ACKNOWLEDGMENTS

Financial support: No financial support was provided relevant to this article.

Potential conflicts of interest: All authors report no conflicts of interest relevant to this article.

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SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit https://doi.org/10.1017/ice.2018.32

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Received December 10, 2017; accepted January 27, 2018; electronically published March 19, 2018
Infect Control Hosp Epidemiol 2018;39:625–627
 0899-823X/2018/3905-0020. DOI: 10.1017/ice.2018.32

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Pneumocystis jirovecii Exhalation in the Course of *Pneumocystis* Pneumonia Treatment

Pneumocystis jirovecii is a transmissible and uncultivable micromycete that causes severe acute pneumonia (ie, *Pneumocystis* pneumonia, PCP) in immunosuppressed patients. *Pneumocystis* spp are host specific, and no exosaprophytic form of *Pneumocystis* sp has been identified so far. Thus, humans may represent the reservoir of *P. jirovecii* and potential infectious sources for susceptible individuals.[1]

Pneumocystis jirovecii DNA has been detected and quantified using quantitative polymerase chain reaction (qPCR) in the air surrounding PCP patients, suggesting exhalation and spread of *P. jirovecii* from infected patients within their environment.[2,3] This finding emphasizes the risk of patient-to-patient transmission of *P. jirovecii* via the airborne route, which was also prompted by investigations of PCP case clusters in hospitals (see the review by Yiannakis et al[4]). Taken together, these data support the maintenance of prevention measures based at least on patient treatment and isolation.[5] Nonetheless, there are no

TABLE 1. Results of Quantification and Genotyping of *Pneumocystis jirovecii* DNA in Pulmonary Samples and 1-m Air Sample From Patients With *Pneumocystis* Pneumonia in the Present Study and Published Elsewhere

Reference	Patients	Pulmonary Sample	*P. jirovecii* Quantification in Pulmonary Sample (*mtLSUrRNA* copy no./µL extracted DNA)	No. of Days Between Treatment Initiation and Air Sampling	*P. jirovecii* Quantification in Air Sample at 1 m From Patient (*mtLSUrRNA* copy no. /m^3)	Match of *Pneumocystis* Genotypes in Pair of Pulmonary and Air Samples (Yes/No)
Choukri et al[2]	P1	Sputum	5.4×10^4	4	4.3×10^4	ND[a,b]
Damiani et al[8]	P2	BAL	1.1×10^7	0	1.0×10^4	No
	P3	BAL	3.5×10^5	4	0	ND[a,b]
	P4	BAL	5.8×10^7	2	7.7×10^5	Yes[a]
	P5	Sputum	2.6×10^7	0	6.8×10^4	Yes[a,c]
	P6	BAL	1.6×10^6	0	4.5×10^6	Yes[a]
	P7	BAL	2.1×10^8	9	2.1×10^4	ND[a,b]
	P8	BAL	9.9×10^6	3	1.4×10^6	Yes[a]
	P9	Sputum	2.0×10^4	2	0	ND[a,b]
	P10	BAL	4.1×10^6	1	1.3×10^4	ND[a,b]
	P11	BAL	2.1×10^4	0	1.7×10^6	Yes[a,c]
	P12	BAL	2.0×10^5	3	0	ND[a,b]
	P13	Sputum	7.0×10^5	2	6.0×10^4	ND[a,b]
	P14	Sputum	1.0×10^3	0	0	ND[a,b]
	P15	Sputum	1.0×10^6	1	7.5×10^3	ND[a,b]
	P16	Sputum	1.1×10^6	0	2.3×10^5	ND[a,b]
	P17	Sputum	2.7×10^5	1	5.5×10^4	ND[a,b]
	P18	BAL	1.5×10^5	0	9.0×10^5	Yes[a]
	P19	BAL	9.2×10^3	1	1.2×10^4	ND[a,b]
Le Gal et al[3]	P1	Sputum	2.4×10^1	4	0	ND[b]
	P2	BAL	4×10^5	0	1.7×10^4	Yes
	P10[d]	Sputum	5.0×10^2	2	0	ND[b]
		BAL	2.4×10^5	9	0	ND[b]
	P13	BAL	3.3×10^5	0	6.3×10^6	Yes
This study	Our patient	BAL	2.97×10^4	1	1.18×10^7	Yes
				2	2.39×10^5	Yes
				3	4.48×10^3	Yes
				4	$<1.3\times10^3$	Yes
				5	$<1.3\times10^3$	Yes

[a]Genotypes at the ITS locus described by Damiani et al[8] for the patients initially studied by Choukri et al.[2]
[b]Not determined.
[c]Partial match.
[d]2 pairs of pulmonary and 1-m air samples during PCP treatment in patient P10 by Le Gal et al.[3] Considering only the results with perfect genotype matching in pairs of pulmonary and air samples, *P. jirovecii* was effectively detected at 1-m from 2 patients (patients P4 and P8 initially studied by Choukri et al.[2]) who had been treated for 2–3 days.

arguments to delineate their duration. In this context, we investigated longitudinal *P. jirovecii* air exhalation by a renal transplant recipient who developed PCP and was efficiently treated with cotrimoxazole (2,880 mg/day). The diagnosis of PCP was based on a positive result of *P. jirovecii* detection in a bronchoalveolar lavage (BAL) sample using microscopy and a qPCR assay targeting the gene encoding the mitochondrial large subunit ribosomal RNA (*mtLSUrRNA*), as previously reported.[2,3] During 5 consecutive days, 5 air samples were collected after treatment initiation in patient's room at 1 m from the patient's head. The samples consisted of 3 m^3 of air collected using the Coriolis µ air sampler (Bertin Technologies, Montigny-le-Bretonneux, France), which concentrates aerial particles in a liquid medium. The DNA extraction of air samples was performed according to the recommendations of Choukri et al.[2] The *P. jirovecii* burden was determined in the BAL and air samples using a qPCR assay amplifying the *mtLSUrRNA* gene as described by Choukri et al.[2] Samples and a negative control were run in triplicate. The quantity of *P. jirovecii* DNA in the samples was determined against a standard curve. The genotyping of *P. jirovecii* from the BAL and air samples was performed by examining *cytochrome b* (*CYB*) and *mtLSUrRNA* genes, as we described elsewhere.[6]

The *P. jirovecii* DNA load was evaluated at 2.97×10^6 copies/mL of native sample in the BAL specimen (2.97×10^4 copies/µL of extracted DNA). The *P. jirovecii* DNA load was 1.18×10^7 copies/m^3 of air in the sample collected 1 day after treatment initiation; 2.39×10^5 copies/m^3 in the sample collected

https://doi.org/10.1017/ice.2018.45 Published online by Cambridge University Press

2 days after treatment initiation; 4.48×10^3 copies/m^3 in the sample collected 3 days after treatment initiation; and $<1.3 \times 10^3$ copies/m^3 in the samples collected 4 and 5 days after treatment initiation. Briefly, a sharp decrease of *P. jirovecii* DNA load was observed between the first and the third air samples (Table 1).

A *CYB2* allele was identified in the BAL and the first 3 air samples, but typing at this locus did not give positive results in the last 2 air samples. *MtLSUrRNA* allele 4 was identified in the BAL and in the 5 air samples. Thus, a perfect match of *P. jirovecii* genotypes was observed. These results are consistent with the fact that *P. jirovecii* detected in air samples was from the patient's source and had been exhaled in his environment.

We obtained the first data on *P. jirovecii* exhalation by a patient during the course of PCP treatment, specifically during the first 5 days but not beyond because the patient was moved from the nephrology unit. The exhalation of *P. jirovecii* decreased dramatically during the first 3 days of treatment. The low fungal burden in the last 2 samples ($<1.3 \times 10^3$ copies/m^3) may be explained by a genuine *P. jirovecii* exhalation but at a low level or by a residual presence of the fungus in the patient's room, even though the patient may no longer have been exhaling *P. jirovecii*. This latter hypothesis may be consistent with the observations by Bartlett et al,[7] who detected *P. jirovecii* DNA in empty hospital rooms within infectious disease and AIDS units.

We compared our results to those previously reported by Choukri et al,[2] Le Gal et al,[3] and Damiani et al,[8] even though their objective was not the analysis of PCP treatment effect on *P. jirovecii* exhalation (Table 1). Considering only the results with perfect genotype matching in pairs of pulmonary and air samples, *P. jirovecii* was effectively detected at 1 m from 2 patients who had been treated for 2–3 days, which is consistent with the results of our present study.[2,3,8]

Available data on PCP case clusters in hospitals combined with recent studies of *P. jirovecii* exhalation by infected patients (the present study, Choukri et al,[2] Le Gal et al,[3] Yiannakis et al,[4] and Damiani et al[8]) support the hypothesis of patient-to-patient transmission of *P. jirovecii* via the airborne route and nosocomial infection occurrence. Although chemoprophylaxis remains essential, the implementation of collective measures is necessary. The putative transmissible stage of *P. jirovecii* is the ascus.[9] Unfortunately, its median size is 5 µm, precisely the threshold value for choosing droplet versus air precaution measures. *Pneumocystis jirovecii* detection in the surrounding air at 5 m reported elsewhere[2,3] provides arguments in favor of air precautions, while the infectivity and viability of *P. jirovecii* in this context remain unknown. At present, the CDC recommends applying standard precautions and avoiding placement of a PCP patient in the same room with an immunocompromised patient.[5] Considering knowledge acquisition on *Pneumocystis* transmission for the past 10 years, these recommendations should be updated.

Finally, our study shows that PCP treatment dramatically decreased *P. jirovecii* exhalation and supports maintaining preventive measures, whatever they may be, over at least 5 days after PCP treatment initiation.

ACKNOWLEDGMENTS

The authors would like to thank Dr D. Quinio and C. Carrou, P. Lecordier, D. Roué, V. Abiven, C. Cam, C. Le Guen, M. Tranvouez, and C. Roger for performing routine diagnoses of *Pneumocystis* infections.

Financial support: This study was supported in part by the European Union (grant no. ERANet-LAC CAPRI-PC HID-0254).

Potential conflicts of interest: All authors report no conflicts of interest relevant to this article.

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Received January 8, 2018; accepted February 5, 2018; electronically published March 19, 2018

Infect Control Hosp Epidemiol 2018;39:627–630

 0899-823X/2018/3905-0021. DOI: 10.1017/ice.2018.45

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