

## Prevention of transmission of potentially pathogenic fungi from the leech *H. verbana* used in hirudotherapy

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Hirudotherapy is often used successfully in modern Medicine, especially in plastic and reconstructive surgery. However, as microbial infections are common complications after leech therapy, patient safety depends to a great degree on the microbiological purity of the animals used. The aim of this study was to find a safe and practical way to eradicate potentially pathogenic fungi colonizing the jaws and body surface of the leech *Hirudo verbana*.

A group of 200 leeches was incubated for 24 hours in aqueous solutions with two different concentrations of clotrimazole (0.6mg/L, 20mg/L) or miconazole (2.5mg/L, 50mg/L). Each leech was immersed separately in the solution using a 120mL sterile plastic tube. The control group (50 animals) had no contact with any of the drugs. The effectiveness of eradicating the fungi colonizing *H. verbana* was evaluated 1, 4, 8, 12 and 16 days after incubation. Samples from the jaws and body surface of the leeches were inoculated on Sabouraud Dextrose Agar with chloramphenicol, incubated at 37°C and examined after 24 and 48 hours. From the 50 control leeches, as well as from 50 water samples in which animals were kept, 152 fungal strains belonging to 13 species and four genera were identified. The most frequently isolated fungi were *Candida albicans* (20.4%), *C. parapsilosis* (17.1%) and *C. tropicalis* (13.2%). In total, 46.7% of all strains from six species belonged to the BSL-2 category: *C. albicans*, *C. ciferrii*, *C. krusei*, *C. tropicalis*, *T. asahii* and *T. asteroides*, while 38.2% strains from six species belonged to the BSL-1 category. Incubating leeches in a solution of clotrimazole or miconazole, both at fungistatic and fungicidal concentrations, significantly inhibited the growth of fungi on the body surface and jaws of the leech. Up to the eighth day after eradication, no fungal growth was observed for any type or concentration of the applied drugs. The most effective eradication of the fungi colonizing the leeches was obtained for a higher concentration of clotrimazole: resulting in no fungal growth for up to 12 days. The results of this study show that medical leeches used in hirudotherapy constitute a reservoir of potentially pathogenic fungi, and therefore, such natural therapy is associated with a high risk for transmission of fungi, especially those from the genus *Candida*. The elimination of fungi from *H. verbana* by the method given above prevents fungal infection for up to eight days for patients undergoing hirudotherapy.