Letter to the Editor

Risk factors for *Haemophilus influenzae* and pneumococcal respiratory tract colonization in CVID

To the Editor:

Disease-specific studies focused on infection risk in common variable immune deficiencies (CVIDs) are needed to define strategies for controlling respiratory infections predominantly due to bacteria such as *Streptococcus pneumoniae* and *Haemophilus influenzae*.[1] Little information is available on the rate of airway bacterial carriage and its consequence in hypogammaglobulinemias. Despite IgG replacement, recurrent respiratory infections are common in CVID, possibly leading to chronic lung damage[2] and poor quality of life.[3] Thus, patients are often prescribed antibiotics and/or long-term antimicrobial prophylactic regimens. Several regimens are used including rotation or periodically changing antibiotics.[4] However, antibiotics influence antimicrobial resistance among airway microbiota. In a recent meta-analysis on patients with chronic lung diseases, 30% of *S pneumoniae* showed resistance to macrolides.[5]

In this observational longitudinal study, we investigated the rate of nasopharyngeal and oropharyngeal mucosal carriage of *S pneumoniae* and *H influenzae* identified by cultural methods and real-time PCR (RT-PCR), the antimicrobial susceptibility patterns, and the antibiotic usage in adult patients with CVID (http://esid.org/Working-Parties/Registry/Diagnosis-criteria). In addition, we verified whether colonization would be associated with risk for respiratory infections.

Exclusion criteria for swabs collection included acute respiratory infections and antibiotic treatment in the month preceding the swabs collection except for antibiotic prophylaxis. Rate of respiratory infections, clinical data, and days of antibiotic usage in the 6 months before swabs collection were recorded. After swabs collection, respiratory infections were recorded for an additional 6 months. Sample collection and identification of *S pneumoniae* and *H influenzae* were performed according to the World Health Organization standard procedures by cultural and molecular methods on nasopharyngeal and oropharyngeal swabs collected on the same day. For both bacterial species, minimum inhibitory concentrations of antimicrobial agents were determined. The interpretative criteria were based on European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (http://www.eucast.org/clinical breakpoints/) (see the Methods section in this article's Online Repository at www.jacionline.org).

The 12-month study included 93 patients with CVID (mean age, 49.4 ± 14.4 years; range, 18-77) with a 1:1 male to female ratio. Participants were on IgG replacement at a monthly cumulative dose of 400 mg/kg. Mean IgG trough levels at the study time were 688.8 ± 156.0 mg/dL. Nineteen patients were exposed to children younger than 6 years at home or through work. Thirty-four (41%) patients had bronchiectasis and 9 (9.6%) were on chronic antibiotic prophylaxis.

The carriage prevalence by culture was 10.8% and 26.9% for *S pneumoniae* and *H influenzae*, respectively, regardless of the isolation site (see Table E1 in this article's Online Repository at www.jacionline.org). Four percent of CVID were cocolonized by both *S pneumoniae* and *H influenzae*. In line with other pneumococcal carriage studies, after the long-term use of glycoconjugate vaccines, we found only 20% of the 13 pneumococcal isolates belonging to vaccine serotypes[6]: 10A and 19A (2 isolates each), 6A, 12F, 23A, 35F, and 37 (1 isolate each); 4 isolates were nontypeable. All 36 *H influenzae* isolates were nontypeable, confirming the absolute prevalence of this serotype, especially among respiratory tract isolates, in the present Hib vaccination era (see Table E2 in this article's Online Repository at www.jacionline.org).

Compared with culture, RT-PCR allowed identifying a higher carriage rate of *Spneumoniae* and/or *H influenzae* (10.8% *vs* 52.7%, $P < .0001$ and 26.9% *vs* 39.8%, $P = .043$, respectively). In the RT-PCR assay, the cycle threshold value was significantly lower in samples found positive by culture compared with those positive by RT-PCR, suggesting that the former samples had a higher *S pneumoniae* and/or *H influenzae* bacterial load. By RT-PCR the percentage of CVID cocolonized carriers significantly increased (4% *vs* 24%; $P = .0001$). *H influenzae*/*S pneumoniae* carriers identified by only RT-PCR had a milder immunological defect than culture-positive patients (IgA, 15.8 ± 3.2 *vs* 2.7 ± 0.9 mg/dL, $P = .0001$; IgM, 22.2 ± 4.4 *vs* 9.9 ± 4.1, $P = .030$).

Risk factors related to colonization were initially identified by binary logistic regression. Low IgM and low IgA serum levels were found to be risk factors for *S pneumoniae* and *H influenzae* colonization. Colonization by culture was associated with IgM serum levels of less than 5 mg/dL (odds ratio [OR], 3.7; 95% CI, 0.86-15.99; $P = .05$; OR, 2.5; 95% CI, 0.95-6.45; $P = .05$, respectively) and with IgA serum levels of less than 7 mg/dL (OR, 5.7; 95% CI, 0.69-47.17; $P = .05$; OR, 8.9; 95% CI, 1.92-40.84; $P = .001$, respectively). Of note, patients cocolonized had particularly low IgA and IgM serum levels (1.3 ± 0.7 mg/dL and 1.7 ± 1.2 mg/dL, respectively). This confirmed data on patients with hyper-IgM syndrome and healthy subjects where IgM antibodies have a distinct anti–*H influenzae* activity in the prevention of respiratory tract bacterial colonization.[7-9]

Other potential risk factors such as age, previous respiratory infections, bronchiectasis, chronic lung diseases, IgG trough level of less than 600 mg/dL, and defects in other immunological parameters were not associated with colonization. Antibiotic use and long-term prophylaxis in the previous 6 months were not associated with *H influenzae* and *S pneumoniae* colonization. However, participants exposed to young children were more likely to be colonized by *H influenzae* (OR, 5.9; 95% CI, 2.00-13.36; $P = .001$), but not by *S pneumoniae*. Multivariate analysis confirmed that IgA serum levels of less than 7 mg/dL (OR, 5.4; 95% CI, 1.08-26.70; $P = .040$) and the presence of children younger than 6 years in the house/workplace (OR, 4.5; 95% CI, 1.37-14.50; $P = .013$) had a direct effect on *H influenzae* colonization (Table I).

Carriers identified by culture, but not by RT-PCR, had a higher risk of upper respiratory tract infection but not lower respiratory

TABLE I. Risk factors for *S pneumoniae* and *H influenzae* colonization as detected by culture

		Univariate analysis						Multivariate analysis		
		S pneumoniae			*H influenzae*			*H influenzae*		
	n (%)	OR	95% CI	*P* value	OR	95% CI	*P* value	OR	95% CI	*P* value
Sex (female)	47 (51.1)	0.95	0.25-3.54	NS	0.70	0.27-1.70	NS	—	—	—
Age < 35 y	12 (12.9)	3.52	0.77-16.10	NS	1.43	0.39-5.23	NS	—	—	—
IgM < 5 mg/dL	34 (38.2)	3.71	0.86-15.99	.05	2.48	0.95-6.44	.05	1.63	0.55-4.79	NS
IgA < 7 mg/dL	58 (65.2)	5.69	0.69-47.17	.05	8.86	1.92-40.84	.001	5.36	1.08-26.7	.040
IgG trough level <600 mg/dL	8 (8.6)	0.60	0.16-2.35	NS	0.71	0.26-1.82	NS	—	—	—
Exposition to children	19 (20.4)	0.42	0.05-3.38	NS	5.89	2.00-17.36	.001	4.45	1.37-14.50	.013
URTI in the previous 6 mo	21 (25.0)	1.74	0.45-6.73	NS	0.93	0.34-2.58	NS	—	—	—
LRTI in the previous 6 mo	32 (38.5)	1.86	0.50-6.96	NS	1.54	0.60-3.92	NS	—	—	—
CLD	49 (53.3)	0.55	0.14-2.09	NS	0.93	0.37-2.34	NS	—	—	—
Bronchiectasis	34 (36.5)	1.08	0.27-4.37	NS	0.82	0.31-2.18	NS	—	—	—
Use of antibiotics in the previous 6 mo	47 (50.5)	0.98	0.26-3.63	NS	0.87	0.35-2.18	NS	—	—	—
Amoxicillin/Clavulanic acid	21 (24.4)	1.20	0.22-6.57	NS	0.97	0.30-3.17	NS	—	—	—
Macrolides	13 (15.1)	1.97	0.35-11.15	NS	1.21	0.33-4.50	NS	—	—	—
Antibiotic prophylaxis	9 (9.7)	1.04	0.12-9.31	NS	0.75	0.15-3.91	NS	—	—	—
CD3+CD4+ <400 cell/mm^3	22 (31.4)	3.41	0.69-16.76	NS	0.81	0.25-2.66	NS	—	—	—
CD19+ <8%	36 (51.4)	1.67	0.37-7.59	NS	0.69	0.23-2.01	NS	—	—	—
CD27+IGD−IgM− <3%	30 (56.6)	1.03	0.21-5.11	NS	2.08	0.60-7.19	NS	—	—	—

CLD, Chronic lung disease; *LRTI*, lower respiratory tract infection; *NS*, not significant; *URTI*, upper respiratory tract infection.

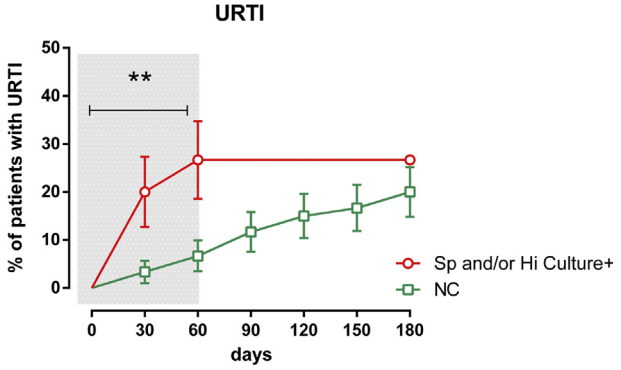

FIG 1. URTIs over the 6 months after colonization status assessment. *URTI,* Upper respiratory tract infection. Red lines: Patients with CVID colonized by *S pneumoniae (Sp)* and/or *H influenzae (Hi)*; Green lines: not colonized.

tract infection within 3 months of swabs collection (hazard ratio, 3.0; 95% CI, 1.0-9.20; Log-rank test $P = .05$), with the highest levels of significance recorded within 60 days (hazard ratio, 4.1; 95% CI, 1.70-10.0; Log-rank test $P = .002$; Fig 1). Thus, the lack of a clinical correlation with RT-PCR data did not allow to suggest to routinely adopt RT-PCR in the colonization assessment of patients with primary antibody deficiencies.

The actual impossibility to replace IgA and IgM at the mucosal level implied the need to consider additional therapeutic interventions in primary antibody deficiencies. such as the use of antibiotic treatment or prophylaxis. However, a major concern related to the use of antibiotic therapy or prophylaxis is that it exerts selective pressure on airway microbiota, facilitating the emergence of antibiotic resistance. Our study added information on rates of antibiotic resistance in *S pneumoniae* and *H influenzae* isolates in CVID. As shown in Table E2, only 3 of 13 pneumococcal isolates were susceptible to all antibiotics tested (penicillin, ceftriaxone, erythromycin, clindamycin, levofloxacin). In particular, 9 of 13 pneumococcal isolates were nonsusceptible to penicillin. However, only 1 isolate was fully resistant, whereas the others showed low-level penicillin resistance, which is considered potentially overcome by high-dose penicillin in case of respiratory infections.

Differently, 19 of 36 *H influenzae* isolates were susceptible to all antibiotics tested. Ampicillin and azithromycin resistance was the most frequently detected: 9 of 36 isolates were resistant to ampicillin and 10 of 36 were resistant to azithromycin. Although the EUCAST breakpoint tables (Version 8, 2018) no longer report clinical breakpoints of macrolide for *H influenzae* in respiratory infections, it indicates the epidemiological cutoffs to detect isolates with acquired macrolide resistance. In the 6 months preceding the swabs collection, beta-lactams were the most frequently administered antibiotic (22 patients), followed by macrolides (14 patients). Carriers of susceptible strains were never treated by beta-lactams and/or macrolides, whereas carriers of strains nonsusceptible to beta-lactams and macrolides were treated for a mean of 14.4 ± 6.6 days ($P = .05$). In conclusion, in CVID very low IgA serum level is a risk factor for carriage and colonization acts as a bacteria reservoir and as a risk factor for respiratory complications.

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METHODS

Nasopharyngeal and oropharyngeal swabs were collected once for each patient and transferred to the laboratory within 4 hours of collection. Aliquots of STGG medium were streaked on 5% sheep blood Columbia agar plates supplemented with 5 μg/mL gentamycin to isolate *S pneumoniae* and on chocolate blood agar containing Vitox, bacitracin, vancomycin, and amphotericin B (*Haemophilus Selective Agar*, Oxoid Ltd, Basingstoke, Hampshire, UK) to selectively culture *H influenzae*. Colonies resembling *S pneumoniae* or *H influenzae* were further identified by standard microbiological tests. *S pneumoniae* isolates were serotyped by the Quellung reaction using commercially available antisera (Statens Serum Institut, Copenhagen, Denmark). The capsular genotype of *H influenzae* isolates was determined by PCR, as previously reported.[E1] For molecular detection of *S pneumoniae* and *H influenzae* in nasopharyngeal and oropharyngeal specimens, genomic DNA was extracted from STGG broths using QIAmp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. *S pneumoniae* was detected by RT-PCR targeting the *lytA* gene and using primers and probes previously described.[E2] For *H influenzae*, RT-PCR was carried out targeting the *hpd* gene with primers hpdF822 and hpdR952 and following conditions previously reported.[E3]

Antimicrobial susceptibility testing

For both bacterial species, minimum inhibitory concentrations (MICs) of antimicrobial agents were determined by E-test (Etest, bioMérieux, Durham, NC). The interpretative criteria were based on EUCAST breakpoints (http://www.eucast.org/clinical breakpoints/). The antimicrobial agents tested in MIC determination were penicillin, ceftriaxone, erythromycin, clindamycin, and levofloxacin for *S pneumoniae* isolates and ampicillin, amoxicillin-clavulanic acid, azithromycin, cefotaxime, ciprofloxacin, and meropenem for *H influenzae* isolates.

Analyses

Patients' demographic characteristics and clinical characteristics were expressed by mean ± SD or by frequencies, where appropriate. The outcome measures in this analysis were colonization by *S pneumoniae* and colonization by *H influenzae* identified by traditional culture and by RT-PCR. Clinical and immunological characteristics were investigated to reveal the relationships between the variable and each outcome measure. Comparison of continuous variables was performed by the unpaired *t* test. We calculated differences in frequencies between groups using Fisher exact test. To determine which variables to consider for building the multivariate logistic regression model for *H influenzae* and/or *S pneumoniae*, a binary logistic regression was run. Variables with a *P* value of less than .25 were considered for the multivariate logistic regression model. The backwards stepwise selection method was used to build the multivariate model. We computed the pooled ORs for end points with 95% CI. Cutoff points for age, CD19+CD27+IgD−IgM− frequency, IgM serum levels, and IgA serum levels were based on the highest sensitivity value plus the specificity identified by the receiver-operating characteristics curve method curve between *S pneumoniae* and/or *H influenzae* colonized and not colonized patients. Infections-survival rates were estimated by using the Kaplan-Meier method. The statistical significance was set at the conventional level of $P < .05$. All statistical analyses were performed using the statistical package STATA 11 (StataCorp LP, College Station, Tex).

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TABLE E1. *S pneumoniae* and *H influenzae* colonization by culture and RT-PCR in CVID

	CVID (n = 93), n (%)	
Species/isolation site	**Culture+**	**RT-PCR+**
S pneumoniae		
Nasopharynx and/or oropharynx	10 (10.8)	49 (52.7)
Nasopharynx	8 (8.6)	32 (34.4)
Oropharynx	6 (6.5)	35 (37.6)
H influenzae		
Nasopharynx and/or oropharynx	25 (26.9)	37 (39.8)
Nasopharynx	22 (23.7)	30 (32.3)
Oropharynx	14 (15.1)	27 (29.0)

TABLE E2. Serotype and antibiotic susceptibility of *S pneumoniae* and *H influenzae* isolates from nasopharynx and oropharynx in patients with CVID

Spneumoniae serotype	Site	n	PG R/I	TX I	EM R	CL R	LV R	*H influenzae* serotype	Site	n	AM R	XL R	CT R	AZ R	CI R	MP R
6A*	NP	1	Yes	Yes	Yes	Yes	No	NTHi	NP	6	No	No	No	Yes	No	No
10A	NP	1	No	No	No	No	No	NTHi	NP	4	Yes	No	No	No	No	No
12F	NP	1	No	No	No	No	No	NTHi	NP	1	Yes	No	No	Yes	No	No
19A	NP	1	Yes	No	Yes	Yes	No	NTHi	NP	1	Yes	Yes	No	No	Yes	No
23A	NP	1	No	No	Yes	Yes	No	NTHi	NP	10	No	No	No	No	No	No
35F	NP	1	Yes	No	No	No	No	NTHi	OP	2	Yes	No	No	No	No	No
NT	NP	1	Yes	No	Yes	Yes	Yes	NTHi	OP	2	No	No	No	Yes	No	No
NT	NP	1	Yes	No	Yes	No	No	NTHi	OP	1	Yes	No	No	Yes	No	No
10A	OP	1	No	No	No	No	No	NTHi	NP	9	No	No	No	No	No	No
19A	OP	1	Yes	No	Yes	Yes	No									
37	OP	1	Yes	Yes	Yes	No	No									
NT	OP	1	Yes	Yes	Yes	Yes	Yes									
NT	OP	1	Yes	No	Yes	No	No									
Total		15	10	3	10	7	2			36	9	1	0	10	1	0

AM, Ampicillin; *AZ*, azithromycin; *CI*, ciprofloxacin; *CL*, clindamycin; *CT*, cefotaxime; *EM*, erythromycin; *I*, intermediate; *LV*, levofloxacin; *MP*, meropenem; *NP*, nasopharynx; *NT*, nontypeable; *NTHi*, nontypeable *H inlfuenzae*; *OP*, oropharynx; *PG*, penicillin; *R*, resistant; *TX*, ceftriaxone; *XL*, amoxicillin-clavulanic acid.
**S pneumoniae* isolate fully resistant to penicillin.