# *Pneumocystis* Colonization in Older Adults and Diagnostic Yield of Single versus Paired Noninvasive Respiratory Sampling

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**(See the article by Ponce et al and the editorial commentary by Calderón, on pages 358–64 and 365–7, respectively.)**

**The presence of *Pneumocystis* was assessed in oropharyngeal wash specimens from 110 adults (median age, 76 years; age range, 69–95 years), 66 of whom had a paired nasal swab specimen. *Pneumocystis jirovecii* DNA was detected in 12.8% of oropharyngeal wash specimens, and the frequency increased to 21.5% in paired specimens. *Pneumocystis* colonization is prevalent in older adults. Double noninvasive sampling increases the diagnostic yield.**

The presence of *Pneumocystis* in respiratory specimens from individuals who do not progress to *Pneumocystis* pneumonia (PCP) has been increasingly recognized in immunocompetent populations since the introduction of highly sensitive DNA amplification methods, such as nested polymerase chain reaction (PCR). The mitochondrial large subunit ribosomal RNA (rRNA) locus is the preferred target, because it is a multicopy gene and its use enhances detection [1, 2]. Characterization of colonization will improve our understanding of *Pneumocystis* reservoirs and foster research to reveal whether it is of clinical significance for immunocompetent individuals [1, 2].

Clusters of PCP have been reported in older individuals who did not have predisposing illnesses [3], and old age has been proposed to be a predisposing factor for *Pneumocystis* colonization [4]. Furthermore, older adults commonly have medical conditions that, if treated with low-dose immunosuppressants, can put them at increased risk of developing PCP [5]. The decreased cellular immunity that may accompany the aging process, also know as *immunosenescence*, may favor *Pneumocystis* colonization in a way similar to HIV infection [6]. However, the incidence of colonization among older adults has not been studied.

An obstacle to accurately diagnosing upper respiratory tract colonization is the need for standardized methods of sampling and detection [1, 7]. The incidences found in different studies of specific populations greatly vary, and the lack of standardized methods hampers comparisons [2]. Furthermore, the relative sensitivity of different specimens for diagnosing colonization with other respiratory pathogens has been reported to vary with age [8]. Therefore, we undertook the present study to describe the incidence of *Pneumocystis* colonization in older adults and to compare the diagnostic yield of nasal swab and oropharyngeal wash specimens as noninvasive sampling methods.

***Methods.*** Older adults attending a preventive health care program at the outpatient clinic of Agustín Cruz Melo, Municipalidad de Independencia, in Santiago, Chile, between October and December 2007 were invited to provide nasal swab and oropharyngeal wash specimens for detection of *Pneumocystis jirovecii*. The Ethics Commission of the North Metropolitan Area of Health in Santiago approved the study, and written informed consent was obtained from all participants. Patients were almost consecutive and were enrolled at a pace of 2–4 patients per day 3 or 4 times per week over a period of 2 months, from 9 October through 12 December 2007. A total of 125 patients were approached, and 15 declined to participate. Inclusion criteria were age of 65 years or more, capacity to provide written informed consent, and being capable of normal-for-age activities. Patients were excluded if they presented with an acute respiratory tract infection, had had any immunocompromising condition during the previous 5 years (eg, cancer), or had malnutrition or had received immunosuppressive drugs for any reason during the previous 3 months (including inhaled or systemic corticosteroids regardless of dose and antibiotics for respiratory conditions or trimethoprim sulfamethoxazole for any reason during the previous 2 months). Age, sex, and inclusion and exclusion criteria were assessed at enrollment by interview and review of the medical records. Patients were contacted by phone 13–18 months after sampling; 42 patients could not be reached by phone, and their medical records were reviewed again to rule out PCP.

Nasal swab specimens were obtained using a sterile, saline-

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moistened, cotton-tipped plastic swab, which was inserted ~4–5 cm into one nostril at an oblique angle toward the turbinates (avoiding the nasopharynx), rotated when resistance was found, extracted, and placed into a tube containing 0.5 mL of sterile saline. Oropharyngeal wash specimens were obtained by having the patient rinse the mouth and gargle the throat for 1 min with 10 mL of sterile saline and recovering the fluid in a screw-capped 50-mL tube [9]. Collected specimens were transported to the laboratory in frozen cool pack–containing insulated boxes. Nasal swab specimens were vigorously vortexed and centrifuged at 20,000 *g* for 5 min, and oropharyngeal wash specimens were centrifuged at 2900 *g* for 5 min. Supernatants were discarded, and the pellets were reconstituted in 200 $\mu$L and stored at −20°C until processing. They were examined individually. DNA was extracted using the QIAmp DNA Mini kit (Qiagen). *Pneumocystis* DNA was identified by a 2-step nested PCR procedure using oligonucleotide primers pAZ102-E and pAZ102-H (which were designed for the gene encoding the mitochondrial large subunit rRNA of *Pneumocystis* and which amplify all *Pneumocystis* species) and internal primers pAZ102-X and pAZ102-Y (which are specific for *P. jirovecii*), as described elsewhere [9–11]. *Pneumocystis*-negative and human $\beta$-globin internal controls were included with each specimen, to monitor for cross-contamination and DNA inhibitors. DNA extraction and preparation of reaction mixtures were performed in separate rooms. Experiments were conducted in duplicate, with blinding to clinical details.

The Stata statistical package, version 10.1 (StataCorp), was used to compare *Pneumocystis* colonization rates as detected by each sampling method alone or in combination in all participants and in subgroups of individuals <80 and ≥80 years old, by the $\chi^2$ association test. Differences for which $P < .05$ were considered significant.

***Results.*** A total of 110 adults (median age, 76; age range, 69–95 years), of whom 79 (71.8%) were female, met the inclusion criteria and were enrolled. One oropharyngeal wash specimen was not evaluable, and the first 44 consecutive nasal swab specimens were discarded before analysis because the swab collection procedure was modified to avoid the nasopharynx. *P. jirovecii* DNA was detected in 14 (12.8%) of 109 oropharyngeal wash specimens, in 7 (10.6%) of 66 nasal swab specimens, and in 14 (21.5%) of 65 paired oropharyngeal wash and nasal swab specimens (Figure 1). No coincidental positive results were detected in paired specimens. When compared by age group, *P. jirovecii* DNA was detected in 10 (13.8%) of 72 oropharyngeal wash specimens and in 6 (14.6%) of 41 nasal swab specimens from individuals <80 years old versus 4 (10.8%) of 37 oropharyngeal wash specimens and 1 (4.0%) of 25 nasal swab specimens from those ≥80 years old ($P \geq .05$). Combining the results increased the diagnostic yield to 10 (25.0%) of 40 and to 4 (16.0%) of 25, respectively.

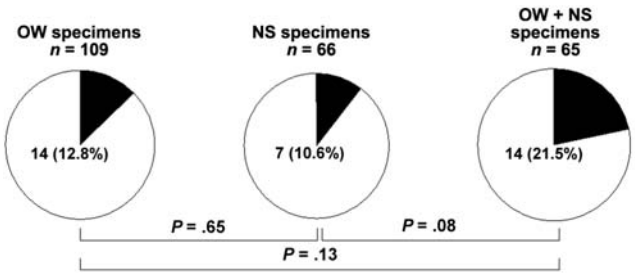


**Figure 1.** *Pneumocystis* colonization in older adults, as detected by nested polymerase chain reaction DNA amplification in nasal swab (NS) specimens, oropharyngeal wash (OW) specimens, and both specimens (combined results).

Phone calls and review of medical records revealed no cases of PCP occurring within 1 year of sampling.

***Discussion.*** This study demonstrates that nonimmunosuppressed older adults are frequently colonized by *Pneumocystis*, suggesting that they participate in the transmission cycle of *P. jirovecii* as a reservoir of this infection for immunosuppressed susceptible individuals. The clinical significance of this finding has not been established. However, none of the participants developed PCP within 1 year of sampling.

The colonization incidence detected by nested PCR DNA amplification of single noninvasive specimens in this study (12.8% for oropharyngeal wash specimens and 10.6% for nasal swab specimens) is comparable to the incidence recently reported by Spencer et al [11] for single oropharyngeal wash specimens from young adults in the community and is inferior to the incidence found by Medrano et al [10] among younger administrative health care workers tested using single oropharyngeal wash specimens. Participants in the present study attended a preventive health program for elderly persons in an outpatient setting; they were in good health overall and did not have respiratory symptoms before or at the time of sampling. Increasing age has been associated with *Pneumocystis* colonization, and older adults without immunocompromising conditions may have decreased T lymphocyte cellular immune responses, predisposing them to *Pneumocystis* colonization [4, 6]. However, an increased incidence of *Pneumocystis* was not documented in participants in the present study relative to reported incidences in younger adults.

Interestingly, pairing 2 specimens almost doubled the yield of *P. jirovecii* DNA detection in this study, from 12.8% to 21.5%. This shows that, given the lack of a microbiological culture method for *Pneumocystis*, the amount of sample is critical for the diagnosis of colonization states in which the load of *Pneumocystis* organisms is minimal. Quantification of *Pneumocystis* copies by real-time PCR normalized on the basis of the number of human genome copies has been shown to be useful to compare the *Pneumocystis* load in bronchoalveolar lavage specimens

obtained for the diagnosis of PCP [12]. Whether normalization on the basis of human DNA may be applicable to establish a minimum amount of specimen required for noninvasive sampling in immunocompetent individuals may deserve further exploration.

Oropharyngeal washing detected a larger (although not statistically significant) proportion of *Pneumocystis*-positive individuals than did nasal swabbing, suggesting that oropharyngeal wash specimens may be preferable for the detection of colonization (Figure 1), as has been suggested for other pathogens [8]. Nasal swab specimens were less sensitive in individuals ⩾80 year old, possibly because of generalized poor tolerance of older persons to the nasal swab collection procedure.

Concomitant positive results for *P. jirovecii* DNA at nasal and oral sampling sites was not found, indicating that approximately half of *Pneumocystis*-colonized individuals would have been missed if only 1 specimen were studied and suggesting that combining sampling results from >1 site can provide a more accurate diagnosis of colonization. Furthermore, combined or mixed sampling may be cost-effective as a strategy to increase the yield of testing in asymptomatic subjects, as has been proposed for other respiratory pathogens [13]. This agrees with the pattern of colonization of *Streptococcus pneumoniae* and *Haemophilus influenzae*, which may reveal different incidences of colonization at different sites within the upper respiratory tract, suggesting that different upper airway anatomical niches may need to be sampled to increase the yield of detection of colonization with *P. jirovecii* [14, 15].

The lack of agreement of positive results for *Pneumocystis* between different specimen types in this population may not be universal for *Pneumocystis* infections; for example, PCP in severely immunocompromised individuals with characteristically higher *Pneumocystis* loads is readily diagnosable by less sensitive methods, such as microscopic analysis or single-round PCR.

In summary, upper respiratory tract colonization by *P. jirovecii* is common in older healthy adults. The clinical significance of this finding remains to be determined. Detection of *P. jirovecii* DNA in oropharyngeal wash specimens does not predict the results for nasal swab specimens and vice versa, indicating that upper respiratory tract colonization by *P. jirovecii* may have different niches. Combining the results for samples of different anatomical sites increases the diagnostic yield, suggesting that mixing specimens before DNA extraction may be a cost-effective strategy to increase the diagnostic accuracy of *Pneumocystis* upper respiratory tract colonization.

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