SAMPLING OF P. JIROVECIIIN THE SURROUNDING AIR OF PATIENTS WITH PNEUMOCYSTIS PNEUMONIA

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/ CONTEXT

Pneumocystis jirovecii is responsible for a disease affecting immuno-depressed patients: Pneumocystis pneumonia (PCP). Airborne transmission of Pneumocystis has been demonstrated in animal models and is highly probable in humans. However, information concerning burdens of Pneumocystis jirovecii in exhaled air from infected patients is lacking due to the technical limitations of the current sampling methods. This fungus is indeed not cultivable, so impaction method based on the cultivability of microorganisms cannot be used. Also, the difficulties of extraction from a solid support or the lengthy conditions of sampling (filtration technique) led us to consider alternative methods that would be suitable for both sampling and quantifying P. jirovecii in air. Our objective is to evaluate P. jirovecii air diffusion in patients with Pneumocystis pneumonia with Coriolis air sampler sampling combined with RT-PCR assays [1].

/ RESULTS

33/56 positive air samples : the more the distance from the

patient is low, the more the P.jirovecii DNA is detected at:

1m - 15/19 samples (78.9%), 3m - 9/13 (69.2%), 5m - 5/12

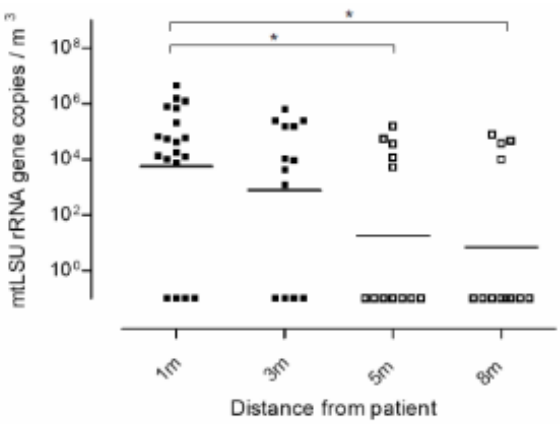
(41.7%), 8m - 4/12 (33.3%)

This study provides the first quantitative data on the

spread of P. jirovecii in exhaled air from infected patients.

/ PROTOCOL

- Collection of air samples in each patient room with the Coriolis ® µ air sampler.
- Collection of 1 air sample of 1.5 m 3 (5 min) at a distance of 1 m from patient's head + air samples inside and outside the room: at the door entrance and in the corridor (3m, 5m and 8m from the patient).
- Centrifugation of the liquid sample at 1300 g for 10 min; removal of the supernatant to leave a 1 mL pellet; storage at +4°C.
- Extraction of DNA from 200 µL of sample pellets using the QIAamp DNA Mini Kit (Qiagen).
- Performance of a real-time quantitative PCR assay targeting the mitochondrial large subunit (mtLSU) rRNA gene of P. jirovecii (Applied Biosystems 7500 PCR system).



Number of *P. Jirovecii* mtLSUrRNA gene copies per m^3 of air sample inside (■) and outside (□) the room. *P<0.05; Dunn's post test.v

/ CONCLUSION

The Coriolis ® samples large volumes of air during a short period and concentrates the airborne microorganisms into a small volume of liquid. This was crucial for efficient capture and detection of Pneumocystis. This facilitates epidemiological investigations with non-cultivable microorganisms (like Pneumocystis sp.) in conjunction with real-time PCR amplification of DNA.

This study leads the way for initiating a quantitative risk assessment for airborne transmission of P. jirovecii and other airborne infectious agents.

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