Original Report

Nasopharyngeal Carriage of Multidrug-Resistant *Streptococcus pneumoniae* in Institutionalized HIV-Infected and HIV-Negative Children in Northeastern Romania

Eugene Leibovitz, MD;* Cristiana Dragomir, MD;† Seli Sfartz, MD;† Nurith Porat, PhD;* Pablo Yagupsky, MD;‡ Simona Jica, MD;† Liliana Florescu, MD;† and Ron Dagan, MD*

ABSTRACT

Objectives: The study compared nasopharyngeal carriage of resistant pneumoniae in human immunodeficiency virus (HIV)-seropositive and -seronegative children.

Methods: Nasopharyngeal colonization with *Streptococcus pneumoniae* was investigated during May 1996 in 162 HIV-negative infants and children (age range, 1–38 mo) and 40 HIV-infected children (age range, 39–106 mo) living in an orphanage in Iasi, northeastern Romania. The HIV-infected children lived separated from the other children and were cared for by a different staff. *Streptococcus pneumoniae* was isolated from 12 of 40 (30%) HIV-infected and from 81 of 160 (50%) HIV-negative children. Antimicrobial susceptibility to penicillin and ceftriaxone was determined by E-test, and to another five antibiotics by disk diffusion. Serotyping was performed by the Quellung method on 81 of 93 (87%) isolates.

Results: Serotypes 6A, 6B, 19A, and 23F together represented 98% of all isolates. Ninety-nine percent of *S. pneumoniae* isolates were resistant to penicillin, and 74% were highly resistant to penicillin (minimum inhibitory concentration [MIC] > 1 μg/mL); MIC_{50} and MIC_{90} to penicillin of the isolates were 2 μg/mL and 8 μg/mL, respectively. Eighty-nine of ninety-one isolates were susceptible to ceftriaxone; 99%, 87%, 87%, 48%, and 21% of the isolates were resistant to trimethoprim-sulphamethoxazole, erythromycin, clindamycin, tetracycline, and chloramphenicol, respectively. Eighty-two (89%) isolates were multidrug resistant (resistant to ≥3 antibiotic classes); 37 of 92 (40%) isolates were resistant to 5 or more antibiotic classes, and 16 of these 37 (43%) belonged to serotype 19A. All serotype 19 isolates were highly resistant to penicillin.

Conclusions: No significant differences were observed in the resistance rates of *S. pneumoniae* in HIV-infected children compared to HIV-negative children. Multidrug-resistant pneumococci were highly prevalent in this Romanian orphanage in both HIV-negative and older HIV-infected children. The observed high prevalence of multidrug-resistant pneumococci (coupled with high penicillin resistance) with a limited number of circulating serotypes emphasizes the need to further evaluate the conjugate vaccines in children at risk for invasive pneumococcal infection.

Key Words: *HIV, nasopharynx, Romania,* S. pneumoniae

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Streptococcus pneumoniae is an important cause of pediatric morbidity and mortality, and resistance of *S. pneumoniae* to antimicrobial agents is increasing worldwide.[1,2] The main reservoir of *S. pneumoniae* is the nasopharynx. Infants and young children have the highest rates of nasopharyngeal colonization with *S. pneumoniae*, and colonization and infection with antibiotic-resistant *S. pneumoniae* (resistant *S. pneumoniae*) occur frequently.[3,4] Risk factors for emergence of resistant *S. pneumoniae* include prior antibiotic treatment; day-care attendance, particularly in children younger than 2 years of age; recent hospitalizations; and recurrent infections.[5–8] Nasopharyngeal colonization with resistant *S. pneumoniae* reaches 59% among children who attend day-care centers.[9–11] Surveillance of antimicrobial susceptibility as well as serogroups of *S. pneumoniae* carried in the upper respiratory tract may be considered a practical way of providing accurate information on the susceptibilty of the *S. pneumoniae* isolates causing disease.[12,13]

Although resistant *S. pneumoniae* is an emerging problem worldwide, there is a marked geographic variability in the levels of penicillin resistance.[1–2] In Europe, a high prevalence of resistant *S. pneumoniae* has been reported in Spain, France, Greece, and eastern Europe.[14–18]

The main purpose of this study was to obtain data on the prevalence and susceptibility patterns of *S. pneumoniae* in Romania, because few data are available from

*Pediatric Infectious Disease Unit, Soroka University Medical Center and Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel; †University of Medicine and Pharmacy, Iasi, Romania; and ‡Clinical Microbiology Laboratories, Soroka University Center and Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel.

Address correspondence to Dr. Eugene Leibovitz, Pediatric Infectious Disease Unit, Soroka Univeristy Medical Center, P.O. Box 151, Beer-Sheva 84101, Israel. E-mail: eugenel@bgumail.bgu.ac.il.

this country in the English-language literature. The objectives of the present study were to study the nasopharyngeal colonization with *S. pneumoniae* in children cared for in an orphanage in Iasi, the capital town of northeastern Romania, and to compare the colonization rates of the HIV-infected children with those of the HIV-negative children. In addition, the prevalence and resistance patterns of *S. pneumoniae* were documented, and the distribution of *S. pneumoniae* serotypes in the institution were determined.

PATIENTS AND METHODS

Patients

The study was conducted in May 1996 in the orphanage ("Leaganul de copii") of Iasi, the capital town of northeastern Romania. All the HIV-negative healthy children younger than 3 years of age and all the HIV-infected children cared for at this orphanage were enrolled in the study. The HIV-infected children were hospitalized in a separate building in a different area of the city. Care for these children was provided by a different team. The HIV-negative children lived in a different building and were grouped according to their age in the three floors of the building.

Human immunodeficiency virus-serologic status was established by an enzyme-linked immunosorbent assay (ELISA) test and, in some cases, by a positive Western blot test. All HIV-infected children were diagnosed as suffering from symptomatic HIV infection. None was receiving anti-retroviral drugs at the time of the study. CD4 T-lymphocyte counts were not obtained in the months preceding the nasopharyngeal sampling.

Bacteriology

Nasopharyngeal samples were obtained with transport swabs and placed in MW173 Amies medium (Transwab; Medical Wire and Equipment, Potley, UK). Swabs were transported via air to Israel and processed within 24 hours at the Clinical Microbiology Laboratory, Soroka University Medical Center. Swabs were plated on trypticase agar media containing 5% sheep blood agar and incubated aerobically at 35°C for 48 hours. Presumptive identification of *S. pneumoniae* was based on the presence of alpha hemolysis and inhibition by optochin. Confirmation was made by positive slide agglutination (Phadebact, Pharmacia Diagnostics, Uppsala, Sweden).

Susceptibility to penicillin, erythromycin, clindamycin, trimethoprim-sulphamethoxazole (TMP/SMX), tetracycline, and chloramphenicol was performed by the disk-diffusion method and interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS).[19] Susceptibility to penicillin and ceftriaxone was performed by E-test (PDM Epsilometer, AB Biodisk, Solna, Sweden). Minimal inhibitory concentrations (MIC) falling between two marks on the E-test strip were rounded up to the next higher twofold dilution, as recommended in the packet insert. Isolates with a MIC over 0.125 μg/mL but less than 1.0 μg/mL were considered intermediately susceptible to penicillin and those with MIC over 1.0 μg/mL were defined as resistant. Ceftriaxone resistance was defined as intermediate if MIC were between 0.5 and 1.0 μg/mL and high for MIC over 1.0 μg/mL.

Serotyping was done by the Quellung reaction, using reagents from the Danish Statens Serum Institut (Copenhagen).[20]

RESULTS

Two hundred and two children took part in this study: 162 were HIV-negative (84 female; 78 male), and 40 were HIV-positive (23 female; 17 male). Of the HIV-negative children, 157 (97%) were younger than 3 years of age. All HIV-infected children were older than 39 months of age (range, 39–106 mo).

Total nasopharyngeal *S. pneumoniae* colonization was 93 per 202 patients (46%): 50% and 30% for HIV-negative and HIV-positive children, respectively (Figure 1). Nasopharyngeal *S. pneumoniae* colonization was age-related in the HIV-negative children. The highest colonization rate (70%) was recorded between 4 and 12 months of age. Nine of the 12 colonized HIV-infected children were over 5 years of age.

Antibiotic susceptibility testing was performed on 92 isolates. No significant differences were observed in the distribution of antibiotic resistance between the HIV-negative and HIV-infected children. The highest resistance rates were recorded against penicillin and TMP-SMX (99% each); 87% of the isolates were resistant to clindamycin or erythromycin. Two isolates were nonsusceptible to ceftriaxone (MIC 1.0 and 2.0 μg/mL, respectively). The cumulative MIC of penicillin and ceftriaxone for the nasopharyngeal *S. pneumoniae* are presented in Figure 2. The MIC values of penicillin for the resistant isolates

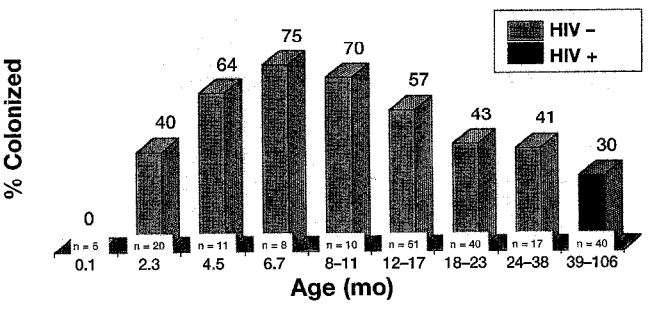

Figure 1. *S. pneumoniae* nasopharyngeal colonization by age group in 162 HIV-negative and 40 HIV-positive infants and children.

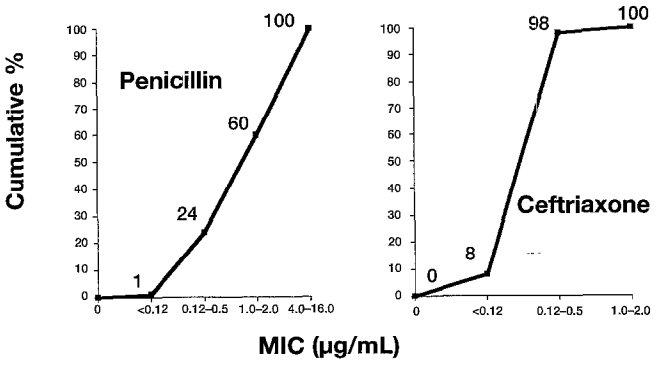

Figure 2. Cumulative MIC of penicillin and ceftriaxone for the nasopharyngeal *S. pneumoniae* isolates.

ranged from 0.125 to 16 μg/mL. MIC_{50} and MIC_{90} of penicillin were 2.0 and 8.0 μg/mL, respectively; 74% (68/92) of all isolates were highly resistant to penicillin (MIC >1.0 μg/mL). MIC_{50} and MIC_{90} of ceftriaxone were 0.25 and 0.5 μg/mL, respectively; 45% (41/92) of the *S. pneumoniae* isolates had a MIC value of 0.5 μg/mL for ceftriaxone.

Typing was performed on 81 of 93 (87%) isolates: 69 from HIV-negative and 12 from HIV-infected subjects. Overall the isolates belonged to six different serotypes (Figure 3). The most common (in order of decreasing frequency) were 23F, 6A, and 19A. Serogroups 23, 6, and 19 together represented 97% of all isolates. No significant difference in the distribution between HIV-infected and HIV-negative subjects was observed.

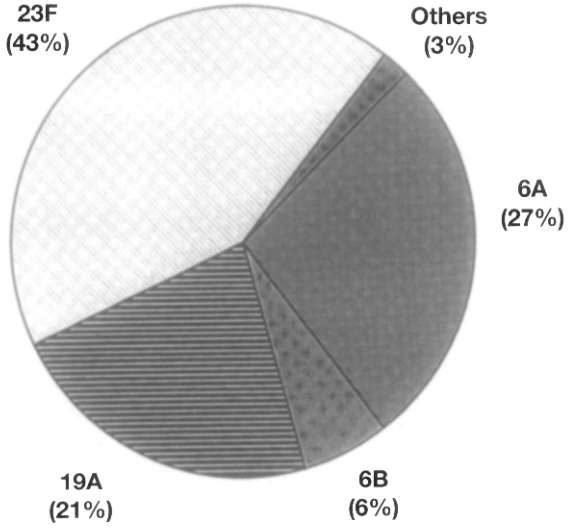

Figure 3. Serotypes found in 81 of 93 nasopharyngeal *S. pneumoniae* isolates.

The resistance patterns by serotype or group are presented in Table 1. Eighty-nine percent (82/92) of the isolates were resistant to three or more drug categories, and 40% (37/92) were resistant to five or more drug categories. Sixteen (43%) of the *S. pneumoniae* isolates resistant to five or more drug categories belonged to serotype 19A. In all, 94% (16/17 isolates) of the serotype 19A *S. pneumoniae* were resistant to five or more drug categories. All serotype 19A *S. pneumoniae* were highly resistant to penicillin. MIC_{50} and MIC_{90} of these isolates were 4 and 8 μg/mL, respectively.

Twenty-three percent of all isolates (types 19A, 11, and 29/34/35/42/47) were not covered by the currently available 7 to 11 valent antipneumococcal conjugate vaccines.

DISCUSSION

Antibiotic-resistant pneumococci have been reported in Romania since 1974, when Vata et al described four cases of meningitis due to resistant organisms in Iasi.[21] A marked increase in the frequency of resistant *S. pneumoniae* has been reported in limited studies published from Romania.[22–24] In a study investigating the nasopharyngeal carriage of resistant *S. pneumoniae* by children hospitalized or attending day-care centers or outpatient clinics in eastern and central Europe, the highest carriage rate (60.9%) was reported in Romania,[25] and in a recently completed study in eastern Europe, *S. pneumoniae* were the most frequently recovered organisms from the middle ear fluid of children with acute otitis media and resistant *S. pneumoniae* represented 42% of the *S. pneumoniae* isolated from Romanian patients.[26] However, these two studies have looked at only a limited number of isolates.

The Romanian orphanage system for abandoned children became open to the Western world after the December 1989 revolution, when an estimated 100,000 to 300,000 children were found placed in "leagane" (institutions taking care of children under 3 years of age), orphanages, and asylums for incurables.[27] Since 1989, it was discovered that more than 1700 institutionalized children in Romania are HIV-infected.[28,29]

Most of them have acquired immunodeficiency syndrome (AIDS) and live in orphanages separated from the other children cared for in these facilities. The prevalence of other infections in these orphanages is incompletely documented.[30–32]

This study documented that multidrug-resistant *S. pneumoniae* were highly prevalent in this orphanage in both HIV-negative and older HIV-infected children. Assuming a similarity between the carried isolates and those causing disease, this finding makes the empirical treatment of infections potentially caused by *S. pneumoniae*

Table 1. Resistance Patterns of Isolates to Various Antibiotics, according to *S. pneumoniae* Serotypes

Serotype	Number of Isolates (n = 92)	Resistance to Antibiotics							Multidrug Resistance	
		PEN (99%)	TMP/SMX (99%)	ERY (87%)	CLIN (87%)	TETR (48%)	CHLOR (21%)	CRO (2%)	3 or More (89%)	5 or More (40%)
6A	22	100	100	100	100	52	0	0	100	36
6B	5	100	100	100	100	100	0	0	100	100
19A	17	100	100	94	94	100	100	0	100	94
23F	35	100	97	74	74	14	3	0	74	11
Others*	13	92	92	92	92	38	8	15	92	42

*One serotype II isolate and one serotype 29/34/35/42/47; 11 were not typed.
PEN = penicillin; TMP/SMX = trimethoprim-sulphamethoxazole; ERY = erythromycin; CLIN = clindamycin; TETR = tetracyclin; CHLOR = chloramphenicol; CRO = ceftriaxone.

in this orphanage extremely problematic. The standard β-lactam antibiotic therapy for *S. pneumoniae* with intermediate resistance to penicillin was found to be effective for nonmeningeal illnesses, although the cerebrospinal fluid (CSF) concentration of penicillin may be not adequate to eradicate the pathogen from the meninges.[6] Because additional updated data on pneumococcal drug resistance in Romania are missing, it is not known whether these findings differ significantly from conditions in other communities and geographic areas in the country. However, the high prevalence of *S. pneumoniae* highly resistant to penicillin in this orphanage may preclude the use of penicillin and possibly many other antibiotics, even in cases of nonmeningeal pneumococcal infections like bacteremia, pneumonia, acute otitis media, and sinusitis, and raises enormous public health concerns regarding the availability, cost, and appropriateness of alternative antibiotic therapies for all pneumococcal diseases in Romania.

Human immunodeficiency virus infection has been implicated as a risk factor for acquisition of invasive infections due to resistant *S. pneumoniae*.[33-36] However, the prevalence of colonization with resistant *S. pneumoniae* in HIV-infected patients and particularly in HIV-infected children has not been systematically studied. The finding in this study, that a high prevalence of colonization with resistant *S. pneumoniae* both in HIV-negative and in the older HIV-infected children, suggests that the high prevalence of colonization and increased exposure to resistant *S. pneumoniae* in the orphanage, rather than the HIV status, represent the major factors determining the carriage status of these children. On the other hand, the high rates of pneumococcal bacteremia among HIV-infected patients was found to be associated with low numbers of CD4+ T cells, and this impaired humoral responses to *S. pneumoniae* rather than to increased exposure to the organism.[33]

Resistant *S. pneumoniae* strains found in this study belonged to six serotypes or serogroups, the most common being 23, 6, and 19. The high prevalence of multidrug resistant *S. pneumoniae* (coupled with high penicillin resistance) with a limited number of circulating serotypes stresses the need to further evaluate the conjugate vaccines in children at risk for invasive *S. pneumoniae* infection. Conjugate pneumococcal vaccine was found to significantly reduce the nasopharyngeal carriage of vaccine-type *S. pneumoniae* in infants and young children.[37-39] However, the vaccine did not have any effect on the carriage of non-vaccine type *S. pneumoniae*, with the exception of serogroup 6A. The fact that in 23% of *S. pneumoniae* isolates in the orphanage the serotypes were not those covered by the currently studied 7 to 11 valent conjugate anti-*S. pneumoniae* vaccines suggests that other serotypes of common antigens should be introduced and studied, to fight antibiotic resistance to *S. pneumoniae* by effective vaccines.

The extremely high incidence of resistant *S. pneumoniae* and the multiple-drug resistance pattern of *S. pneumoniae* in the orphanage of Iasi emphasize the need for broader and continuous surveillance of the prevalence and antimicrobial susceptibility profiles and serotype or serogroup distribution of *S. pneumoniae* in Romania, to better understand the evolution and spread of resistance all over the country and to guide patient therapy and development of effective vaccines.

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