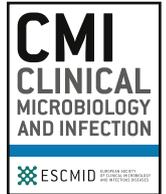




Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Letter to the Editor

A microbiome assessment of medical marijuana

Dear Sir,

To reduce the risk of infection in highly immunocompromised patients, prophylactic antibacterial, antifungal and antiviral agents are frequently prescribed and patients are routinely advised to reduce their risk of exposure by avoiding soil, plants and cut flowers due to the presence of *Aspergillus* and other moulds and *Nocardia* spp. [1,2]. Other recommendations to limit exposures include the avoidance of water-retaining materials given their association with *Pseudomonas aeruginosa*, raw vegetable sprouts (*Escherichia coli*), undercooked eggs (*Salmonella enteritidis*), fresh salsa, and berries (*Cyclospora* etc.) among others [1]. These recommendations do not comment on the infectious risks of medical marijuana—a substance now legal in 11 countries—and used by ~13% of the US population yearly.

Medical marijuana obtained from dispensaries does not differ in form from recreational marijuana and consists of dried material from the *Cannabis* plant purchased in a variety of preparations. Patients seek out marijuana (or related products) in an attempt to control nausea or vomiting due to cytotoxic chemotherapy, to control pain or neuropathy, or to stimulate appetite. Legalization has impacted the public's perception of safety [3], and inferences on safety are implied by the ability of physicians to write prescriptions for medical marijuana.

The potential infectious risks of marijuana obtained from dispensaries has not been systematically evaluated and we believe that this unduly places patients (unknowingly) at risk for acquisition of severe infections. Using both 16S/internal transcribed spacer community analysis and metagenomics approaches we sought to define the potential infectious risks of marijuana.

Twenty cannabis samples from different dispensaries in northern California were collected by Steep Hill Laboratories (Berkeley, CA) and catalogued, and their DNA was extracted using the PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA). The fungal communities (assessed by internal transcribed spacer gene sequence analysis) and the bacterial communities (from 16S rRNA gene) were analysed using published methods [4–6]. More specific whole metagenome analyses were used to better understand the presence of distinct species within the microbiome [6].

Internal transcribed spacer analysis resulted in ~4000 total fungal taxonomic classifications. Of the 20 fungal genera observed at >0.1% frequency, many were genera that contain opportunistic pathogens (e.g. *Cryptococcus*, *Mucor*, *Aspergillus*) (Appendix 1). Cross-amplification of the 16S primers with plant chloroplast and mitochondria DNA resulted in limited bacterial 16S amplification, as previously reported in microbiome assessments of plant

material [7]. However, samples yielded enough 16S data for reliable bacterial community analysis (>5000 operational taxonomic units) in at least one sample (MJ150-008).

Species identification using GOTTCHA [8] on the whole metagenome data for this sample returned multiple pathogens that would probably be attributed to hospital-acquired infection rather than to exposure to marijuana obtained from a dispensary: *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Pseudomonas putida*, *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* (Appendix 2) [9]. Although limited in coverage, WMS reads from the samples analysed mapped to the fungal genomes targeted: sample 138-011 read ($n = 534$) mapping supports the presence of *Alternaria alternata* and *Cladosporium sphaerospermum*. Whole metagenome sequence reads from sample MJ150-008 ($n = 658$) indicated the presence of *Aspergillus fumigatus*, *Cryptococcus laurentii* and *Mucor circinelloides*, all well-known causes of invasive fungal infections in immunocompromised hosts.

We found numerous Gram-negative bacilli and fungal pathogens contaminating medical marijuana. These pathogens potentially pose a grave risk to our patients, particularly the immunosuppressed. Although our report is limited to genomic analyses, a few previous studies have cultured multiple fungi (*Aspergillus* and *Penicillium* spp.) and bacteria (*Klebsiella*, *Enterobacter*, *Salmonella* and *Bacillus*) [10] confirming that viable organisms can be recovered from cannabis.

Some may argue that the temperature of inhaled samples (50–60°C) suggests that inhaled smoke may be sterile. However viable organisms have been cultured from smoke even after water filtration [11], suggesting that temporary exposure to elevated temperatures or attempts at filtration are probably insufficient to protect the compromised host.

The legalization of medical marijuana has been followed by a shift in both patient and physician attitudes with 76% of physicians in favour of marijuana usage for medicinal purposes in a recent survey [12]. Respondents focused primarily on their responsibility as caregivers to alleviate suffering. However, we contend that these well-intentioned motives to relieve suffering by practitioners and patients alike have unknowingly ignored a product that can be contaminated with infectious agents and so harbour potentially lethal risks. These non-regulated products are often sold under the auspices of a dispensary, which many patients may confuse with pharmacies inappropriately giving patients the sense of security they have with regulated medical products.

It is our hope to educate practitioners about the potential risks until a comprehensive risk analysis can be performed. Our results

<http://dx.doi.org/10.1016/j.cmi.2016.12.001>

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suggest that handling marijuana in any form might expose the patient to a number of both bacterial and fungal pathogens well-known to cause serious infections in the immunocompromised population. Smoking or vaporization provides a direct portal of entry into the terminal bronchioles and alveoli. Moreover, the recovery of these organisms in a symptomatic patient would be unlikely to initiate a search for unusual exposures. *Aspergillus* and other moulds may therefore be attributed to breakthrough infection, and recovery of Gram-negative bacilli would be attributed to healthcare-associated pneumonia and/or a failure of prophylaxis.

Future work focusing on the presence of viable organisms, and susceptibility profiles of these organisms, will need to be performed. These findings will allow us to more accurately predict the microbial risks posed by medical marijuana to our patients. Until this work is completed it seems prudent to advise immunocompromised patients against the use of vaporized or inhaled marijuana.

Transparency declaration

There are no conflicts of interest in connection with this article. RG and AT are employed by a commercial cannabis testing laboratory. All other authors have no disclosures.

Funding

Existing departmental funds were used for this study.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.cmi.2016.12.001>.

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21 November 2016

Available online xxx

Editor: L. Leibovici