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First data on *Pneumocystis jirovecii* colonization in patients with respiratory diseases in North Lebanon

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Abstract

Pneumocystis colonization may play a role in transmission and local inflammatory response. It was explored in patients with respiratory diseases in North Lebanon. Overall prevalence reached only 5.2% (95% CI 2.13–10.47) but it was higher (17.3%) in the subpopulation of patients with chronic obstructive pulmonary disease (COPD). COPD was the only factor associated with a significantly increased risk of colonization. *mtLSU* genotyping revealed predominance of genotype 2, identified in five patients (71.4%), including one patient who had co-infection with genotype 3. These first data in North Lebanon confirm *Pneumocystis* circulation among patients with respiratory diseases and the potential for transmission to immunocompromised patients.

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Pneumocystis colonization occurs in both immunocompetent and immunocompromised individuals, reaching 0–65% in the general population [1,2], 20–69% in human immunodeficiency virus (HIV) –infected patients [3] and 16–55% in patients with chronic obstructive pulmonary disease (COPD) [1,3,4]. Colonized individuals may be at risk of developing *Pneumocystis* pneumonia (PcP) or serve as a reservoir for transmission [3]. Moreover, *Pneumocystis* may stimulate the host inflammatory response, lead to lung damage and play a role in the progression of lung diseases such as COPD [3].

In this study, which was approved by institutional review boards of the Lebanese university and of the different hospitals, *Pneumocystis* colonization was prospectively explored in 134 patients with community-acquired respiratory diseases (33 rhinopharyngitis, 27 bronchitis, 23 COPD, 17 influenza, 14 asthma, eight respiratory infections, four pneumonia, four respiratory distress syndromes, two lung cancers, one fibrosis, one acute pulmonary oedema). Exclusion criteria were hospital-acquired respiratory infection and anti-*Pneumocystis jirovecii* treatment (with sulfamethoxazole or atovaquone) in the preceding 6 months. Patients were enrolled from July 2012 to October 2013 in four hospitals of Tripoli (Tripoli governmental (n = 13), Nini (n = 69), al Mazloum (n = 14), and el Monla (n = 12) hospitals) and in local medical care centres (n = 26). Among hospitalized patients, 92 were recruited during their stay in Pneumology departments and 16 were recruited from Oncology-Haematology departments. Standardized forms were filled with clinical, biological and demographic data for each patient: age, sex, presence of chronic pulmonary disease or other respiratory disease, immune deficiencies (cancer, HIV), corticotherapy and any other immunosuppressive medications, or antibiotherapy and smoking habits were recorded. Collected samples (one per patient) included 56 oropharyngeal washes, 37 sputa, 15 tracheal aspirations, 14 bronchoalveolar lavages and 12 nasal swabs. DNA extraction was performed using the Nucleospin tissue Kit (Macherey-Nagel, Hoerdt, France). *Pneumocystis* DNA was detected using an *mtLSU* nested-PCR assay [5]. Positive samples were sequenced and further processed to determine fungal load using a quantitative *mtLSU* PCR assay [6]. *Pneumocystis jirovecii mtLSU* sequences were deposited

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in GenBank under Accession Numbers KM023735 to KM023742.

Nested-PCR assay was positive in seven specimens from patients with COPD (n = 4), rhinopharyngitis (n = 1), bronchitis (n = 1) and influenza (n = 1) (Table 1). Prevalence of *Pneumocystis* colonization reached 5.2% (95% CI 2.13–10.47), which was within the same range as immunocompetent patients with various lung diseases in Iran (7.3%), a neighbouring country [7]. The *mtLSU* quantitative PCR confirmed *Pneumocystis* DNA detection for six out of seven samples, with a 7.97 to 3.51 × 10^4 copies/μL fungal burden (Table 1), which was consistent with previously reported loads in colonized patients and with the 2 × 10^4 upper cut-off value that was proposed by Damiani *et al.* for differentiation of colonized patients and patients with PcP [8]. The prevalence was similar in patients with rhinopharyngitis (3.0%), bronchitis (3.7%), and influenza (5.9%), but it was higher in patients with COPD (17.3%, Table 1), confirming previous data in this population [1,3,4] and potential occurrence of *Pneumocystis* in patients with influenza [9]. *Pneumocystis* was not detected in patients with asthma, supporting a lower risk for colonization in these patients, despite previous reports of an association between PcP and asthma [10]. No lung cancer patient was colonized, but only two such patients were included. This low number of cancer patients, the absence of patients with cystic fibrosis or interstitial lung diseases, and the high number of patients with rhinopharyngitis, asthma or influenza, could explain our lower overall prevalence when compared with previous studies in Spain (27.1% in patients with lung cancer, cystic fibrosis, interstitial lung disease or COPD) [11], or in the UK (18% in patients with mainly lung cancer or pneumonia) [12]. As these studies used a nested-PCR assay without real-time PCR confirmation, an additional explanation for higher *Pneumocystis* colonization could be false-positive results due to carryover contamination. As the prevalence of PcP in HIV-infected patients in Lebanon is low (10.9%) [13], the lower prevalence of *Pneumocystis* colonization in Lebanon could further be explained by a lower overall burden of *Pneumocystis* that could result from the influence of climatic factors [14]. Finally, differences in genetic susceptibility have been recently suggested in the Netherlands, where a significant lower incidence of PcP was found among HIV-positive Africans compared with Western patients [15]. Hence, the role of genetic factors would be interesting to explore in Lebanese patients.

When potential risk factors and sample types were analysed (Table 2), the frequencies were similar or lower in colonized patients for all the examined criteria, except sex ratio and COPD. As the low number of colonized patients induced a high risk of falsely supported null hypothesis, statistical analyses were only performed for these criteria. Fisher Exact test revealed a significantly higher prevalence of *Pneumocystis* colonization in patients with COPD (p 0.019). The higher proportion of males in the *Pneumocystis*-positive group was not statistically significant (p 0.238), but this result could be due to a lack of statistical power and should be interpreted carefully. Nevertheless, *Pneumocystis* association with COPD confirmed a previous multivariate analysis that identified COPD as the only important predictive factor of colonization [16], but were in contrast to another study [12]. However, this last study included only subjects with very mild airway obstruction. Our study also agreed with this previous study, which did not identify immunosuppressive treatment and cancer as risk factors for *Pneumocystis* colonization [12]. Despite previous reports of association between corticotherapy and *Pneumocystis* colonization [12], this criteria did not appear as a risk factor in our study. This result, which supports data from Morris *et al.* [16], could be related to the low number of Lebanese patients undergoing corticotherapy in our study. Another specific feature of our population was the high frequency of smokers or

TABLE 1. Characteristics of patient with *Pneumocystis jirovecii* carriage: epidemiological, biological and clinical data; quantitative PCR and *mtLSU* genotyping results

Patient identification	Localization	Date of sample collection	Age	Sex	Sample type	Underlying respiratory disease or infection	Smoking habits	Other risk factors[a]	Microbiological findings	*P. jirovecii* DNA loads (copies/μL)	mtLSU genotype
O3	Nini hospital	11/07/13	57	M	Sputum	COPD SI	Yes	No	Neg	7.97 × 10^0	Mixture (2 & 3)
X3	Nini hospital	04/06/13	78	M	Nasal swab	COPD SIII	Yes	No	Neg	3.95 × 10^1	2
33	Nini hospital	30/10/12	75	F	OPW	COPD SIII	Yes	No	NA	4.47 × 10^3	1
V1	Monla hospital	12/09/13	75	M	Sputum	COPD SIII	Yes	No	NA	9.71 × 10^0	
P3	Mazloum hospital	25/08/13	70	M	BAL	Flu	NA	No	Neg	Neg	2
47	Local medical care centre	01/03/13	53	M	OPW	Rhino-pharyngitis	Yes	No	Neg	5.37 × 10^2	1
41	Tripoli Governmental hospital	16/11/12	75	M	OPW	Acute bronchitis	Passive	No	NA	3.51 × 10^4	2

Abbreviations: BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease; mtLSUrRNA, mitochondrial large subunit ribosomal RNA; NA, not available; OPW, oropharyngeal wash.
[a]Other risk factors: cancer, immune deficiency, corticotherapy, immunosuppressive treatment, antibiotherapy.

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TABLE 2. Comparison of patient localization, underlying respiratory diseases, other potential risk factors, and sample type between *Pneumocystis jirovecii* colonized and non-colonized patients

		P. jirovecii DNA detected ($n = 7$)	*P. jirovecii* DNA not detected ($n = 127$)
Mean age		69.0 ± 9.9	51.0 ± 20.9
Sex ratio		6	1.3
Localization	Tripoli Governmental hospital	1 (14.3%)	12 (9.4%)
	Nini hospital	3 (42.8%)	66 (52.0%)
	Monla hospital	1 (14.3%)	11 (8.7%)
	Mazloum hospital	1 (14.3%)	13 (10.2%)
	Local medical care	1 (14.3%)	25 (19.7%)
Population characteristics	COPD[a]	4 (57.1%)	19 (14.9%)
	Acute bronchitis	1 (14.3%)	26 (20.5%)
	Pulmonary fibrosis	0 (0%)	1 (0.8%)
	Influenza	1 (14.3%)	16 (12.6%)
	Rhinopharyngitis	1 (14.3%)	32 (25.2%)
	Asthma	0 (0%)	14 (11.0%)
	Pneumonia	0 (0%)	4 (3.1%)
	Respiratory infection	0 (0%)	8 (6.3%)
	Acute pulmonary oedema	0 (0%)	1 (0.8%)
	Respiratory distress syndrome	0 (0%)	4 (3.1%)
	Lung cancer	0 (0%)	2 (1.6%)
Other risk factors	Cancer	0 (0%)	16 (12.6%)
	Immune deficiency	0 (0%)	1 (0.8%)
	Corticotherapy	0 (0%)	5 (3.9%)
	Immunosuppressive treatment	0 (0%)	16 (12.6%)
	Antibiotherapy	0 (0%)	10 (7.9%)
Type of samples	OPW	3 (42.8%)	53 (41.7%)
	Sputum	2 (28.6%)	35 (27.5%)
	BAL	1 (14.3%)	13 (10.8%)
	Nasal swab	1 (14.3%)	11 (8.7%)
	Tracheal aspiration	0 (0%)	15 (11.8%)

Abbreviations: BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease; OPW, oropharyngeal wash.
[a]Because the number of colonized patients was low, the statistical analyses were not performed for criteria with similar or lower frequencies in colonized and non-colonized patients. Among the risk factors tested (i.e. sex ratio and COPD), COPD was the only one associated with a significant increased risk of *Pneumocystis* colonization (p 0.019).

passive smokers in both colonized and non-colonized individuals (100% and 81.5% in six colonized and 65 non-colonized individuals for whom smoking habit was successfully determined, respectively). This result supported previous data reporting that smoking is not a risk factor for *Pneumocystis* colonization [16]. Lastly, the similar frequency of positivity of bronchoalveolar lavages, sputa, oropharyngeal washes and nasal swabs (Table 2) confirmed the usefulness of non-invasive samples, especially nasal swabs, which are not usually used for *Pneumocystis* detection [17].

The *mtLSU* genotyping revealed predominance of genotype 2 (85A; 248C) (71.4%). One patient had co-infection with genotype 3 (85T; 248C). Genotype 1 (85C; 248C) was found in two patients (Table 1). The very low number of samples in which *Pneumocystis* colonization was detected limits comparison with other studies. However, it should be noted that genotype 2 was also found to be predominant in Italy (39%) [18] and in Cuba (48%) [19]. Nevertheless, our results contrasted with a Spanish study that reported a predominance of genotype 1 in patients with pulmonary diseases (45%) [11]. Seasonal variation in the occurrence of *Pneumocystis* genotypes, which has been reported in the UK [20], was suggested by identification of most genotype 2 isolates during the dry season (June–September) whereas both patients with genotype 1 isolates were sampled during the wet season (March–October) (Table 1).

To our knowledge, this is the first investigation of *Pneumocystis* carriage in Lebanon. These first data from North Lebanon confirm colonization of patients with respiratory diseases, which may evolve into PcP if the underlying disease reaches a severe stage or in the absence of appropriate treatments. Furthermore, the circulation of *P. jirovecii* among patients with respiratory diseases indicates its potential for transmission to immunocompromised patients.

Transparency declaration

The authors declare no conflicts of interest.

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