *C. albicans*, *C. tropicalis*, *C. guilliermondii*, *C. kefyr*, *C. stellatoidea*, *Trichosporum asahii*, *Trichosporum ovoides*, *Trichosporum cutaneum*, *Saccharomyces cerevisiae*. These yeast cells were isolated and identified in a previous study (Bonassoli 2005). Nowadays these isolates are part of a yeast bank maintained in the Medical Mycology Division of the Laboratory of Teaching and Research in Clinical Analysis at the State University of Maringá, Paraná, Brazil. The isolates have been stored in dehydrated gelatin drops (Marangon 2003) at room temperature since their identification. For the tests, the yeasts were reactivated in Sabouraud Dextrose Broth (Difco, USA) and, then, subcultured in CHROMagar Candida® (CHROMagar BioMerisc, France) to assess the purity of the culture and the color of the colonies.

Emulsion with zinc oxide (ZnO): The emulsion used in this study was developed and characterized at the Dispersed Systems Laboratory (LASID – UFRN) (data not shown) (Silva 2009; Silva 2010).

Inoculum preparation: A small amount of yeast, Sabouraud Dextrose agar (SDA) (Difco, USA), 24 h at 35 ºC culture, was suspended in sterile saline (NaCl) 0.85 %, and adjusted to 0.5 McFarland scales, which equates to a concentration of 1.0 – 5.0 x 10^6 CFU/mL. A dilution of 1:50 in NaCl 0.85 % was performed followed by 1:20 dilution in Muller-Hinton Broth (MHB) (Difco, USA) in order to obtain a final concentration between 1.0 – 5.0 x 10^3 CFU/mL.

Microbiological assay: The emulsion with ZnO and emulsion base (EM_{ZnO} and EMB, respectively) was diluted in MHB $1{:}2_{(w/v)}$ and placed in contact with each inoculum also in a $1{:}2_{(v/v)}$ getting the final concentration of 25 $\%_{(w/v)}$. An inoculum in MHB (1:2) was used as a control. After inoculation and incubation at 35 °C for 24 h, subcultures of 10 µL were inoculated onto SDA and incubated at 35 °C for 24 h for colony counting.

RESULTS AND DISCUSSION

The study was carried out with healthy people. Therefore, they did not present any symptom of fungal infections. However, it is known that the yeasts found in those individuals are able to cause infections in susceptible patients. Thus, a product with antifungal properties and easy to handle can be important in preventing those infections.

For this study with 10 yeasts, *C. parapsilosis* may be considered the most important specie in both NICUs and hands of health professionals. However, other nine species were evaluated corresponding to the most frequent ones in our hospital.
Due to the difficult of determination of the MIC (minimum inhibitory concentration) by visual analysis of the emulsion cream, it was chose to determine the CFUs

(colony forming units) for a fixed concentration (25 $\%_{w/v}$) of EM_{ZnO} and EMB, separately. The CFU values obtained for these yeasts are shown in Table 1.

Table 1: Colony-forming unit values for some species of yeasts isolated from hands, compared to preparations with EM_{ZnO} and EMB.

Isolates	EM_{ZnO}	EMB	Positive Control
C. parapsilosis	8.85×10^4	1.43×10^6	4.09×10^6
C. albicans	5.00×10^3	5.50×10^5	4.18×10^6
C. guilliermondii	1.20×10^5	9.90×10^5	4.75×10^6
C. kefyr	0	0	3.60×10^6
C. stellatoidea	8.50×10^4	2.90×10^5	4.90×10^6
C. tropicalis	2.00×10^4	9.05×10^5	5.36×10^6
S. cerevisiae	4.33×10^5	2.16×10^6	4.73×10^6
T. asahii	0	7.50×10^5	4.70×10^6
T. cutaneum	0	1.35×10^6	3.30×10^6
T. ovoides	1.00×10^5	1.50×10^5	4.35×10^6

*EM_{ZnO} = emulsion with ZnO; EMB = emulsion base (without ZnO); Positive control = yeast not exposed to the EMB.

For *C. parapsilosis,* the EM_{ZnO} provided an average reduction of 97.84 % of the CFU/mL as compared to the positive control, and 100 % for *C. kefyr*, *T. asahii* and *T. cutaneum*. A slightly increased strength was found for *S. cerevisiae* (90.85 %) in relation to the positive control (Figure 1). This yeast is usually considered non-pathogenic, but it has been responsible for more than 5.0 % of bloodstream isolated yeasts (Swinne 2009), especially those admitted to NICUs.

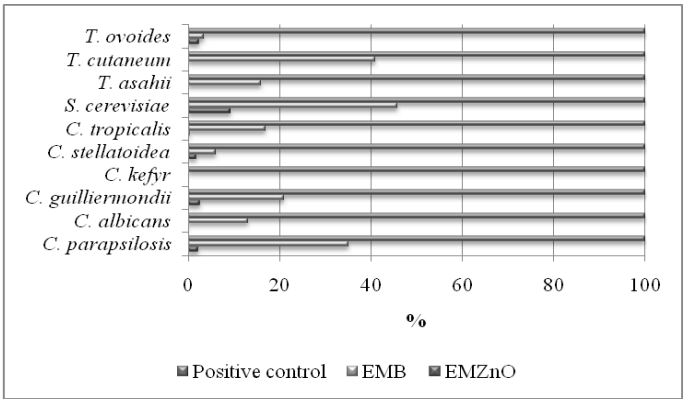

Figure 1: Reduction of the number of colony-forming unit in different yeast strains isolated from hands after contact with EM_{ZnO} and EMB.

To verify whether the antifungal activity was due to the presence of ZnO in the formulation, EMB was also tested. Although less significant, EMB also showed antifungal activity, reducing more than 50 % of the amount of CFU for all studied yeasts. The greatest resistance was also seen for *S. cerevisiae* (54.23 %), but other yeasts showed a more sensitive profile, such as *C. kefyr* that has no grown in the presence of EMB, demonstrating that in this case, there was no need of ZnO. However, its important role should be highlighted. When comparing the results of EM_{ZnO} to EMB, it can be seen that the presence of ZnO promotes a reduction of 40.91 % for *T. cutaneum* (Figure 1).

Overall, comparing the results of EM_{ZnO} to EMB, it can be concluded that the ZnO significantly improves the action of the cream base, causing a reduction of 80.20 ± 15.90 to 98.12 ± 2.78 (%), demonstrating the antifungal potential of ZnO. Therefore, its incorporation in a cream base not only facilitates its use, but also confers other properties such as hydration.

CONCLUSIONS

It is well known that hand washing and glove use reduces the risk of contamination, particularly in facilities of the NICUs. However, concerning the control of transmission of a fungal infection, the use of a product with antifungal properties could be a good alternative. Considering the results presented, this formulation might be a good tool against hospital infections, especially in NICUs. Because the *in vitro* results here presented were promising, a randomized clinical trial can provide conclusive information about the use of this cream. Should be the next step of investigation by our group.

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