Eswab™ for the Collection of Fungal Surveillance Culture from Immunocompromised Patients

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ABSTRACT

Introduction:

Fungal infections are an important cause of morbidity and mortality in immunocompromised patients especially when they are undergoing chemotherapy treatment for cancer or are immune suppressed because of bone-marrow recipients or solid organ transplantation. *Candida* and *Aspergillus* species are the most common fungal pathogens responsible for invasive fungal infection (IFI). Unfortunately, the diagnosis of IFIs remains difficult and it is often confirmed late. The value of fungal surveillance cultures (SCs) as predictors of IFIs is well known and consists of swabs such as throat, nasal and rectal. Copan ESwab is a Liquid Based Microbiology device used in our institution for the collection of all clinical specimens for the diagnosis of infectious diseases including fungal. The objective of this study was to demonstrate the performance of ESwab for the collection of clinical specimens for the detection of fungal and yeast in SCs.

Methods:

The study was carried out on 23148 samples received by the microbiology laboratory of the Tor Vergata Polyclinic starting from May 2009 to December 2015. All samples were collected in Eswab from immunocompromised patients (hematology, oncohematology, Mediterranean Institute of hematology and stem cell transplantation). The SCs for the detection of yeasts and filamentous fungi were performed on nasal swabs, throat swabs and rectal swabs. In particular, 12,499 nasal swabs (54%), 8796 throat swabs (38%) and 1852 rectal swabs (8%) were processed. ESwab specimens were loaded on the WASP, plated on Sabouraud Gentamycin Chloramphenicol 2 agar (SGC2), incubated at 37° C for the first 24h, then maintained at 28° C, plates were observed daily and after 15 days they were reported as negative. If positive, isolates were identified by Vitek 2 System cards (YST, bioMérieux) for yeasts and by microscopic exam (using lactophenol cotton blue stain) as well as MALDI-TOF System (Bruker Daltonics) for filamentous fungi (FI). In some occasion FI required identification by gene sequencing, which was carried out by performing amplification and nucleotide sequencing of 18S rDNA partial gene (using ABI 310 genetic analyzer; Applera). The identifications were achieved by sequence alignment (searching the best consensus sequences) to the universal databases in the NCBI data bank.

Results:

The positive samples were 260 (1.12%), namely: 80.1% throat swabs, 8.1% rectal swabs and 11.8% nasal swabs. The yeasts were mainly represented by *Candida* spp. (90.7%). *Candida albicans* was the most common specie (59.5%) followed by *Candida tropicalis* (15.8%), *Candida glabrata* (9.7%) and *Candida parapsilosis* (5.1%). *Penicillium* was isolated in 2.2% of specimens, 3.2% of the isolates were *Aspergillus* (especially *niger*, *fumigatus* and *nidulans)* while less than 1% (0.8%) was represented by other filamentous fungi. **Two clinical cases were highly significant**: i) a fatal case of invasive fungal sinusitis due to *Scopulariopsis acremonium* in a bone marrow transplant recipient in which the fungus was detected 14 days after the admission of the patient; ii) three clinical cases of *G. clavatum* isolated from central venous catheter and peripheral blood cultures in which the surveillance cultures (particularly the rectal swabs) remained consistently negative even during the invasive infection.

Conclusions:

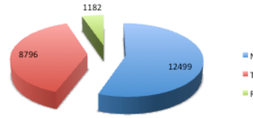
Both *Candida* and FI were easily detected (in an elapsed time ranging from 24-48h and 48h-5 days for yeast and fungi, respectively) from all clinical specimens submitted in Eswab, demonstrating that is a good system for preserving the viability of yeasts and fungi. The very low percentage of positive suirvellance cultures ought to impose a meditation on the real microbiological value of such practice.

METHODS

Polyclinic Tor Vergata
400 beds, teaching Hospital

Hematology, Oncohematology 19 bedsStem Cell Transplantation 13 beds.

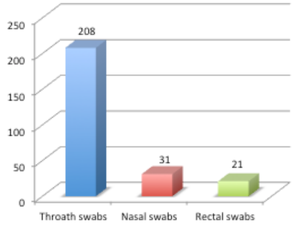
A total of 23148 samples were processed, an average of 345 samples/month. Samples were collected using Eswab™ (Copan) loaded on WASP and plated on Saboraud agar

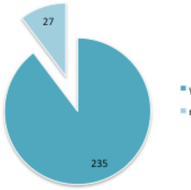


Observed for 15 days

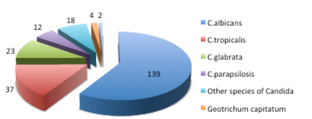
Identification pathway

RESULTS

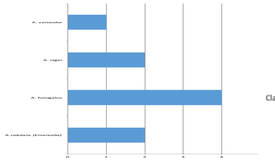


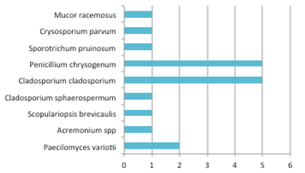


Some specimens contains two isolates



Other species of *Candida*: (*C.guillermondii, C,rugosa, C.kefyr, C.krusei, C.dublinensis, C.incospicua, C.norvegensis, C.shaerica (Kluyveromyces)*)

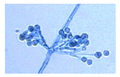




CONCLUSIONS

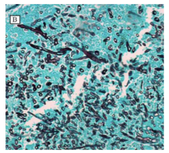
Yeast and molds can be easily detected (in an elapsed time ranging from 24-48h and 48h-5 days for yeast and fungi, respectively) from all clinical specimens submitted in Eswab, demonstrating that is a good system for preserving the viability of microorganisms. However, the result of the rectal swabs in our second clinical case as well as the overall low percentage of positive suirvellance cultures ought to impose a meditation on the real microbiological value of such practice.

CLINICAL CASES



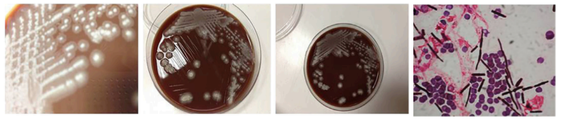
A fatal case of Scopulariopsis acremonium sinus infection in an allogeneic hema-topoietic stem cell transplant patient

Fourteen days after admission, a culture from the sinus secretion was positive for *Scopulariopsis acremonium*



Septate hyphae (Gomori methenamine silver stain) magnification ex.

Rapid vascular diffusion of the fungus to the major head vessels was observed, which led to subsequent repeated cerebral ischemia and death. The presence of hyphae in the right carotid wall might be considered an indirect sign of fungal blood diffusion in the absence of positive blood cultures. The infection developed during the course of prolonged voriconazole prophylaxis, which was found to be effective in the in vitro antifungal drug assay. This finding induced us to consider the capacity of this drug to reach infected paranasal sinuses, and the need in cases such as this of a combined systemic and local pharmacological therapy or a combined medical and surgical approach



Gram stain preparation of blood culture showing arthroconidia and hyphae of *Geotrichum clavatum* (*Saprochaete clavata* syn.) Magnification 1000 X

Figure 3 Forty-eight-hour old culture of *Geotrichum clavatum* on chocolate agar (subculture from blood culture vials).

Here we report the clinical history of three patients affected with hematologic malignancies who developed an infection caused by *Geotrichum clavatum*. Two of 3 patients were affected by acute myeloid leukemia (AML), 1 by mantle cell lymphoma (MCL). In all cases, *G. clavatum* was isolated from central venous catheter and peripheral blood cultures, while the surveillance cultures (particularly the rectal swabs) remained consistently negative even during the invasive infection. *In vitro* susceptibility test confirmed an intrinsic resistance to echinocandins and, in all cases visceral localizations (spleen, liver and lung) were documented by total body CT-scan. A prolonged antifungal therapy with high doses liposomal amphotericin-B was necessary to obtain fever resolution

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