

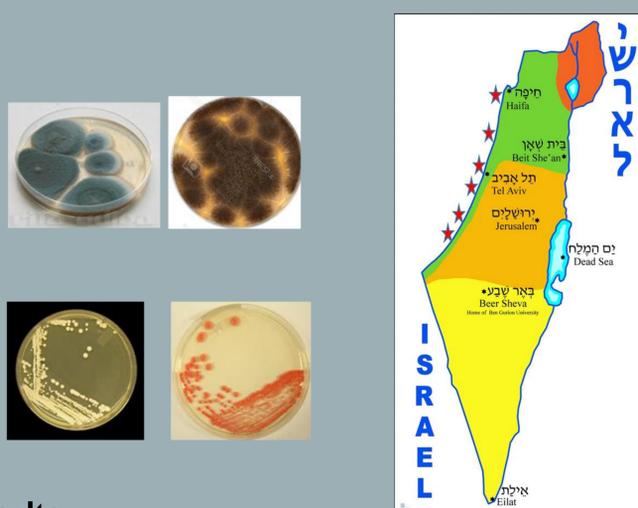
M. Frenkel<sup>1</sup>, Y. Yunik<sup>1</sup>, S. Blum<sup>2</sup>, D. Elad<sup>2</sup>, E. Sionov<sup>3</sup> E. Segal<sup>1</sup>

<sup>1</sup>Department of Clinical Microbiology and Immunology, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel;  
<sup>2</sup>Department of Clinical Bacteriology and Mycology, The Kimron Veterinary Institute, Bet Dagan, Israel;  
<sup>3</sup>Institute for Postharvest and Food Sciences, Agricultural Research Organization, The Volcani Center, Rishon LeZion, Israel.

**Introduction and Aim :** This study is part of a collaborative research in the frame of an ECMM-EFISG-ISHAM working group consisting of over 20 laboratories, which aims to explore fungal contamination in beach-sands around the Mediterranean Sea and other water bodies, in view of possible impact on human health. Fungal sand contamination and the possible impact on human health were explored regarding three aspects: 1. Fecal contamination, as judged by presence in sand of the human GI commensal – *Candida albicans* and other *Candida species*; 2. Contamination by fungi known for involvement in dermal infections, such as Dermatophyte species; 3. Presence in sand of various molds which may possibly be causes of respiratory allergies, such as Aspergillus, Penicillium, Mucorales species and/or other molds.

The present report relates specifically to the presence of Aspergillus and Mucorales species in sands of beaches around the Mediterranean Sea in Israel.

**Methods:** Six beaches from North to South (Haifa, Keisaria, Tel Aviv, Palmachim, Ashdod, Ashkelon) were examined. Based on a technique described previously by Brandao and colleagues, water- extracts of sand samples collected at the 6 sites were cultured on standard mycological media. The fungal load in sand was evaluated quantitatively by enumeration of colony forming units (CFU) of the fungi isolated from the sand samples. The fungi were identified phenotypically, spectrally by MALDI –TOFF mass spectroscopy using the Bruker system (with two data bases), and by ITS sequencing (not all isolates).



## Results

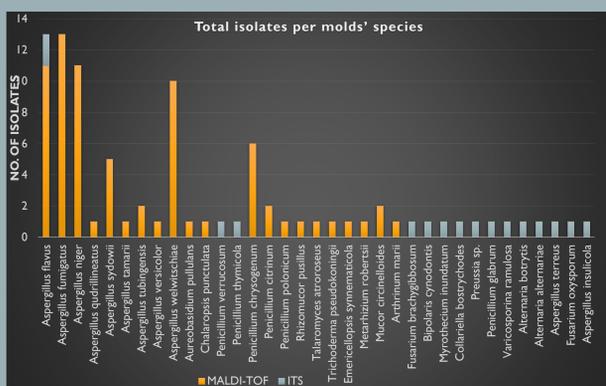
The 3 rounds of samplings of the sands from the 6 beaches during one year yielded 157 fungal isolates, consisting of 125 molds (79.6% of total isolates) and 32 yeasts (21.4%). The major genera of the molds were Aspergillus and Penicillium consisting of a total 71 isolates.

### Fungal isolates

	Identified	Non-Identified (level of species)	Total	Growth at 37 °C
Molds	83 (66% of total)	42	125	38 (30.4%)
Yeasts	24 (75% of total)	8	32	15 (46.8%)
Total	105 (67% of total)	52	157	53 (33.7%)

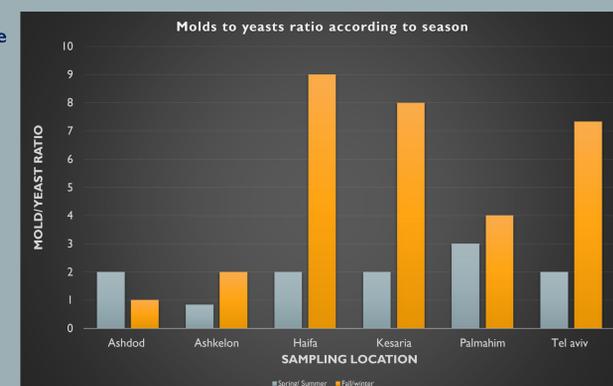
#### Comments :

- Identification of environmental fungi is difficult, particularly for molds, even by MALDI-TOF system, as it depends on the available data bases, which generally are for clinical use.
- Use of ITS sequencing increases percentage of identification
- The majority of the isolates are molds (79.6% of total isolates)
- Most of the molds (69.6%) do not grow well at 37°C and may be considered environmental fungi, while almost half of the yeast can grow at 37°C.



The identified molds could be assigned to 35 fungal species, out of which were 11 Aspergillus species, including a total of 60 Aspergillus isolates. The major 4 Aspergillus species found in sand were *A. fumigatus* (13 isolates), *A. flavus* (13 isolates), *A. niger* (11 isolates), and *A. welwitschiae* (10 isolates) and a single isolate of *A. terreus*. Most of the Aspergillus isolates grew well at 28°C and 37°C. Mucorales species, including *Mucor circinelloides* and *Rhizomucor pusillus* and a variety of other mold species were also found in the sands.

In 5 of the 6 screened beaches the proportion of molds to yeasts was higher in the fall/winter period. Fall/winter in Israel is generally characterized by lower temperature and humidity, which may contribute to the increased presence of molds.



### Summary and Conclusions:

All these species are known human opportunist pathogens and also known as potential allergens. Thus, these findings may have relevance as potential environmental source for infections in immunocompromised individuals and be of significance as exposure source for asthmatic individuals. Furthermore, while regulations for beaches are partially available regarding bacterial contamination, they are non-existent for fungal contamination. Hence, data on fungal contamination of beaches may serve as a basis for development of regulatory measures.