A European ECMM-ESCMID survey on goals and practices for mycobiota characterization using Next Generation Sequencing

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A European ECMM-ESCMID survey on goals and practices for mycobiota characterization using Next Generation Sequencing

Short title: NGS for mycobiota characterization in Europe

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Abstract

Background. Although substantial efforts have been made to investigate about the composition of the microbiota, fungi that constitute the mycobiota play a pivotal role in maintaining microbial communities and physiological processes in the body.

Objectives. Here, we conducted an international-survey focusing on laboratory's current procedures regarding their goals and practices of mycobiota characterization using NGS.

Methods. A questionnaire was proposed to laboratories affiliated to working groups from ECMM (NGS study group) and ESCMID (ESGHAMI and EFISG study groups). Twenty-six questionnaires from 18 countries were received.

Results. The use of NGS to characterize the mycobiota was not in routine for most of the labs (N=23, 82%) and the main reason of using NGS were primary to understand the pathophysiology of a dysbiosis (N=20), to contribute to a diagnosis (N=16), or to implement a therapeutic strategy (N=12). Other reported reasons were to evaluate the exposome (environmental studies) (N=10), or to investigate epidemics (N=8). Sputum is the main sample studied, and cystic fibrosis represent a major disease studied via the analysis of pulmonary microbiota. No consensus has emerged for the choice of the targets with 18S, ITS1 and ITS2 used alternatively among the labs. Other answers are detailed in the manuscript.

Conclusions. We report a photography of mycobiota analysis that may become a major tool in the near future. We can draw some conclusions on the diversity of approaches within the answers of the 27 labs and underline the need for standardization.

Key words: Microbiota, Mycobiota, fungi, Europe, Lung infections, Digestive tract infections

Main text

The human body hosts billions of commensal, symbiotic and pathogenic microorganisms, mainly bacteria, known as the human microbiota. In recent years, substantial efforts have been made to investigate about the composition of the microbiota in the different body sites [1,2]. Although the contribution of fungi is limited to approximately 0.1 % of the total microbiome (Mycobiota has been evaluated at less than 0.1% in human feces upon the European MetaHIT project), it is thought that fungi play a pivotal role in maintaining microbial communities and physiological processes in the body [3]. Host-microbiome interactions are responsible for development of several disorders. It becomes clearer that microbiota, including fungi, are in balance with each other as well as with the host and that disturbance of this balance can be involved in numerous disease development including the Gut-Brain axis [4,5]. Next generation sequencing (NGS) techniques have clearly renewed methodology to characterize host-associated microbiota in a more comprehensively way, and have consequently revealed a much more diverse microbiota than was previously thought to exist [5-7]. Full characterization of the microbial community in different sites of the body can give indications on the severity of fungal infections [8].

As human microbiome has emerged as an important but complex trait influencing health and diseases, we aim at generating new efficient knowledge on mycobiome. Therefore, we constituted a panel of experts from the European Society of Clinical Microbiology and Infectious Diseases (ESCMID, ESGHAMI and EFISG study groups) and the European Confederation of Medical Mycology (ECMM). As a primary objective, we addressed an international-survey focusing on laboratory's current procedures regarding their goals and practices of mycobiota characterization using NGS. Secondly, we propose a prospective multicenter study, Stand-MYC project, that will address key points regarding fungal targeted metagenomics. Here we summarized the survey data. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as the research in this article related to a survey.

The survey included twenty questions, was addressed by internet, and completed by a total of 27 laboratories from 14 European countries (Figure 1), Iran, Mexico, Singapore and Thailand. The laboratories reported their main reason of using NGS to study the mycobiota primary to understand the pathophysiology of a dysbiosis (N=20), at a second level, to contribute to a diagnosis (N=16), or to implement a therapeutic strategy (N=12)(Table 1). Other reported reasons were to evaluate the exposome (environmental studies) (N=10), or to investigate epidemics

(N=8). The use of NGS to characterize the mycobiota was not in routine for most of the labs (N=23, 82%), while among the remaining (N=5, 18%) who were using it in routine, their main objective was to improve diagnosis. For about half of the labs (N=13, 46%), NGS was used in experimental studies on mycobiota mainly to improve diagnosis (N=12), or to implement a therapeutic strategy (N=7), to evaluate the exposome (N=7), and investigate epidemics (N=5).

Respiratory tract represented the most studied site, followed by the digestive tract, the distribution of the different diseases addressed by NGS in both lung and digestive tract are shown in Table 1, and were in agreement with the recent bibliography highlighting the NGS use in chronic respiratory diseases and inflammatory bowel disease. Sample originated in half of the laboratories from sputum (N=14), or from stool (N=12), broncho-alveolar lavage (N=11), environmental (N=6), oral wash (N=4) and skin (N=1). A majority of labs (N=22) considered that bacterial microbiota must be associated when studying the mycobiota, similarly for the viral microbiota, which they considered that should be associated as well (N=15).

Considering the technical level, our survey exhibited a huge diversity on molecular methods and protocols, as previously reported [7,9]. Target used for mycobiota analysis was equal among 18S rRNA (N=11), ITS1 (N=11) and ITS2 (N=11), while some centers used the shotgun approach (N=5). When asked about data analysis,, database used for alignment were mostly UnitE (N=8 labs), and both Genebank and CBS databases (N=7 labs), while 3 only labs reported that they excluded the reads shorter than 150 nucleotides from the bioinformatics analysis, as usually proposed [8, 10–12]. Finally, only one laboratory declared using propidium monazide pre-treatment. This last point underlined the weakly opened debate on valuable pretreatment and extraction methods in order to guarantee the best analysis of the results.

Therefore, the Stand-MYC study proposal will be made by the ECMM NGS working group and ESCMID Fungal Infection Study Group (EFISG) & ESCMID Study Group for Host and Microbiota Interaction (ESGHAMI) study groups. The aims of the Stand-MYC study are: 1) to compare the DNA extraction protocols used by the different teams involved in the project on the same human samples as well as on an artificial community of fungi (fungal mock), and a negative control of DNA extraction, 2) to quantify differences in observed microbial community compositions according to the different extraction methods used, and ultimately 3) to recommend an efficient consensual DNA extraction protocol that will help to confidently assess the contributions of mycobiome to human health or diseases.

In conclusion, NGS to study mycobiota is a great challenge for understanding pathophysiological questions in experimental studies as well as for driving diagnostic strategies or investigate an

environmental source of exposure [13]. Techniques will rapidely develop, moving from targeted NGS to shotgun approaches and whole genome sequencing will also be very useful in the next years in the field of mycology, but also for an holistic reasoning. The diversity of technical approaches underline the need for standardization if we want to compare comparable data. Results of the Stand-MYC study will also help laboratories to standardize their protocol and to facilitate inter-laboratory comparisons of the results that may become essential for the robustness of such analysis. Efforts must also be done to standardize the bio-informatic step.

Appendice 1 : The authors want to warmly acknowledge colleagues from the ECMM-ESCMID (ESGHAMI & EFISG) NGS study groups who accepted to answer to the survey (27 labs from 18 countries) : **Austria** (Michaela Lackner, Innsbruck), **Czech Republic** (Tomas Freiberger, Brno), **Denmark** (Rasmus Hare, Copenhagen; Klaus Leth Mortensen, Aarhus), **Iran** (Mohammad T. Hedayati, Sari), **Ireland** (Gary Moran, Dublin), **Germany** (Maria Vehreschild, Cologne; Jörg Steinmann, Nueremberg), **France** (Laurence Delhaes, Bordeaux; Françoise Botterel, Paris; Jean-Pierre Gangneux, Rennes)**, Greece** (Emmanuel Roilides, Thessaloniki), **Israel** (Ronen Ben-Ami, Tel Aviv), **Italy** (Giuliana Lo Cascio, Veneto), **Mexico** (Rogelio de Jesús Treviño-Rangel, Monterrey, Nuevo León), **Netherlands** (Jacques Meis, Nijmegen; Willem Melchers, Nijmegen; Bastian Hornung, Leiden), **Portugal** (Raquel Sabino, Lisboa), **Serbia** (Aleksandra Barac, Belgrade), **Singapore** (Sanjay H Chotirmall), **Spain** (Jordi Rello, Barcelona; M.Teresa Martin-Gomez, Barcelona; Ana Alastruey-Izquierdo, Madrid), **Thailand** (Thanwa Wongsuk, Bangkok), **Turkey** (Zeynep Ceren Karahan, Ankara)

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Table 1. Results of the survey on goals and practices for mycobiota characterization using Next Generation Sequencing in European laboratories (N=27 laboratories in 18 countries).

Questions	**Answers (%)**
Goals of using NGS to study the mycobiota	- Understand the pathophysiology of a dysbiosis : 71% - Contribute to a diagnosis : 57% - Implement a therapeutic strategy : 43% - Evaluate the exposome (environmental studies) : 36% - Investigate epidemics : 33%
Mycobiota sites studied or planned to be studied	- Lower respiratory tract : 54% - Digestive tract : 39% - Upper respiratory tract : 36% - Environmental samples : 25% - Oral cavity : 11% - Skin : 3%
Samples used for NGS processing	- Sputum : 50% - Stool : 43% - Broncho-alveolar lavage : 39% - Environment samples : 21% - Oral wash : 11% - Skin 3%
Lung diseases to be studied by NGS	- Cystic fibrosis : 50% - Lung transplantation : 32% - COPD : 29% - Asthma : 29% - Pneumonia : 29%
Digestive tract diseases to be studied by NGS	- Crohn disease : 18% - Pseudomembranous colitis : 14% - Chronic ulcerative colitis : 11% - Other (<10%) : rheumatoid polyarthritis, diabetis mellitus, AIDS…
Target used	- 18s rRNA : 40% - ITS1 : 40%

	- ITS2 : 40% - Shotgun approach : 18% - 28s rRNA : 0%

Figure 1. Map of the 14 European countries that participated in the ECMM-ESCMID survey.