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RESEARCH PAPER

Nasopharyngeal pneumococcal carriage rates among HIV-infected adults following widespread pediatric use of conjugate pneumococcal vaccine-13

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ABSTRACT

Background: Nasopharyngeal pneumococcal carriage rates among HIV-infected adults has not been described since conjugate pneumococcal vaccine-13 (PCV13) was added to the universal infant and childhood vaccination schedule in 2010. *Methods*: HIV-infected adults presenting for routine health care visits to the Designated AIDS Center in Syracuse, NY between December 2013 and June 2015 were eligible for enrollment. Demographic, medical, and social history were recorded after obtaining informed consent. Nasopharyngeal samples were collected and cultured for the presence of Streptococcus pneumoniae using standard microbiologic techniques. Antibiotic susceptibility testing was performed using E-test→. *Results*: 707 nasopharyngeal samples were collected from 414 HIV-infected adults. 18 samples were culture positive for *S. pneumoniae*; 1 (6%) isolate was of vaccine-type, 9 (50%) were non-vaccine types, and 8 (44%) were non-typeable. The 18 isolates were recovered from 15 different patients (4% of those enrolled). Three patients were culture positive for pneumococcus isolated from 2 consecutive samples, with non-typeable pneumococci identified consecutively from 2 patients and serotype 35B identified consecutively from 1 patient. The most commonly identified non-vaccine serotypes were 35B and 15B/C. Identified pneumococci were penicillin and cefotaxime susceptible. *Conclusion*: Four percent of HIV-infected adults in our study population were colonized with *S. pneumoniae*. The non-vaccine serotypes 35B and 15B/C predominated.

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Introduction

Streptococcus pneumoniae is a leading cause of pneumonia, bacteremia, and meningitis among all age groups.[1] Adults with chronic illnesses, including those with HIV infection, are at a high risk for developing invasive pneumococcal disease (IPD) and its complications.[2] Following the introduction of highly active antiretroviral therapy (HAART) for the treatment of HIV infection in the mid-1990s, reductions in IPD among HIV-infected adults was observed.[3] Despite this decline, the incidence of IPD in HIV-infected adults remained approximately 60 times higher than that seen in the general population, independent of the patient's CD4 count.[4] For these reasons, the 23-valent polysaccharide pneumococcal vaccine (PPV23) has been recommended for all HIV-infected individuals since 1997.[5]

Nasopharyngeal carriage of pneumococci occurs when an individual is exposed to someone already colonized. While acquisition of and colonization with pneumococcus usually occurs without sequelae, it is considered a necessary prerequisite for the development of invasive disease.[6] Prior to the introduction of conjugate pneumococcal vaccines, nasopharyngeal carriage rates ranged from 45% in pre-school aged children to 6% in adults without children in the home.[5,6] Rates among HIV-infected adults were reported to be between 7-23%.[7,8]

The addition of conjugate pneumococcal vaccine-7 (PCV7) to the US universal pediatric immunization schedule in 2000 led to an expected decline in vaccine-type IPD among immunized children. Two years after the introduction of PCV7, nasopharyngeal carriage rates of vaccine-type pneumococci were reduced from 15% to 2.6% in all age groups in the UK and from 52% to 31% in Alaskan children.[9,10] Seven years after the introduction of PCV7, nasopharyngeal carriage rates among HIV-infected adults in the United States were reported to be at 3%.[11] Conjugate vaccine-associated reductions in nasopharyngeal carriage contributed to decreased transmission throughout the community as evidenced by a clear decline in the incidence of IPD among adults following routine use of conjugate vaccine in children.[12]

By 2004, serotype replacement with non-PCV7 vaccine strains emerged resulting in both nasopharyngeal carriage and IPD among children and adults, including those infected with HIV.[13-17] Infections caused by the more tenacious and antibiotic resistant serotype 19A had become particularly problematic. In 2010, PCV13, offering protection against an additional 6 of the most common pneumococcal serotypes, including serotype 19A, replaced PCV-7 in the universal pediatric immunization schedule allowing an opportunity for further reductions in IPD.[18] In 2012, the Advisory Committee on Immunization

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Practices expanded the recommendation for PCV13 vaccination to include high risk adults, including those with HIV-infection.[19]

Recently published studies describe nasopharyngeal pneumococcal carriage rates of 10-22% among HIV-infected adults in Malawi and Indonesia.[20,21] The lack of conjugate pneumococcal vaccine use in these communities during their study periods likely contributes to the higher carriage rates in these developing countries when compared to the carriage rates in the US at a similar time.

Data describing nasopharyngeal pneumococcal carriage rates following the use of PCV13 are primarily reported from the European pediatric population. Three years after introduction of PCV13, pneumococcal carriage among European infants and toddlers dropped from 71% to 56%.[22] Similarly, carriage in French children decreased from 54% to 46% in a 3 year span.[23] In this work, we characterized pneumococcal nasopharyngeal carriage rates in HIV-infected adults following the introduction of PCV13 to the universal pediatric immunization schedule. In addition, we describe the serotype distribution and antibiotic susceptibility pattern for each of the isolates.

Results

414 HIV-infected adults were enrolled in this study. 301 (73%) of the adults were male. The age range was 18-78 years, with a median of 48 years with most identifying their race as Caucasian (243, 59%) or African American (134, 32%). Half of the subjects had at least 1 medical co-morbidity known to predispose to IPD. 220/414 (53%) had received PPV-23 vaccine in the past 5 years, while only 14 (4%) of 389 eligible for PCV13 (as determined by the timing of PPV23 receipt) had received PCV13 (Table 1). The 25 subjects who had been immunized with PPV23 within the past year were not yet eligible to receive PCV13.

707 nasopharyngeal samples were collected from 414 HIV-infected adults. 18/707 (3%) samples were culture positive for *S. pneumoniae*; 1 (6%) was vaccine-type 3, 9 (50%) were non-vaccine types and 8 (44%) were non-typeable. The 18 isolates were recovered from 15 different patients (4% of those enrolled). There were no association between gender, race, or social/behavioral risk factors and pneumococcal colonization (Table 2). Of the 15 HIV-infected subjects colonized, 9 (60%) had no other identified medical co-morbidity placing them at risk for IPD.

Of the 707 swabs collected from 414 subjects, 196 (28%) were obtained from individuals with respiratory symptoms, 674 (95%) from individuals receiving anti-retroviral therapy, and 68 (10%) from individuals taking TMP/SMX prophylaxis. The presence of respiratory symptoms was the only patient factor associated with pneumococcal colonization (61% vs 27%, $p = 0.001$). The presence of pneumococcal colonization was not associated with any of the patients' most recent laboratory test results, including the absolute neutrophil count ($p = 0.59$), CD4 count ($p = 0.74$), and HIV viral load ($p = 0.45$)(Table 3).

Only 1 of the 18 (6%) isolates identified was determined to be a serotype included in the PCV13 vaccine (serotype 3). Nine (50%) were non-PCV13 vaccine serotypes, and 8 (44%) were non-typeable (Table 4). The non-PCV 13 vaccine serotypes identified include 10A ($n = 1$), 15B/C ($n = 3$), 17F ($n = 1$), 31 ($n = 1$), and 35B ($n = 3$). There was no association between receipt of PPV23 and serotype isolated from nasopharyngeal sample ($p > 0.05$). All 9 (100%) typeable pneumococci were penicillin susceptible, and all 18 (100%) typeable and non-typeable isolates were cefotaxime susceptible. Eight (44%) of the isolates were trimethoprim-sulfamethoxazole (TMP/SMX) resistant; 7 (88%) of the TMP/SMX resistant isolates were not typeable. There was no association between antibiotic use in the past 4 weeks, TMP/SMX prophylaxis, anti-retroviral therapy, medical co-morbidities, the presence of children younger than 5 years in the household, cigarette smoking, or alcohol use and pneumococcal antimicrobial susceptibility ($p > 0.05$ for each).

Of the 414 patients enrolled, 115 (28%) had 2 swabs obtained, 48 (12%) had 3 swabs obtained, and 22 (5%) had at least 4 swabs obtained during the study period. Three patients had pneumococcus isolated from 2 consecutive nasopharyngeal samples separated by 3-6 months. In 2 cases, the pneumococcus identified from both swabs were non-typeable. In the third case, both isolates were identified as serotype 35B. No cases of IPD occurred among enrolled subjects during the study period.

Table 1. Subject demographics.

Demographics	Enrolled subjects	% of subjects
Total enrolled	414	100
Gender		
Male	301	73
Female	113	27
Age (years)		
Range	18-78	
Median	48	
Race		
Caucasian	243	59
African American	134	32
Hispanic	27	7
Mixed	3	1
Medical co-morbidities		
None	204	49
$\geq$ 1 medical co-morbidity[a]	210	51
Social/Behavioral Risk Factors		
Children < 5 years in the house	30	7
Cigarette smoker	189	46
Alcohol use	175	42
PCV13 eligible[b]	387	
Received PCV13	11	3
PPV23 eligible	414	
PPV23 receipt in past 5 years	220	53

[a]including medical co-morbidities predisposing to IPD (asthma, chronic obstructive pulmonary disease, liver cirrhosis, cancer, splenectomy, diabetes mellitus, chronic heart disease, chronic kidney disease, hepatitis B, and hepatitis C)
[b]patients were eligible for PCV13 if they had not received PPV23 or if had been over 1 year since PPV23 receipt.

Discussion

Data describing nasopharyngeal pneumococcal carriage among adults in the United States are sparse. To our knowledge, this is the first report to describe pneumococcal colonization among HIV-infected adults following the 2010 introduction of PCV13 vaccine into the pediatric immunization schedule. Four percent of the 414 subjects enrolled were colonized with pneumococcus at least once during the 15-month study period, and only 1 of the isolates was a serotype included in PCV13 isolated from a subject who was eligible for but had not received PCV13

Table 2. Characteristics of patients colonized with pneumococcus at least once during study period.

	No carriage identified N (%)	Carriage identified at least once N (%)	P
Total enrolled	399 (96)	15 (4)	
Gender			
Male	292 (73)	9 (60)	0.26
Female	107 (27)	6 (40)	
Age (years)			
Range	18-78	24-65	
Median	48	50	
Race			
Caucasian	233 (58)	10 (67)	0.34[a], 0.43
African American	131 (33)	3 (20)	0.16[c]
Hispanic	25 (6)	2 (13)	
Mixed race	3 (<1)	0	
Medical co-morbidities			
None	195 (49)	9 (60)	0.40
≥ 1 medical co-morbidity	204 (51)	6 (40)	0.40
Social/Behavioral Risk Factors			
Children < 5 years in the house	29 (7)	1 (7)	0.93
Cigarette smoke	180 (45)	9 (60)	0.26
Alcohol use	168 (42)	7 (47)	0.73
PCV13 eligible	374	15	
Received PCV13	11 (3)	0	0.43
PPV23 eligible	399	15	
PPV23 receipt in past 5 years	209 (52)	11 (73)	0.11

[a]comparison of colonization rates between Caucasian and African American.
[b]comparison of colonization rates between Caucasian and Hispanic.
[c]comparison of colonization rates between African American and Hispanic.

vaccine. Only 4% of eligible HIV-infected subjects had been immunized with PCV13 despite clear ACIP recommendation in place for 12 months prior to initiating study enrollment. The virtual absence of PCV13 serotype colonization in this population, that at the time was largely unimmunized with PCV13, suggests and supports that vaccine serotypes are circulating at very low levels in our community because of the successful immunization of other groups, especially young children.

Our overall pneumococcal carriage rate of 4% (15/414) in HIV-infected adults following the introduction of PCV13 to the pediatric immunization schedule in the US was similar to Onwubiko's findings in 2008 which showed pneumococcal colonization rates of 3% (6/175) among HIV-infected adults following the widespread use of PCV7 in the US.[11] Of note, 1

Table 3. Impact of current respiratory symptoms, medication use, and laboratory results obtained within 3 months of nasopharyngeal swab collection on pneumococcal carriage.

	All samples n(%)	Not colonized n(%)	Colonized n(%)	p
Total collected	707	689 (97)	18 (3)	
Clinical symptoms				
Respiratory symptoms present[a]	196 (28)	185 (27)	11 (61)	0.001
Medication use				
Recent antibiotic use	60 (8)	59 (9)	1 (6)	0.65
Anti-retroviral therapy	674 (95)	658 (96)	16 (89)	0.19
TMP-SMX[b]	68 (10)	66 (10)	2 (11)	0.83
Laboratory results				
ANC[c3]	11 (2)	11 (2)	0	0.59
CD4 < 200 cells/mm^3	63 (9)	61 (9)	2 (11)	0.74
Viral load ≥ 1000 cells/mm^3	78 (11)	77 (11)	1 (6)	0.45

[a]As defined by runny nose, ear pain, cough, or sore throat.
[b]Trimethoprim-sulfamethoxazole prophylaxis.
[c]absolute neutrophil count.

Table 4. Pneumococcal serotypes and antibiotic susceptibilitya patterns.

Serotype	Number of samples	Penicillin susceptible	Cefotaxime susceptible	TMP-SMX[b] susceptible
3[c]	1	1 (100)	1 (100)	1 (100)
10A	1	1 (100)	1 (100)	0
15B/C	3	3 (100)	3 (100)	3 (100)
17F	1	1 (100)	1 (100)	1 (100)
31	1	1 (100)	1 (100)	1 (100)
35B	3	3 (100)	3 (100)	3 (100)
Non-typeable	8	7 (88)	8 (100)	1 (13)
Non-PCV13 serotype	9	9 (100)	9 (100)	8 (89)

[a]CLSI breakpoints used for determination of susceptibility (≤2 μg/mL penicillin, ≤2 μg/mL cefotaxime, ≤2 μg/mL trimethoprim-sulfamethoxazole).
[b]TMP-SMX: trimethoprim-sulfamethoxazole.
[c]in PCV13 vaccine.

(17%) of the pneumococcal isolates in Onwubiko's study was serotype 19A, a type that has since been added to the conjugate pneumococcal vaccine and was not isolated in our population. Based on that study and our work, pneumococcal colonization rates in HIV-infected adults are clearly lower than the rates described in the pre-conjugate vaccine era. Prior to the use of conjugate pneumococcal vaccine, Janoff, in 1993, described pharyngeal pneumococcal colonization in 8 (14%) of 56 HIV-infected men, and in 1997, Rodriguez-Barradas followed 103 HIV-infected adults in Brazil for 6 months and found carriage rates ranging from 7% to 23% depending on CD4 count.[7,8] While it is likely that immunization with conjugate vaccine eradicated both nasopharyngeal colonization with and transmission of pneumococci throughout the community, other factors may also have contributed to the low carriage rate in our study, including the high number of participants receiving TMP-SMX for PCP prophylaxis.

We found no association between demographic, medical, social/behavioral risk factors, CD4 counts, viral load, ANC, and pneumococcal carriage in our study population, likely due to low carriage rates. Similarly, Onwubiko found no differences between the HIV-infected adults who were and were not colonized with pneumococcus following use of PCV7.[11] Interestingly, while low CD4 count (≤200 cells/mm^3), no or intermittent use of HAART, smoking, intravenous drug use, and chronic hepatitis have been associated with the development of invasive pneumococcal disease, these factors are not reported to be associated with nasopharyngeal carriage.[24-29] One logical explanation is that although these are not risk factors for carriage, once colonized, those with low CD4 count or inconsistent use of HAART are more likely to develop IPD secondary to immune dysfunction.

Forty-four percent of our isolated pneumococci were non-typeable. While non-typeable pneumococcal isolates are more commonly associated with carriage rather than invasive disease, non-typeable pneumococci has been isolated from children with IPD.[17,30,31] Furthermore, recent evidence suggests that non-typeable pneumococci have high genetic recombination potential leading to the possibility of spreading antibiotic resistance making their identification important for surveillance studies[32,33,34]

Approximately 50% of the pneumococcal isolates found during our study were non-PCV13 serotypes (with an additional 44% of the types being non-typeable). One-third of the

non-PCV13 strain isolates were serotype 35B, one-third were serotype 15B/C, and the last third were an isolate each of type 10A, 17F, and 31. Published reports describe similar increases in these serotypes in children following PCV13 implementation.[17,22,31,35-38] McElligot found serotype 35B to be among the leading source of penicillin non-susceptible pneumococci in both carriage and non-invasive pneumococcal infection in children in Ireland.[36] Cohen noted that serotypes 15B/C and 35B were among the non-PCV13 serotypes identified in carriage among children in France, with serotype 15B/C reaching the "emergent serotype" threshold of >5% in their study population.[22] Mameli, in a study of nasopharyngeal pneumococcal carriage among Italian children, also found a shift toward non-PCV13 serotypes, with serotype 15B among the most commonly isolated type.[31] Similarly, 3 years following the introduction of PCV13, Dunais showed that non-PCV13 strains accounted for 94% of nasopharyngeal carriage among healthy French children, with 15B/C and 35B among the most commonly identified types.[23] A recent US study showed 15% and 14% of pneumococcal isolates from the nasopharynx of 656 children in Atlanta were 35B and 15B/C, respectively.[35]

Serotypes 35B and 15B/C have also been identified as increasing causes of IPD. Adult pneumococcal surveillance data are limited, however, between 2009 and 2012, Mendes observed an increase in prevalence of pneumococcal infections in hospitalized adults in the US due to serotypes 15B/C from 2.7% to 6.3% and 35B from 3.6% to 8.2%.[39] Richter describes an increase in serotype 35B pneumococcal infection in children from 2% in 1999-2000 to 9.1% in 2012-2013.[40] Similarly, Desai found that 9% and 11% of IPD in studied children in Atlanta were caused by serotypes 35B and 15B/C, respectively.[35] By 2015, the CDC's Active Bacterial Core Surveillance program showed that serotypes 15B/C and 35B have emerged as 2 of the top 4 most frequent causes of IPD in children less than 5 years of age.[41] Furthermore, as serotypes 15B/C and 35B emerged, it became clear that many isolates are β-lactam resistant.[36,38,41]

Strengths of this study include the prospective collection of a large number of nasopharyngeal samples from a high risk population for pneumococcal isolation. Limitations of this study include the low carriage rates resulting in low number of pneumococci isolated, limiting the generalizability of serotype and antibiotic susceptibility results and factors associate with carriage.

We describe low nasopharyngeal pneumococcal carriage with antibiotic susceptible isolates among HIV-infected adults in central New York, likely secondary to widespread use of conjugate pneumococcal vaccine-13 among children in our community. Serotypes 15B/C and 35B accounted for more than half of the non-vaccine type isolates, further supporting the growing concerns of serotype replacement. Unlike reports by others, we did not see evidence of antibiotic resistance in these isolates. Future efforts to modify or enhance existing pneumococcal vaccine strategies should take these observations under consideration.

Methods

HIV-infected individuals, 18 years of age and older, seeking care at the adult or pediatric Designated AIDS Center in Syracuse, New York between December 2013 and March 2015 were eligible for enrollment. After obtaining informed consent, data were collected from each subject's medical record including age, gender, race/ethnicity, the presence of other medical comorbidities predisposing to IPD (asthma, chronic obstructive pulmonary disease, liver cirrhosis, cancer, splenectomy, diabetes mellitus, chronic heart disease, chronic kidney disease, hepatitis B, and hepatitis C), current respiratory symptoms (as defined by runny nose, ear pain, cough, or sore throat), antibiotic use in the previous 4 weeks (including trimethoprim-sulfamethoxazole (TMP-SMX) use for *Pneumocystis* prophylaxis), anti-retroviral therapy, presence of children in the household, cigarette smoking, alcohol use, intravenous drug use, HIV viral load, CD4 count, absolute neutrophil and lymphocyte counts, and number and types of pneumococcal vaccines received.

A nasopharyngeal sample was obtained by passing a sterile nasopharyngeal swab with calcium alginate tipped applicator and aluminum handle (Puritan Calgiswab, Guilford, ME) through the anterior nares to the posterior nasopharynx. Swabs were placed immediately into skim milk-tryptone-glucose-glycerol transport media and stored at −80 degrees Celsius until inoculation onto a blood agar plate. Plates were incubated overnight at 37°C, 5% CO_2. Pneumococci were identified by standard techniques including morphology, α-hemolysis, bile solubility, and optochin susceptibility. Serotyping of the isolates was performed at Wadsworth Center, Bacteriology Laboratory, New York State Department of Health (NYSDOH) by Quellung reaction in the presence of serotype-specific antisera prepared at NYSDOH and Statens Serum Institut (Copenhagen, Denmark).[42,43] Penicillin, cefotaxime, and TMP-SMX minimal inhibitory concentrations were determined for each isolate by Etest, (Biomerieux, Durham, NC).

Collection of medical, demographic, and social history and nasopharyngeal swab occurred at each office visit for the subject during the study period. The study protocol was approved by the local SUNY Upstate Medical University Institution Review Board (#510633).

We used counts and percentages to describe the sample for relevant demographic, behavioral, and medical characteristics. Chi-square and Fisher's exact tests were used to test for associations between subject characteristics and pneumococcal colonization. We used SPSS version 22 for all statistical analysis with a priori $\alpha = .05$.

Abbreviations

HIV	human immunodeficiency virus
HAART	highly active antiretroviral therapy
IPD	invasive pneumococcal disease
PPV23	23-valent polysaccharide pneumococcal vaccine
PCV7	pneumococcal conjugate vaccine-7
PCV13	pneumococcal conjugate vaccine-13
TMP/SMX	trimethoprim-sulfamethoxazol

Disclosure of potential conflicts of interest

MS receives research funding from Pfizer and GlaxoSmithKline. JD is a consultant to GlaxoSmithKline, Merck, Sanofi, and AstraZeneca and receives research funding from GlaxoSmithKline, Pfizer, and AstraZeneca. For the remaining authors, none were declared.

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