MULTICENTER STUDY ON ISOLATION PROCEDURE, SPECIES IDENTIFICATION AND CLINICAL SIGNIFICANCE OF ASPERGILLUS SPP., Scedosporium Spp., AND OTHER FILAMENTOUS FUNGI (FF) IN ITALIAN PATIENTS WITH CYSTIC FIBROSIS: PRELIMINARY RESULTS.

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ITALY

First Meeting of the ISHAM Working Group on "Fungal respiratory infections in Cystic Fibrosis"
Angers (France), 2009, June 7th - 8th
Aspergillus spp. and other FF are known to be colonizers of the respiratory tract of CF patients and can cause several forms of pulmonary disease ranging from the allergic bronchopulmonary aspergillosis (ABPA), Aspergillus bronchitis to invasive fungal disease in lung transplant patients.

very few data about prevalence, species distributions and clinical significance of FF in pulmonary infections in CF are available in Italy.
In Italy are increasing:

• the reports of isolation rate of FF from CF specimens is in the clinical microbiology laboratories

• the number of CF pts with predisponing factors, increasing susceptibility to fungal colonization/infection (median pts age, frequent and prolonged of antibiotic therapy courses, therapy with corticosteroid)

• the interest of clinicians to better understand the possible role of FF pathogens in pulmonary infections, particularly in CF pts with decreased pulmonary functions and exacerbations
Variation of FF occurrence in specimens are described in Italian CF clinical microbiology laboratories

- Absence of standardised mycological diagnostic protocols specifically addressed for CF specimens
- Type and organisation of the CF microbiology labs, as usually part of a general clinical microbiology laboratory routine service, with low familiarity with mycological procedures
- Different mycological skill/attitude of microbiologists for mycology
- Different species identification level among laboratories: some report only generically FF, the most recognise only the genera level, only few identified FF isolates at species level
- The results is a difficulty to assess and to compare the prevalence of FF in pts attending different CF Care Centres at national and international level, as well to study the importance of these pathogens on clinical course in the colonised pts.
The Italian Society for the Study of CF (SIFC) with the Working Group for CF Microbiology (Coordinator G. Manno, Genova) recently is promoting a national program with the aim to:

• improve the microbiological diagnosis of FF from CF specimens in all Clinical Microbiology Laboratories working for the 39 CF Care Centres located in Italy.

• Set up a collaborative study between clinical microbiologist and clinicians involved in the care of Pulmonary Infections in CF in order to better understand the clinical significance of FF recovered in pts.

• Standardisation of test and criteria for the ABPA diagnosis.

• Determine the epidemiology of FF infections in italian CF pts.

• Compare the italian data with the epidemiology of others european and non-european countries.

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Steps of the Italian program for study FF Infections in CF

• Distribution of a questionnaire to assess the current mycological procedures used in CF microbiology labs

• Issue and distribution of recommendation for microbiological procedures in CF, including appropriate mycological methods

• Arranging at least three training courses/years located in different Italian area, for CF microbiology including a session specifically addressed for Mycology procedures in CF

• QC samples distribution to laboratories for FF species identification

• Foster FF strains collections among laboratories for future studies and strains genotyping
Mycology Reference Centres for FF species identification in CF

Prof. Viviani - University of Milan

Dott. E. Manso - Ancona

Hospital

Prof. M.T. Montagna - University of Bari

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Preliminary data about FF prevalence in specimens of 870 Italian CF pts

- CF Care Centre of G. Gaslini Institute in Genova: 220 pts regularly in follow up

- CF Care Centre of Maggiore Hospital - Policlinics Mangiagalli of Milano: 650 pts regularly in follow up
Procedure for mycological examination of sputum samples used

• Sputum cultures were cultivated with conventional protocols for CF specimens

• pre-treatment of the sample for 30 min at 37°C, in the presence of an equal volume of an aqueous solution of ditiotreitole (Sputasol)

• quantitative inoculation: 20 µl per plate for pre-treated samples in 3 Sabouraud+gentamycin+CAF agars plates incubated at 37° and 20 - 25° for 14 days

• species identification was determined by macro and microscopic morphological examination.
## FF species (%) distributions in CF specimens - 6 months (from 1 December 2008- to 31 May 2009)

<table>
<thead>
<tr>
<th>Species</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. fumigatus</td>
<td>63</td>
</tr>
<tr>
<td>A. flavus</td>
<td>10</td>
</tr>
<tr>
<td>A. terreus</td>
<td>9</td>
</tr>
<tr>
<td>A. niger</td>
<td>1</td>
</tr>
<tr>
<td>A. nidulans</td>
<td>0,4</td>
</tr>
<tr>
<td>Scedosporium spp.</td>
<td>7</td>
</tr>
<tr>
<td>Mucor spp.</td>
<td>0,3</td>
</tr>
<tr>
<td>Penicillium spp.</td>
<td>6</td>
</tr>
<tr>
<td>Alternaria spp.</td>
<td>1,25</td>
</tr>
<tr>
<td>Scopulariopsis spp.</td>
<td>0,4</td>
</tr>
<tr>
<td>Pecilomices spp.</td>
<td>0,85</td>
</tr>
<tr>
<td>Rhizopus oryzae</td>
<td>0,25</td>
</tr>
<tr>
<td>Cladosporium spp</td>
<td>0,25</td>
</tr>
<tr>
<td>Sporotricum pruinosum</td>
<td>0,25</td>
</tr>
</tbody>
</table>
FF species distributions in Genova and Milan

Others: Mucor, Alternaria spp., Scopulariopsis spp., Pecilomices spp., Rhizopus oryzae, Cladosporium spp. Sporotrichum pruinosum

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FF in CF pts in Genova

• 35/220 (15.9%) of pts where colonized by filamentous fungi:
• In 5 pts with FF and bacterial chronic colonization the recovering of FF in high number in sputa was correlated with pulmonary exacerbations and received antifungal with antibacterial therapy
  • A. fumigatus (3 pts)
  • A. flavus (1pts)
  • and Sporotrichum pruinosum (1 pt).
Sporotrichum pruinosum
an unusual chronic colonisation in FC pts

reported to be allergenic
• observed in the respiratory secretions of some human patients, indicating a possible ability to colonize the bronchopulmonary pathways
• rarely found in cooler regions, Sporotrichum is prevalent in warm-temperate and tropical zones, and grows (rapidly) on soils, decaying plant matter, wet or rotting wood, grasses, and landscaping mulch
• colonies may appear white, rosy-beige, or orange, and have a velvety to granular texture

S. pruinosum was repeatedly isolated in high amount and iphae observed directly in sputa of a 16 years old female patient (also chronically colonized by P. aeruginosa and S. maltophilia) from 2006 to 2009 concomitantly with pulmonary infection exacerbation; the clinical condition patients improved after antifungal therapy (voriconazole).

Sporadically recovered together with ad S. apiospermum and A. fumigatus and Candida spp.

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Conclusions

• These preliminary data confirm that fungal colonization is common in CF pts in Italy.

• This colonization may leads to clinical deterioration when allergy develops.

• The significant rate of *Aspergillus* non-*fumigatus* and *S. apiospermum* recovered, confirm that these species, resistant to antifungal drug, are increasing in CF.

• More study are needed in order to compare mycological results with the clinical outcome of colonized patients. Fungal infections was rare, but may be underdiagnosed
THANKS

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Lisa Cariani - Milano
Arianna Biffi - Milano
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Priscilla Cocchi - Firenze
Nicoletta Rivenni - Fierenze
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Dianella Savoia - Torino
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Fulvia Gioffrè - Soverato
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