Arch Clin Med Case Rep 2022; 6 (2): 229-233 *DOI: 10.26502/acmcr.96550472*

Research Article

Mycobiome Analysis Reveals High Fungal Richness in Lungs of Acute Respiratory Distress Syndrome after Septic Tank Incident

Hedda Luise Verhasselt[1], Matthias Kudla[1], Frank Herbstreit[2], Sophie Weidener[2], Florian Reckinger[3], René Scholtysik[4], Jan Buer[1], Jan Kehrmann[1*]

[1]Institute of Medical Microbiology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany

[2]Department of Anesthesiology and Intensive Care Medicine, University Hospital Essen, University of Duisburg-Essen, Essen, Germany

[3]Faculty of Agricultural Sciences and Landscape Architecture, Osnabrück University of Applied Sciences, Osnabrück, Germany

[4]Institute of Cell Biology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany

***Corresponding author:** Jan Kehrmann, Institute of Medical Microbiology, University Hospital Essen, University of Duisburg-Essen, Hufelandstr. 55, 45122 Essen, Germany, Tel: +4920172385913.

Received: 23 February 2022; **Accepted:** 16 March 2022; **Published:** 29 March 2022

Citation: Hedda Luise Verhasselt, Matthias Kudla, Frank Herbstreit, Sophie Weidener, Florian Reckinger, René Scholtysik, Jan Buer, Jan Kehrmann. Mycobiome Analysis Reveals High Fungal Richness in Lungs of Acute Respiratory Distress Syndrome after Septic Tank Incident. Archives of Clinical and Medical Case Reports 6 (2022): 229-233.

Abstract

We present a fatal septic tank incident developing acute respiratory distress syndrome and multi-organ dysfunction syndrome. Fungal pathogens complicated the course of disease which were detected by bronchoscopy, microbiologic culture, testing of serum galactomannan and Next-generation sequencing from bronchoalveolar fluid. Mycobiome analysis revealed an unexpected high fungal phylogenetic richness and detected several fungal pathogens reported as causative pathogens for human lung infections that were not detected by traditional microbiological culture. Antifungal microbial therapy

should be considered to be included for empiric antimicrobial treatment for septic tank incident patients.

Keywords: Mycobiome; Mould; Microbiome; Septic tank

1. Introduction

Septic tanks are simple onsite sewage facilities to settle and reduce solids and organics from domestic wastes by microbial processes. During this process, a mixture of sewer gases accumulates containing also hydrogen sulphide. This is of particular risk by its ability to depress the central nervous system and to rapidly cause unconsciousness [1] potentially resulting in aspiration of tank liquid and induction of pulmonary infections due to microorganisms. Calculated antibiotic treatment after incidents with liquid organic animal waste has so far focused on bacterial pathogens [2, 3]. We present a fatal case of an accident resulting in aspiration of domestic septic tank effluents.

2. Case Presentation

Here, a 70-year old farmer plunged into the third reservoir of his biological septic tank and aspirated liquid. Brown liquid was recovered during bronchoscopy in the hospital the patient was initially admitted to. Developing a severe acute respiratory distress syndrome, the patient was transferred to the University Hospital Essen, a few hours after the incident. Antibiotic therapy with piperacillin/tazobactam was continued. Infiltrates in both lungs were detected in the chest CT scan. Macroscopic growth of moulds in the airways during bronchoscopy (Figure 1A) and increasing signs of (mucosal) inflammation were determined. Due to further deterioration, extracorporeal membrane oxygenation therapy and continuous venous-venous hemodialysis were started. Antimicrobial therapy was expanded by voriconazole after detection of fungal growth during bronchoscopy. The patient died three days after the accident in septic shock with multi-organ dysfunction.

3. Material and Methods

The microbial approach described herein was to quantify Aspergillus-Antigen from serum and Bronchoalveolar Fluid (BAL) using Platelia® galactomannan assay (Biorad, Hercules, CA, US) and to perform fungal culture on universal agars, Candida selective agar and Malt Extract agar (Oxoid, Wesel, Germany). Grown moulds were identified by microscopic and macroscopic features and sequencing of the Internal Transcribed Spacer (ITS) region 1 after DNA-extraction. DNA from BAL was isolated after a bead-beating step with the Fast-Prep24 device using the ZymoBiomics DNA Miniprep Kit (Zymo Reseach, Freiburg, Germany). Amplicon PCR was performed with 30 cycles using ITS2-region primers with Illumina adapter overhangs [4]. After purification using the Qiagen PCR purification kit (Qiagen, Hilden, Germany), the PCR product and Nextera XT Index Kit (Illumina, San Diego, CA) were used for index PCR and run on an Illumina Miseq as described [5]. Data analysis was performed using QIIME2. Adaptors were trimmed using Cutadapt and quality filtering and ASV picking was performed using DADA2 wrapped in QIIME2. Fungal taxonomy was assigned using the UNITE database, QIIME release for Fungi 2. version 04.02.2020 [6].

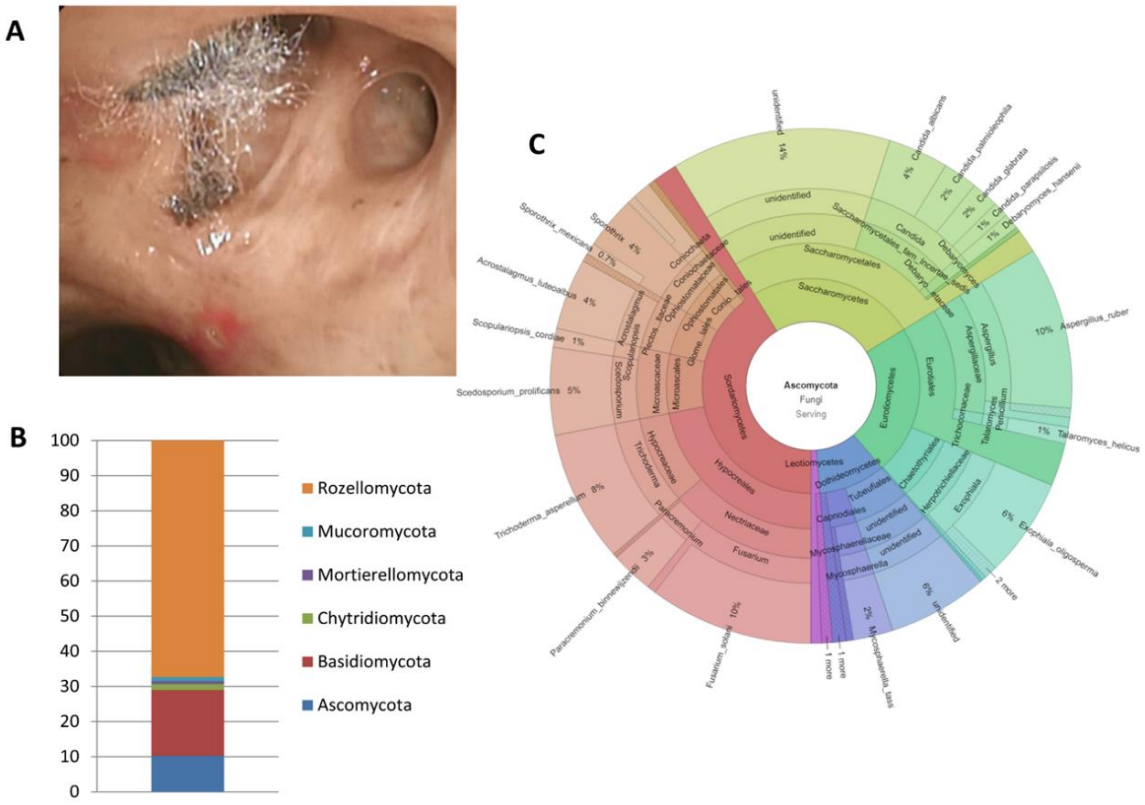

Figure 1: Macroscopic visible fungal growth during bronchoscopy **(A)**. Relative abundance of sequences that were assigned to fungal phyla **(B)**. Krona plot illustrating the proportion of sequences assigned to fungal classes, orders, families, genera and species of the phylum Ascomycota **(C)**.

4. Results and Discussion

In this case, the detection of galactomannan from serum, which was negative at day of admission and increased to 6.0 index (threshold index 0.5) at day two after admission is a serologic indicator for an invasive fungal infection. BAL culture identified *Aspergillus fumigatus, Lichtheimia ramosa, Lycoperdon umbrinoides, Mucor circinelloides* and *Trichoderma asperellum*. Mycobiome analysis using NGS of ITS2-region from BAL revealed a high fungal phylogenetic richness with detection of six fungal phyla. Of 93,585 quality filtered sequences of the kingdom of fungi, 49.1% of the sequences could not be assigned to fungal phyla. Surprisingly, 67.3% of reads that were assigned to fungal phyla belonged to the phylum Rozellomycota (Figure 1B), an ancestor of the microsporidia [7]. About 18.8% of sequences that were assigned to fungal phyla were reads belonging to Basidiomycota and 10.2% to Ascomycota that are the dominating fungal phylum detected in the respiratory tract of healthy individuals. The phyla Mortierellomycota, Mucoromycota and Chytridiomycota exhibited 0.7% - 1.7% each of reads assigned to fungal phyla. Several fungal species or genera

that have been reported to cause fungal infections in humans were detected by NGS for the phylum Ascomycota including Candida spp., *Exophiala oligosperma*, *Aspergillus*, *Scedosporium prolificans* and *Fusarium solani* (Figure 1C). Besides the risk of toxic gas inhalation, aspiration of septic tank effluents may cause severe lung infections. Guidelines for management of septic tank gas poisoning as well as case reports are scarce. Initial antimicrobial therapies of published slurry incidents have focused on bacterial pathogens [2, 3] and few reports have included fungal pathogens for initial antimicrobial therapy [8] taking into account that manure contains highly diverse bacterial and fungal communities depending on the animal species housed [9]. In this report, the combination of fungal growth seen during bronchoscopy with detection of serum galactomannan was indicative for a fungal infection. Clinical and serological findings in combination with the detection of several fungal pathogens from BAL by culture and NGS suggested that antimycotic drugs supplementing the anti-infective therapy may be advisable to prevent tissue inflammation and further infectious complications. Both were initiated in our case and its use has been reported in the literature [8].

Interestingly, a high proportion (67%) of reads that were assigned to fungal phyla belonged to the Rozellomycota that comprises the class Microsporidia, a group of unicellular intracellular parasites closely related to fungi. The phylum has a remarkable high biodiversity that is poorly characterized so far [7]. Although a high abundance of fungal reads were assigned to the phylum Rozellomycota, its relevance on local inflammation and complications for this case is unknown, as well as antiinfective treatment options. Unfortunately, autopsy was not performed to confirm invasive fungal infection and to further pathologically determine the relevance of the presence of the highly abundant fungi of the phylum Rozellomycota. Whereas *L. umbrinoides* causes hypersensitivity pneumonitis after inhaling of spores, members of the genus Trichoderma, important decomposers of decaying plant material, are an infrequent but emergent human pathogen [10]. Fungal infections with *A. fumigatus*, *Lichtheimia* spp. and Mucor spp. are frequently described in immunocompromised patients but they can occur also in previously immunocompetent patients undergoing surgery, critically ill patients or patients with co-infections (influenza virus or severe acute respiratory syndrome coronavirus 2) [11]. Furthermore, fungal pathogens that may cause severe infections in humans, were detected by NGS only and not by culture (*Candida* spp., *Exophiala oligosperma*, *Scedosporium prolificans* and *Fusarium solani*). Resulting from the variety of detected fungal species it is difficult to assign (the) most relevant fungal species and also to infer a targeted antifungal therapy. This is the first case of liquid aspiration from a septic tank reported in the literature using an NGS approach for detection of fungal pathogens from BAL.

5. Conclusion

In conclusion, this report shows that fungal pathogens may complicate septic tank accidents and indicates that fungal pathogens should be considered for initial anti-infective treatment. In addition, NGS revealed a high richness of fungal pathogens of various phyla and improved the sensitivity for detection of fungal pathogens from BAL that may be missed by conventional culture.

Potential Conflicts of Interest

All authors declare that they have no conflict of interest.

Patient Consent Statement

A patient's relative gave written informed consent.

Funding

This research received no external funding.

Data Availability

Sequencing data are available in the National Center for Biotechnology Information (NCBI) Sequence Read Archive under BioProject accession PRJNA813019.

Author Contributions

HLV, JK, MK and RS designed and performed the analysis. FH, SW, FR and JB collected and contributed data. JK and HLV wrote the manuscript.

References

1. Milby TH. Hydrogen sulfide intoxication. Review of the literature and report of unusual accident resulting in two cases of nonfatal poisoning. J Occup Med 4 (1962): 431-437.
2. Fahy JV, Walley T, Gibney RT, et al. Slurry lung: a report of three cases. Thorax 46 (5): 394-395.
3. Osbern LN, Crapo RO. Dung lung: a report of toxic exposure to liquid manure. Ann Intern Med 95 (1981): 312-314.
4. Hoggard M, Vesty A, Wong G, et al. Characterizing the Human Mycobiota: A Comparison of Small Subunit rRNA, ITS1, ITS2, and Large Subunit rRNA Genomic Targets. Front Microbiol 9 (2018): 2208.
5. Kehrmann J, Menzel J, Saeedghalati M, et al. Gut Microbiota in Human Immunodeficiency Virus-Infected Individuals Linked to Coronary Heart Disease. J Infect Dis 219 (2019): 497-508.
6. Koljalg U, Nilsson HR, Schigel D, et al. The Taxon Hypothesis Paradigm-On the Unambiguous Detection and Communication of Taxa. Microorganisms 8 (2020): 1910.
7. Corsaro D, Walochnik J, Venditti D, et al. Solving an old enigma: Morellospora saccamoebae gen. nov., sp. nov. (Rozellomycota), a Sphaerita-like parasite of free-living amoebae. Parasitol Res 119 (2020): 925-934.
8. Ugras M, Guraksin O, Sen TA, et al. Surfactant replacement therapy in a pediatric near-drowning case in manure. Pediatr Emerg Care 28 (2012): 913-914.
9. Sun L, Han X, Li J, et al. Microbial Community and Its Association with Physicochemical Factors During Compost Bedding for Dairy Cows. Frontiers in microbiology 11 (2020): 254.
10. Sandoval-Denis M, Sutton DA, Cano-Lira JF, et al. Phylogeny of the clinically relevant species of the emerging fungus Trichoderma and their antifungal susceptibilities. Journal of clinical microbiology 52 (2014): 2112-2125.
11. Koehler P, Bassetti M, Chakrabarti A, et al. Defining and managing COVID-19-associated pulmonary aspergillosis: the 2020 ECMM/ISHAM consensus criteria for research and clinical guidance. Lancet Infect Dis 21 (2020): 149-162.