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

S. Khalife1,2, E. M. Aliouat1, C. M. Aliouat-Denis1, N. Gantois1, P. Devos1, H. Mallat2, E. Dei-Cas1,4, F. Dabboussi2, M. Hamze2 and E. Fréalle1,4

1) Biology and Diversity of Emerging Eukaryotic Pathogens (BDPEE), Pasteur Institute of Lille, Centre for Infection and Immunity of Lille, University of Lille, Lille, France, 2) Health and Environment Microbiology Laboratory, AZM Centre for Research in Biotechnology and its Application, Doctoral School of Sciences and Technology, Lebanese University, Tripoli, Lebanon, 3) Department of Research, Lille University Hospital and 4) Parasitology-Mycology Laboratory of Lille University Hospital Centre & Faculty of Medicine of Lille, University of Lille, Lille, France

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 sub-population 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.

New Microbes and New Infections © 2015 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases.

Keywords: Chronic obstructive pulmonary disease, Lebanon, *mtLSU* genotype, *Pneumocystis* colonization, respiratory diseases

Original Submission: 28 November 2014; Revised Submission: 15 February 2015; Accepted: 24 February 2015

Available online 4 March 2015

*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 antibiotic therapy 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.
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 × 10⁶ copies/μL fungal burden (Table 1), which was consistent with previously reported loads in colonized patients and with the 2 × 10⁶ 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 [16].

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 | 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 × 10⁶                  | Mixture (2 & 3) |
| X3                    | Nini hospital | 04/06/13                   | 78  | M   | Nasal swab  | COPD SIII                                   | Yes           | No                | Neg                      | 3.95 × 10⁶                  | 2              |
| 33                    | Nini hospital | 30/10/12                   | 75  | F   | OPW         | COPD SIII                                   | Yes           | No                | NA                       | 4.47 × 10⁶                  | 1              |
| V1                    | Monla hospital| 12/09/13                   | 75  | M   | Sputum      | COPD SIII                                   | Yes           | No                | NA                       | 9.71 × 10⁶                  | 1              |
| P3                    | Msdoum hospital| 25/08/13                   | 70  | M   | BAL         | Flu                                         | NA            | No                | Neg                      | 2                           |                |
| 47                    | Local medical care centre | 01/08/13 | 53  | M   | OPW         | Rhino-pharyngitis                           | Yes           | No                | Neg                      | 5.37 × 10⁵                  | 1              |
| 41                    | Tripoli Governmental hospital | 16/11/12 | 75  | M   | OPW         | Acute bronchitis                            | Passive       | No                | NA                       | 3.51 × 10⁶                  | 2              |

Abbreviations: BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease; mtLSU-RNA, mitochondrial large subunit ribosomal RNA; NA, not available; OPW, oropharyngeal wash.

*Other risk factors: cancer, immune deficiency, corticotherapy, immunosuppressive treatment, antibiotic therapy.
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 reporting that smoking is not a risk factor for Pneumocystis determined, respectively). This result supported previous data colonized individuals for whom smoking habit was successfully determined (100% and 81.5% in six colonized and 65 non-smokers in both colonized and non-colonized patients (100% and 81.5% in six colonized and 65 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).

Other risk factors

| Type of samples | P. jiroveci DNA not detected |
|-----------------|-----------------------------|
| Antibiotherapy  | 0 (0%)                      |
| OPW            | 3 (42.8%)                    |
| Sputum         | 2 (28.6%)                    |
| BAL            | 1 (14.3%)                    |
| Nasal swab     | 1 (14.3%)                    |
| Tracheal aspiration | 0 (0%)                     |

Abbreviations: BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease; OPW, oropharyngeal wash.

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. jiroveci among patients with respiratory diseases indicates its potential for transmission to immunocompromised patients.

Transparency declaration

The authors declare no conflicts of interest.

Acknowledgements

This work was supported by grants from the Pasteur Institute of Lille, University of Lille, ‘Centre National de la Recherche Scientifique’ (CNRS), and ‘Institut National de la Santé et de la Recherche Médicale’ (Inserm). SK was supported by a PhD fellowship from the Azm & Saade Association from Lebanon.

References

[1] Nevez G, Magois E, Duwat H, Gouilleux V, Jounieaux V, Toret A. Apparent absence of Pneumocystis jirovecii in healthy subjects. Clin Infect Dis 2006:42:e99–101.
[2] Ponce CA, Gallo M, Bustamante R, Vargas SL. Pneumocystis coloni- zation is highly prevalent in the autopsied lungs of the general population. Clin Infect Dis 2010:50:347–53.
[3] Morris A, Wei K, Afshar K, Huang L. Epidemiology and clinical sig- nificance of pneumocystis colonization. J Infect Dis 2008:197:10–7.
[4] Calderón EJ, Rivero L, Respaldiza N, Morilla R, Montes-Cano MA, Friaza V, et al. Systemic inflammation in patients with chronic obstructive pulmonary disease who are colonized with Pneumocystis jiroveci. Clin Infect Dis 2007:45:e17–19.
[5] Wakefield AE, Pixley FJ, Banerji S, Sinclair K, Miller RF, Moxon ER, et al. Amplification of mitochondrial ribosomal RNA sequences from Pneumocystis carinii DNA of rat and human origin. Mol Biochem Parasitol 1990;43:69–76.
[6] Alario A, Desoubeaux G, Sarfati C, Hamane S, Bergeron A, Azoulay E, et al. Real-time PCR assay-based strategy for differentiation between active Pneumocystis jiroveci pneumonia and colonization in immuno- compromised patients. Clin Microbiol Infect 2011:17:1531–7.
[7] Khodadadi H, Mirhendi H, Mohebeli M, Kordbacheh P, Zarrinfar H, Makumira K. Pneumocystis jiroveci colonization in non-HIV-infected patients based on nested-PCR detection in bronchoalveolar lavage samples. Iran J Public Health 2013;42:298–305.

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

| Localization | P. jiroveci DNA detected | P. jiroveci DNA not detected |
|--------------|-------------------------|------------------------------|
| Tripoli Governmental hospital | 6 (14.3%) | 1 (9.4%) |
| Nini hospital | 3 (42.8%) | 66 (52.0%) |
| Monia hospital | 1 (14.3%) | 11 (8.7%) |
| Mazloum hospital | 1 (14.3%) | 13 (10.2%) |
| Local medical care COPD | 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%) |
| 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%) |
| Type of samples | | |
| Antibiotherapy | 0 (0%) | 10 (7.9%) |
| 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.

This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
[8] Damiani C, Le Gal S, Da Costa C, Virmaux M, Nevez G, Totet A. Com-
bined quantification of pulmonary Pneumocystis jirovecii DNA and serum
(1->3)-β-D-glucan for differential diagnosis of pneumocystis pneumonia
and Pneumocystis colonization. J Clin Microbiol 2013;51:3380–8.

[9] Pulcini C, Hasseine L, Mondain V, Baudin G, Roger P-M. Possible
pandemic H1N1 influenza complicated by Pneumocystis jiroveci pneu-
monia in an HIV-infected patient. J Mycol Med 2012;22:88–91.

[10] Sy ML, Chin TW, Nussbaum E. Pneumocystis carinii pneumonia asso-
ciated with inhaled corticosteroids in an immunocompetent child with
asthma. J Pediatr 1995;127:1000–2.

[11] Montes-Cano MA, de la Horra C, Martin-Juan J, Varela JM, Torronteras R, Respaldiza N, et al. Pneumocystis jiroveci genotypes in
the Spanish population. Clin Infect Dis 2004;39:123–8.

[12] Maskell NA, Waine DJ, Lindley A, Pepperell JCT, Wakefield AE, Miller RF, et al. Asymptomatic carriage of Pneumocystis jiroveci in subjects undergoing bronchoscopy: a prospective study. Thorax 2003;58:594–7.

[13] Naba MR, Kanafani ZA, Awar GN, Kanj SS. Profile of opportunistic
infections in HIV-infected patients at a tertiary care center in Lebanon.
J Infect Public Health 2010;3:130–3.

[14] Varela JM, Regordán C, Medrano FJ, Respaldiza N, de La Horra C, Montes-Cano MA, et al. Climatic factors influencing Pneumocystis jiroveci infection in southern Spain. Clin Microbiol Infect 2004;10:770–2.

[15] Schoffelen AF, van Lelyveld SFL, Barth RE, Grass L, de Wolf F, Netes MG, et al. Lower incidence of Pneumocystis jiroveci pneumonia among Africans in the Netherlands host or environmental factors? AIDS 2013;27:1179–84.

[16] Morris A, Scirba FC, Lebedeva IP, Githaiga A, Elliott WM, Hogg JC, et al. Association of chronic obstructive pulmonary disease severity and Pneumocystis colonization. Am J Respir Crit Care Med 2004;170: 408–13.

[17] Vargas SL, Pizarro P, López-Vieyra M, Neira-Avilés P, Bustamante R, Ponce CA. Pneumocystis colonization in older adults and diagnostic yield of single versus paired noninvasive respiratory sampling. Clin Infect Dis 2010;50:e19–21.

[18] Dimonte S, Berrilli F, D’Orazio C, D’Alfonso R, Piacco F, Bordi E, et al. Molecular analysis based on mtlSU+RNA and DHPS sequences of Pneumocystis jiroveci from immunocompromised and immunocompe-
tent patients in Italy. Infect Genet Evol 2013;14:68–72.

[19] Monroy-Vaca EX, de Armas Y, Illnait-Zaragozí MT, Toraño G, Diaz R, Vega D, et al. Prevalence and genotype distribution of Pneumocystis jiroveci in Cuban infants and toddlers with whooping cough. J Clin Microbiol 2014;52:45–51.

[20] Miller RF, Evans HER, Copas AJ, Cassell JA. Climate and genotypes of Pneumocystis jiroveci. Clin Microbiol Infect 2007;13:445–8.