Non-culture-based methods for diagnonsis of invasive candida infection

Rehab Yousry Beltagy Badr

The increasing frequency of invasive fungal infections and the high mortality associated with disseminated fungal disease have underscored the importance of rapid detection of pathogenic fungi. Candida species account for the vast majority of fungal infections. Approximately 80 % of systemic fungal infections are caused by Candida species, with 60% of these infections caused by C. albicans, although there are an increasing number of cases of infection caused by non-C. albicans species such as C. tropicalis, C.parapsilosis, C. glabrata and C. krusei. It was therefore crucial to design a system hat would be able to detect all these species.Also identification of the infecting organism to the species level has become increasingly important as the Candida species distribution changed in recent years with different patterns in their susceptibility to antifungal agents and also the risk of developing deep organ involvement and the severity of Clinical manifestations, differs depending on the infecting species. Also, species-specific identification is relevant for epidemiological purposes.Prompt detection and accurate speciation may help to improve fungal disease management as a whole and lead to more rational use of antifungals. Traditional identification methods based on phenotypic features are often time—consuming and depend largely on the skill and experience of the technician .Diagnosis of fungal infections in immunocompromised hosts has been difficult because of insufficient sensitivity and specificity of conventional culture methods, and also by procedures that depend on the host functioning immune system, but within recent years, novel serological and molecular methods have been developed to improve the early diagnosis of fungal infections which is essential for adequate therapeutic management. This study was designed to evaluate the abilities of non-culture based methods (Enzyme linked immunosorbent assay For detection of Candida antigen mannan and Multiplex,real-time PCR for detection and identification of the five common species of Candida in blood) in the diagnosis of invasive candidiasis in patients with hematological malignancy and to compare their results with those obtained by conventional methods (Blood culture, Germ tube test and Auxacolor method. Forty five patients with hematological malignancies who had febrile neutropenia (1000 neutrophile/ L with a temperature of 38 oC) that had failed to respond to broad spectrum antibiotic therapy after 2 days of treatment (28 males and 17 females). Their age ranged from 15 to 74 years with mean age of 43.3 11.2 years. Blood, urine, sputum and oropharyngeal swabs samples were subjected to Fungal cultures for blood, urine,

sputum and oropharyngeal swabs. Blood culture was done on BD BACTECTM Mycosis-IC/F culture vials(Becton, Dickinson and Company, Sparks, Maryland 21152 USA) and incubated in the BACTEC 9050 continues monitoring system then subcultured on solid media as the other specimens; sputum, urine, and oropharyngeal swabs, all were cultured on plates of Sabouraud's dextrose agar(OXOID LTD., BASINGESTOKE, HAMP SHIRE, ENGLAND) media at 37°C. The results of fungal cultures for blood, urine, sputum and oropharyngeal swabs were as follow 4 out of 45 blood culture (8.9%) were positive for Candida by blood culture technique , 5out of 45 urine samples (11.1%) were positive for Candida by urine culture, 9 out of 45 oropharyngeal swabs (20%) were positive for Candida by culture and 5out of 45 sputum specimens (11.1%) were positive for Candida by culture of sputum. yeast isolates from different culture specimen were identified to the species level by germ tube test and Auxacolor test. The germ tube test could identify 12 (52.2%) Candida isolates out of 23 as Candida albicans and 11 (47.8%) as non-albicans Candida species. The Auxacolor test could identify 22 out of 23 Candida isolates to the species level (11 (47.8%) Candida isolates out of 23 as Candida albicans and 11 (47.8%) as non-albicans Candida species) yeast isolates from different culture specimen were identified to the species level by germ tube test and biochemically by the use of Auxacolor Tm 2 (Bio- Rad Sanofi pasture, FRANCE, Fax 433 (0) 147419133). from the selected 45 patients, 13 (29%) were positive for Candida by Mannan antigen detection test in serum by Enzyme linked immunosorbent assay (Bio-Rad sanofi pasture, FRANCE). The results of Multiplex,real-time PCR for the selected 45 patients showed that 14 out of 45 (31%) whole EDTA (K3E-EDTA K3; Greiner bio- one) blood samples were positive for Candida by by using Light Cycler 1.5 system (Roche) and LightCycler- DNA Master SYBR Green I PCR Kit (Roche Diagnostics). C.albicans (50%) was the most commonly isolated Candida species by Multiplex,real-time PCR followed by C.tropicalis (14.3%), C. glabrata (14.3%), C. parapsilosis (14.3%) and C.krusei (7.1%). Considering Multiplex,real-time PCR as a gold standard, the sensitivity of blood culture technique and Candida Mannan antigen detection test was 28.6% and 92.2% respectively, while the specificity of blood culture technique and Candida Mannan antigen detection test was 100% for both Blood culture method took the most time to detect Candidemia (up to 15 days) while Multiplex,real-time PCR took the least time after extraction procedures (only less than 1 hour)It can be concluded from this study that:• Rapid diagnostic techniques with high degree of sensitivity and specificity should be used for detection of the different Candida species causing invasive candidiasis.• Candida Mannan antigen detection test shows promising results and are easy to perform during a routine day work in most laboratories and the results are obtained within 4 hours after taking samples. But this test can,t differentiate between Candida species.• Concerning the time consumed to diagnose candidemia; blood culture technique took the most time and Multiplex,real-time PCR took the least time taking into account the extraction procedures.• Multiplex,real-time PCR is superior to conventional methods for the diagnosis of candidemia. Provides accurate and reproducible method that combines the enhanced sensitivity and increased specificity, and it is clearly advantageous regarding speed, handling and number of samples that can be analyzed per run and

fulfills the need for rapid identification.• The traditional rapid-screening tests, such as germ tube test remain the initial procedures of choice for identification of clinical yeast specimens.• Auxacolor test may be a useful tool for use in routine clinical microbiology laboratories, due to its ease of setting up and reading, the cost per test, and its performance .• Although C.albicans is the most common isolated Candida species, other species of Candida as C. glabrata, C. tropicalis, C.parapsilosis and C. krusei have emerged as important pathogens.