21st ISHAM Congress Delhi, India September 2022

Session 3.5: <u>Environmental Exposure - Risk for Human Disease</u>



Fungal contamination in sand and water of sea, lakes and river beaches: risk factor for fungal human disease

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INTRODUCTION – A short history of the "Environmental Fungi and Health Risk" Group

- In 2018 at an ECMM Council Meeting, the Israeli delegate, Esther Segal, proposed a "new study topic":
 "Exploration of Fungi in Sand around the Mediterranean coast"
- □ The suggestion was accepted by the ECMM Council and "A call" for participation was published
- Very soon a large group of members from the different ECMM countries joined that Group and it became known as the "Sand Project Group"
- Today the group includes: ~26 members representing 12 countries from various areas in Europe and Australia, exploring various sea and fresh waterbodies as to mycobiota in sand and water, and having plans for future research in this area
- The "Sand Group" received inspiration from an environmentalist acquainted with Esther Segal, Joao Brandao, who was employed at the National Health Institute in Portugal
- Joao worked on various regulatory issues in regard to health safety of beaches around Portugal, both in regard to sand and water
- So Joao became one of the "Trio" leading the sand-water group
- > Jean-Pierre Gangneux, a physician at the University of Rennes, France, is the third corner of the "Trio"
- Jean-Pierre' interests are focused, in addition to the Sand-Water Mycobiota, also on environmental issues of the hospital environment







I would like in the first part of my talk to introduce the Mycosands initiative.

Between 2018 and 2020 a pan-European team covering:

- ***** The Mediterranean sea
- ***** The Atlantic Ocean
- ***** the Black Sea
- ***** The Baltic Sea
- * The Adriatic Sea
- ***** The Italian Lakes
- Plus one team in Sydney (Pacific Ocean), who covered three local bathing sites, including Bondi beach.

All collected samples, and processed them to explore fungi in sand of all the beaches; and partially also in water

Mycosands -2/5 (Sampling sites)



Geographical distribution of the sampling points using mapping with QGIS (Version 3.10.0-A Coruña). Circles correspond to urban beaches and diamonds to non-urban beaches. Dots within the shapes indicate water-sampling sites. Red=Northwest Europe, Green=Southwest Europe, Blue=Mediterranean, Brown=Black Sea and Purple=Sydney (Australia)

Mycosands - The Working Procedure

Sampling, culture, fungal identification



Mycosands – Results of sand

- Median number of all fungi in any beach sand ('All Fungi') is 89.2 CFU/g*
- All Fungi and Yeasts correlate negatively to the hours of sunshine
- Inland beaches have higher counts than coastal beaches (2017.0 vs 76.7 CFU/g)
- Composition of mycoflora differs between coastal and inland beaches.
- Hotter climates favour presence of fungi in sand.
- Fall/Winter present higher counts of fungi in sand than Spring/Summer.
- Urban and non-urban beaches have different mycoflora composition

*Integrated (rounded to 90 CFU/g) in the new WHO guidelines – chapter 8

Mycosands – Results of water

- Inland beach waters have higher presence of fungi then coastal waters, including *C. albicans* (informal mainly human faecal pollution indicator)
- Fungi and Yeasts correlate negatively to the hours of sunshine
- Fall/Winter present higher counts of fungi in water than Spring/Summer.
- Higher prevalence of 'All fungi' in non-urban waters

Mycosands – Conclusions

- Both sand and water should be monitored for fungi
- The median value of 89 CFU/g of all fungi may serve as a reference for sand regulation
- *Candida albicans*, dermatophytes, endemic fungi and other fungi should be considered
- Fungal analysis of water needs more data before reference values can be established

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Mycosands: Fungal diversity and abundance in beach sand and recreational waters - Relevance to human health

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Mycoses

ORIGINAL ARTICLE

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Fungi in sands of Mediterranean Sea beaches of Israel—Potential relevance to human health and well-being

Michael Frenkel, Yaron Yunik, Marcelo Fleker, Shlomo E. Blum, Edward Sionov, Daniel Elad, Hanan Serhan, Esther Segal 🔀

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Part II: WHAT IS HIDING IN THE ISRAELI MEDITERRANEAN SEA-WATER AND BEACH SAND

Sampling of sand from beaches representing various areas of the Israeli Mediterranean Sea coast, as shown in the map, has been carried out –continuing the preliminary former publication.

North to South screened beaches:

- ✤ Haifa
- Kesaria
- Tel Aviv
- Palmahim
- Ashdod
- Ashkelon



The Survey

Samples of sand and water were collected and processed by the following procedure:

- Isolation of fungi from sand and from water
- Identification of the isolated fungi from sand and water (MALDI TOFF-MS, partially by ITS)
- Quantitation of fungi in sand and water by Enumeration of CFU/gr sand and CFU/ml of water

The following slides will detail the results of this survey







Total No. of fungal isolates in sand & water

Some comments to fungi in sand and water

As judged by these data, the two major conclusions are:

- ✓ Sand harbor more fungi than water, possibly due to effect of salinity or dilution effect
- ✓ Both sand and water harbor more molds than yeasts

7 6 6 No.of Isolates 5 4 4 3 3 3 3 2 2 2 2 2 2 1 0 Candida Clavispora... CNPTOCOCCUS... Candida Buillermondii Rhodotorula mucilaginosa Collariella bostrychodes Candida tropicalis candida peliiculosa Candida Parapsilosis Candida lipolytica CNPtococcus Albidus Rhodotorula Blutinis Candida metapsilosis candida albicans candida atricana candida lusitaniae vodaneae Ohimeri Rhodotorula SP.

No. of isolates/ Yeasts species in Sand & water

Sand Water

Some Comments on Yeasts in Sand and Water

- Sand harbored yeasts of the genera Candida, Cryptococcus, Rhodotorula and Kodamea. From water only 3 Candida species were isolated.
- In sand 10 species of Candida, including *C. albicans* were found. In water only 3 species were found, not including *C. albicans*.
- The environmental Cryptococcus albidus found in sand, is known as rare cause of cryptococcosis in immunocompromised patients. An even rarer Cryptococcus species, Cr. uzbekistanensis was also found in sand.
- Kodamea ohmeri is an emerging fungal pathogen in immunocompromised patients. Kodamea ohmeri was isolated from sand.



Molds in Sand & Water



Some comments on molds in sand and water

- ✓ Molds constitute >85% of fungal isolates from sand and water
- ✓ The largest group of the isolated molds were the Aspergilli
- ✓ The Aspergilli were the only group of fungi isolated both from sand and from water
- ✓ The second large group found in sand were the *Penicillium species*
- ✓ They were followed by *Fusarium species* and Mucorales
- ✓ Of note, a single isolate of Mucorales was found in water
- ✓ The rest of the molds found only in sand were single isolates representing 7 different species

Aspergillus species	Molds in water	Molds in sand	Mucorales spices	Molds in water	Molds in sand	Penicillium species	Molds in water	Molds in sand	Fusarium species	Molds in water	Molds in sand
Asp.tubingensis	13	47	Rhizopus arrhizus	1	1	Pen. chrysogenu m		5	Fusarium		1
Asp. welwitschiae	10	31	Mucor circinelloides		1	Pen. citrinum		1	Fusarium petroliphilum		1
Asp.niger	4	17	Rhizomucor pusillus		1	Penicillium/ Paecilomyce s		1	Fusarium brachygibbosum		1
Asp. flavus	4	17				Pen. rubens		2	Fusarium solani		1
Asp. fumigatus	1	5				Pen. polonicum		1			
Asp. oryzea		1				Pen. thymicola		1			
Asp. astellatus		1				Pen. verrucosum		1			
Asp. cs.costaricrnsis		1									
Asp. flavus\oryzae		2									
Asp. luchuensis		1									
Asp. puniceus		1									
Asp. sydowii		3									
Asp. tamarii		1									
Asp. terreus		4									
Asp. versicolor		1									

More comments on molds in sand and water

Table 1 provides some further details on molds in sand and water:

- The largest group among the Aspergilli are the "black" species: A. tubengensis, A. welwitschiae,, A. niger
- All these species were found both in sand and in water, albeit in lower numbers in water than in sand
- Another significant group of Aspergilli found both in sand and in water are isolates of A. flavus and A. fumigatus.
- > Penicillium chrisogenum was the most common Penicillium species found in sand
- > The genus Fusarium was represented by single isolates of 4 species found in sand









F. solani

FUNGAL LOAD

Fungal load was assessed by enumeration of colony forming units (CFU) in all the six beaches surveyed. This is demonstrated in the next slides of beaches: in the combined panel of beaches and in the slides of individual beaches (from north to south)















Comments on fungal load in sand and water

- The CFU determination of fungi in the two systems: sand and water, strengthens the qualitative observation, that sand is the medium that harbors higher levels of fungal units (CFU), e.g. higher fungal load.
- Thus, sand is of increased relevance as source of potential human contamination by fungi than recreational seawater.
- Furthermore, although there are differences between the beaches, the fungal load is higher for molds in all screened beaches than for yeasts, which may indicate the increased relevance of human contamination by mold species over that of yeast species.

SUMMARY

The survey resulted in 232 fungal isolates:

□ Molds were more abundant than yeasts in sand and in water

CFU counts indicated higher load of molds than yeasts in sand and in water

□ Fungal species diversity of mold and yeast species in water was lower than in sand

U Yeasts and molds may cause disease in humans

□ Molds may also cause human allergies

CONCLUSIONS

- * Taken together, the data of this survey indicate that recreational water bodies and surrounding beach - sands may harbor fungi with potential risk for human morbidity, particularly for immunocompromised individuals.
- Hence, beaches and water bodies should be monitored in reference to fungal contamination

THANK YOU for your attention

