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Epidemiological trends in *Aspergillus* spectrum involved in human aspergillosis in region of Sousse, Tunisia : A 33-year retrospective study (1986-2019).

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INTRODUCTION AND OBJECTIVE

Epidemiological aspects of aspergillosis are constantly changing. They concern the diseases incidence and their magnitude in terms of public health, the risk factors, the spectrum of species involved in each type of infection and their susceptibility to the antifungal agents. Here, we aimed to study the spectrum of *Aspergillus* involved in human pathology in various clinical samples received in the laboratory of Parasitology-Mycology at Farhat Hached Teaching hospital of Sousse, central Tunisia.

METHODS

Our work is a a retrospective cross-sectional study covering a period of 33 years from January 1st, 1986 to October 31st, 2019.

The study population included all *Aspergillus* strains isolated from human biological samples in the laboratory of Parasitology-Mycology of Farhat Hached University Hospital of Sousse, Tunisia, during the study period. Mycological diagnosis was based on direct microscopy of biological specimens and species identification by culture on Sabouraud-Chloramphenicol and Czapek mediums.

RESULTS

Over the 33-year study period (1986-20119), 1040 *Aspergillus* isolates were recovered from 954 biological samples collected from 879 patients.

The age of these patients ranged from 1 to 94 years with a mean of 46.3 ± 20.3 years and a median of 47 years. Men accounted for 52.5% of the patients.

The *Aspergillus* isolates were recovered from the external auditory canal (48.74%), the lower respiratory tract (36.7%), toenails (8%) and other sites (6.56%) (Table 1).

The direct examination was positive for 516 (54.1%) samples. It showed only mycelial filaments for 350 (36,7%) specimens (Figure 1), yeasts for 85 (8,9%) specimens, mycelial filaments with yeasts for 64 (6.7%) specimens, mycelial filaments with *Aspergillus* heads for 17 (1.8%) specimens.

A single *Aspergillus* species was isolated in 725 samples (76%), two species in 65 samples (6.8%) and three species in samples (1.26%). The most frequently isolated species were *Aspergillus* (*A*). *flavus* (40.6%) (Figure 2) and *A. niger* (34.6%), followed by *A. fumigates* (8.9%), *A. terreus* (5.4%) , *A. nidulans* (4.7%) and *A. versicolor* (1.5%). The species was not identified for 16 (1.5%) isolates (Table 2).

Table 1 : Distribution of samples according to the anatomic site of isolation.

Sampling site	Number (%)
External auditory canal	465 (48.74%)
Lower respiratory tract	350 (36.7%)
Toenails	77 (8%)
Nose	13(1.4%)
Surgical wound	7(0.7%)
Oropharynx	7(0.7%)
Ocular	9(0.9%)
Sinus	6(0.6%)
Peritoneal fluid	4(0.4%)
Brain	2(0.2%)
Paraspinal abscess	1(0.1%)
Cutaneous	1(0.1%)
Total	942

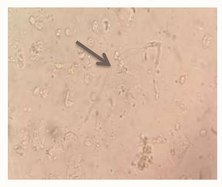

Figure 1 : Direct examination showing mycelial filament.

Figure 2 : Colony of *A. flavus*.

Table 2 : Distribution of *Aspergillus* species.

Species	Number (%)
A. flavus	422 (40,6%)
A. niger	360 (34.6%)
A. fumigatus	93 (8.9%)
A. terreus	56 (5.4%)
A. nidulans	49 (4.7%)
A. spp	16 (1.5%)
A. versicolor	16 (1.5%)
A. ochraceus	7 (0.7%)
A. sclerotium	6 (0.6%)
A. tamarii	4 (0.4%)
A. candidus	3 (0.3%)
A. sydowi	2 (0.2%)
A. clavatonanicus	2 (0.2%)
A. alliaceus	1 (0.1%)
A. avenaceus	1 (0.1%)
A. glaucus	1 (0.1%)
A. chevaleni	1 (0.1%)
Total	1040

DISCUSSION AND CONCLUSION

Aspergillus spp. are ubiquitous molds widely distributed in the environment worldwide. In human, they can be responsible of syndromes with a remarkable diversity. In fact, the spectrum of aspergillosis includes subacute or chronic localized infections (pulmonary aspergilloma, sinusitis, external otitis, etc.), allergic disorders (allergic bronchopulmonary aspergillosis, extrinsic allergic alveolitis, eosinophilic rhinosinusitis), locally invasive infection (keratitis) and invasive life-threatening infections in immunocompromised patients [1].

In our work, the two main sampling sites were the external auditory canal and the lower respiratory tract but unusual sites have been also rarely observed such us brain, peritoneum or paraspinal location. The culture of *Aspergillus* is becoming increasingly important for the diagnosis but must be interpreted in context of the risk for the patient [1]. Direct examination was positive in only 516 (54.1%) samples. Other tests such as PCR and serological markers (galactomannan, B-glucan) are needed to better diagnosis [2].

In developed countries, *A. fumigatus* causes the majority of aspergillosis cases, followed by *A. flavus*, *A. niger*, *A. terreus* and *A. nidulans* [1]. However, in many developing countries, *A. flavus* and *A. niger* are the predominant species. We found that *A. flavus* is the most common specie in our region followed by *A. niger* and *A. fumigatus*.

These findings are very useful to more accurate selection of empirical antifungal therapy in aspergillosis management. Antifungal susceptibility of different species is not the same, but testing techniques are not always available.

This study highlights the remarkable diversity of manifestations caused by *Aspergillus* species in human and the prevalence of different species in our region. The noteworthy differences in the frequency of *Aspergillus* species, their degree of involvement in the various type of infections emphasize the importance of the local epidemiology assessment so as to develop more adapted therapeutic strategies in so far as a new therapeutic alternatives are offered.

Thus, there is a need to develop a greater understanding of the pathogenesis of the disease, formulate better and more sensitive diagnostic techniques, develop superior antifungal agents and increase an awareness of the disease among clinicians [3].

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