JOURNAL OF CLINICAL MICROBIOLOGY, June 2006, p. 2032–2038 Vol. 44, No. 6
0095-1137/06/$08.00+0 doi:10.1128/JCM.00275-06

Distribution and Invasiveness of *Streptococcus pneumoniae* Serotypes in Switzerland, a Country with Low Antibiotic Selection Pressure, from 2001 to 2004

Andreas Kronenberg,[1] Phillip Zucs,[2] Sara Droz,[1] and Kathrin Mühlemann[1,3]*

Institute for Infectious Diseases, University Bern, CH-3010 Bern, Switzerland[1]; Federal Office of Public Health, Bern, Switzerland[2]; and Department for Infectious Diseases, University Hospital Bern, Bern, Switzerland[3]

Received 8 February 2006/Returned for modification 17 March 2006/Accepted 27 March 2006

To describe the serotype-specific epidemiology of colonizing and invasive *Streptococcus pneumoniae* isolates, which is important for vaccination strategies, we analyzed a total of 2,388 invasive and 1,540 colonizing *S. pneumoniae* isolates collected between January 2001 and December 2004 within two nationwide surveillance programs. We found that the relative rank orders of the most frequent serotypes (serotypes 1, 3, 4, 6B, 7F, 14, 19F, and 23F) differed among invasive and colonizing isolates. Serotypes 1, 4, 5, 7F, 8, 9V, and 14 had increased invasive potential, and serotypes/serogroups 3, 6A, 7, 10, 11, 19F, and 23F were associated with colonization. The proportion of pediatric serotypes was higher among children <5 years old (48.5%) and persons >64 years old (34.1%) than among other age groups (29.1%); it was also higher in West Switzerland (40.2%) than in other geographic regions (34.7%). Likewise, serotype-specific proportions of penicillin-resistant isolates for types 6B, 9V, 14, and 19F were significantly higher in West Switzerland. The relative frequency of pediatric serotypes corresponded with antibiotic consumption patterns. We conclude that the epidemiology of invasive and colonizing *S. pneumoniae* isolates is influenced by the serotype-specific potential for invasiveness, and therefore, surveillance programs should include colonizing and invasive *S. pneumoniae* isolates. Antibiotic selection pressure determines the serotype distribution in different age groups and geographic regions and therefore the expected direct and indirect effects of the 7-valent conjugate vaccine.

Streptococcus pneumoniae is a common cause of severe invasive disease and upper respiratory tract infections. For example, the annual incidence of otitis media in Switzerland is 22,500/100,000 children <2 years of age and 18,000/100,000 children between 2 and 5 years of age (9). The virulence of *S. pneumoniae* is largely determined by its polysaccharide capsule, which is also the target of pneumococcal vaccines in current use. More than 90 serotypes have been identified based on the antigenic composition of the polysaccharide capsule. Some of these serotypes exhibit a distinctive epidemiology with regard to their potential to cause invasive disease, their occurrence in specific age groups or geographic regions, their association with antibiotic resistance, and their epidemic potential (16, 29).

The introduction of the 7-valent conjugated pneumococcal polysaccharide vaccine (PCV-7; serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) stresses the importance of surveillance of pneumococcal serotype-specific epidemiology. Replacement of vaccine serotypes with nonvaccine serotypes has been observed in several vaccine studies and also shortly after the introduction of universal vaccination of infants in the United States (16, 32).

A precise estimation of the prevalence of drug-resistant invasive *S. pneumoniae* isolates is hampered by the relatively low numbers of cases in young age groups. Therefore, in 1996, the Centers for Disease Control and Prevention recommended that nasopharyngeal isolates be used for the surveillance of pneumococcal resistance (6). This approach assumes that the serotype-specific epidemiology of invasive and colonizing pneumococci is largely comparable or at least correlated in a predictable manner. Few studies have compared the serotype distribution and antibiotic resistance prevalence of invasive and nasopharyngeal pneumococci (20, 21, 24, 26, 27). More recently, several studies have estimated the invasiveness of prevalent pneumococcal serotypes based on the relative serotype prevalence in invasive and colonizing isolates (1, 14, 28). Low numbers of pneumococcal isolates and/or a limited comparability of study groups from which invasive and noninvasive *S. pneumoniae* isolates were collected hampered a detailed analysis in many of these studies.

This study made use of two nationwide Swiss surveillance systems, one monitoring nasopharyngeal pneumococcal carriage and the other focusing on invasive pneumococcal disease. Invasive and colonizing *S. pneumoniae* isolates collected prospectively between January 2001 and December 2004 were compared with regard to their relative serotype distribution and antibiotic resistance prevalence. In addition, the relative invasiveness of pneumococcal serotypes was estimated as described previously by Brueggemann et al. (1, 2).

(Part of the data was presented at the Annual Assembly of the Swiss Society for Infectious Diseases, Basel, Switzerland, June 2005, and the Meningitis Research Foundation Conference, London, United Kingdom, November 2005.)

* Corresponding author. Mailing address: Institute for Infectious Diseases, University of Bern, Friedbühlstrasse 51, 3010 Bern, Switzerland. Phone: 41 31 632 32 59. Fax: 41 31 632 87 66. E-mail: kathrin.muhlemann@ifik.unibe.ch.

MATERIALS AND METHODS

Invasive and colonizing pneumococcal isolates. Data on invasive *S. pneumoniae* were obtained from the Swiss National Reference Center for Invasive Pneumococci (NRCP). Reporting of invasive pneumococcal infection has been

mandatory in Switzerland since 1999 (http://www.bag.admin.ch). In March 2002, the NRCP (http://www.ifik.unibe.ch) was set up and has since been prospectively collecting clinical pneumococcal isolates from normally sterile body sites (blood, cerebrospinal fluid, and joint, pleural, and peritoneal fluid but not middle-ear fluid) sent by Swiss clinical microbiology laboratories. The referral of invasive pneumococcal isolates to the NRCP is voluntary. Nevertheless, it is estimated that the center receives >95% of all invasive isolates cultured in Switzerland. For instance, the number of invasive infections reported to the Swiss Federal Office of Public Health during the years 2003 and 2004 was 924 and 1,000, respectively. During the same time, the NRCP received 901 and 968 isolates, respectively. In this study, data on invasive isolates from March 2002 to December 2004 were included.

Data on colonizing pneumococci were obtained from the Pneumococcal Resistance Study that is being conducted within the Swiss Sentinel System. This nationwide, ongoing, prospective pneumococcal surveillance study has been described in detail previously (25). In brief, nasopharyngeal swabs are collected from all outpatients who present with acute otitis media or pneumonia to practitioners participating in the sentinel network. *S. pneumoniae* is being cultured from the swabs at the Institute for Infectious Diseases, University of Bern, Bern, Switzerland, as previously described (25). In this study, data for nasopharyngeal isolates from January 2001 to December 2004 were included.

Serotyping and resistance testing were done for all invasive and colonizing bacterial isolates included in this study at the Institute for Infectious Diseases, University of Bern, Switzerland, as previously described for the colonizing isolates (25). In brief, capsular serotyping was done on all isolates by a Quellung reaction using antiserum from the Statens Serum Institute (Copenhagen, Denmark). All isolates were tested against oxacillin (1-μg disk), erythromycin, cotrimoxazole, and levofloxacin by the disk diffusion method. For isolates with reduced susceptibility to any of these four antibiotics, MICs were determined by use of the E test method (AB Biodisk) according to Clinical and Laboratory Standards Institute (CLSI) (formerly NCCLS) guidelines (7). Data on patients' age and gender were available for invasive and colonizing isolates, whereas patients' canton (state) of residence was derived from the sending laboratory's or sentinel physician's address. Also, for invasive isolates, the anatomical site of isolation was known.

Antibiotic consumption data. Data on outpatient antibiotic sales were provided by IMH-IMS Health Market Research and have partially been published previously (11). Defined daily doses (DDD) per 1,000 inhabitants daily were calculated using WHO standard doses and demographic information from the last census in 2000.

Statistical analysis. Serotype distribution was analyzed for invasive and colonizing isolates by age group and geographic region. For the purpose of geographic comparisons, Switzerland was divided into two regions, the French-speaking West and the remaining parts of the country (designated "other"), as described previously (25).

Serotype/serogroup-specific penicillin resistance was calculated as the proportion of penicillin-nonsusceptible *S. pneumoniae* (PNSP) isolates in each serotype/serogroup.

An odds ratio (OR) was calculated for the likelihood of an individual serotype/serogroup being isolated from a sterile site compared to merely colonizing a patient, as described previously (1). For the main analysis, all other serotypes/serogroups served as the reference group. For comparison with the literature, the analysis was repeated with serotype 14 as a fixed reference (2). Age and geographic region were added to the model as potential confounders.

Descriptive analysis, analysis of variance, and logistic regression analysis were performed using StatView (version 5.0; SAS Institute). Proportions were compared with the chi-square or Fisher's exact test as appropriate. A cutoff *P* value of ≤0.05 (two-tailed) was used for all statistical analyses.

RESULTS

A total of 2,388 invasive *S. pneumoniae* isolates collected by the NRCP between March 2002 and December 2004 were analyzed. Invasive isolates were obtained mainly from blood cultures (90.8%) or cerebrospinal fluid (3.5%). In addition, the study included data on 1,540 nasopharyngeal pneumococcal isolates collected between January 2001 and December 2004 within the Swiss Sentinel Surveillance Network, designated as colonizing *S. pneumoniae* isolates. Invasive and colonizing study isolates showed different distributions for patients' age and geographic provenance: colonizing isolates were cultured more often from children <5 years of age and were from the Western part of Switzerland (Table 1). These potentially confounding variables were taken into account in the multivariate analysis.

TABLE 1. Characteristics of 2,388 invasive pneumococcal isolates from the Swiss National Reference Center (March 2002 to December 2004) and 1,540 pneumococcal nasopharyngeal isolates from the Swiss Sentinel Surveillance Network (January 2001 to December 2004)

Parameter	No. (%) of pneumococcal isolates		*P*
	Invasive	Colonizing	
Total	2,388 (100)	1,540 (100)	
Age groups (yr)			<0.001
<5	204 (9.3)	972 (63.3)	<0.001
5–64	869 (39.6)	538 (35.0)	0.005
>65	1,121 (51.1)	26 (1.7)	<0.001
Male	1,249 (56.8)	833 (54.3)	NS[a]
West Switzerland	763 (32.0)	735 (48.0)	<0.001
Antibiotic nonsusceptibility			
Penicillin (MIC > 0.06 mg/liter)	257 (10.8)	217 (14.1)	0.002
Penicillin (MIC > 2 mg/liter)	33 (1.4)	8 (0.5)	0.010
Erythromycin	320 (13.4)	200 (13.0)	NS
Cotrimoxazole	454 (19.0)	290 (18.8)	NS
Levofloxacin	28 (1.2)	1 (0.1)	<0.001
Serotypes[b]			
1	131 (5.5)	26 (1.7)	<0.001
3	252 (10.6)	181 (11.8)	NS
4	182 (7.6)	18 (1.2)	<0.001
5	8 (0.3)	1 (0.1)	NS
6	82 (3.4)	93 (6.0)	<0.001
6A	0 (0.0)	6 (0.4)	0.004
6B	105 (4.4)	116 (7.5)	<0.001
7	40 (1.7)	33 (2.1)	NS
7F	151 (6.3)	14 (0.9)	<0.001
8	101 (4.2)	11 (0.7)	<0.001
9	93 (3.9)	52 (3.4)	NS
9V	99 (4.1)	21 (1.4)	<0.001
10	27 (1.1)	31 (2.0)	0.030
11	48 (2.0)	70 (4.5)	<0.001
14	366 (15.3)	108 (7.0)	<0.001
15	27 (1.1)	56 (3.6)	<0.001
18C	72 (3.0)	74 (4.8)	0.004
19A	68 (2.8)	41 (2.7)	NS
19F	93 (3.9)	252 (16.4)	<0.001
22	72 (3.0)	28 (1.8)	0.022
23	23 (1.0)	32 (2.1)	0.005
23F	162 (6.8)	123 (8.0)	NS
Other	132 (5.5)	88 (5.7)	NS
Not typeable	54 (2.3)	65 (4.2)	<0.001

[a] NS, not significant ($P > 0.05$).
[b] Numbers for serogroups 6, 7, 9, and 23 do not include serotypes 6B, 7F, 9V, and 23F.

Serotype/serogroup distribution among invasive and colonizing isolates. Capsular serotyping of isolates yielded 41 different serotypes/serogroups. A small percentage of isolates (3.1%) were nontypeable. With the exception of serotypes 5 and 6A, serotypes/serogroups that accounted for <1% of all isolates were combined into a group designated "other" for further analyses (Table 1). Serotype/serogroup distribution differed between invasive and colonizing isolates (Table 1). In the univariate analysis, serotypes/serogroups 1, 4, 7F, 8, 9V, 14, and 22 occurred significantly more often among invasive iso-

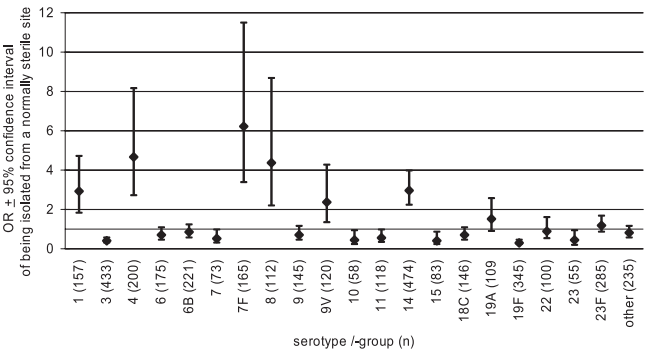

FIG. 1. ORs and 95% confidence intervals for the probability of *S. pneumoniae* being isolated from a normally sterile site compared to being isolated from the nasopharynx for different serotypes/serogroups adjusted for age and geographic region. The numbers in parentheses next to the serotypes/serogroups on the *x* axis indicate the number of isolates.

lates. Serotypes/serogroups 6, 6B, 10, 11, 15, 18C, 19F, and 23 and nontypeable isolates were more frequent among colonizing isolates. Among the rare serotypes/serogroups (<1% of all isolates), serotype 5 was represented by eight (0.3%) invasive isolates and one (0.1%) colonizing isolate ($P > 0.05$), serotype 6A was found only among colonizing isolates (0.4%, $P = 0.004$), serotype 12F occurred only among invasive isolates (0.7%, $P < 0.001$), and serotype 38 was less frequent among invasive isolates (0.1%) than among colonizing isolates (0.6%) ($P = 0.002$).

Association of serotypes/serogroups with invasiveness. ORs were calculated for individual serotypes/serogroups in a logistic regression model adjusting for age and geographic region (Fig. 1). Serotypes 1 (OR, 2.9; 95% confidence interval [CI], 1.8 to 4.7), 4 (OR, 4.7; 95% CI, 2.7 to 8.1), 7F (OR, 6.2; 95% CI, 3.4 to 11.5), 8 (OR, 4.4; 95% CI, 2.2 to 8.7), 9V (OR, 2.4; 95% CI, 1.3 to 4.3), and 14 (OR, 3.0; 95% CI, 2.2 to 4.0) were significantly associated with invasive isolates (Fig. 1). These six serotypes contributed 43.1% of all invasive isolates compared to 12.9% of all colonizing isolates ($P < 0.001$). Serotype 14 was the most prominent invasive serotype, comprising 15.3% of invasive isolates, but it also belonged to the more prevalent colonizing serotypes (7% of all colonizing isolates). Serotypes 3 (OR, 0.4; 95% CI, 0.3 to 0.6), 7 (OR, 0.5; 95% CI, 0.3 to 0.95), 10 (OR, 0.5; 95% CI, 0.2 to 0.9), 11 (OR, 0.6; 95% CI, 0.3 to 0.95), 15 (OR, 0.4; 95% CI, 0.2 to 0.8), 19F (OR, 0.3; 95% CI, 0.2 to 0.4), and 23 (OR, 0.4; 95% CI, 0.2 to 0.9) were significantly associated with colonizing isolates (Fig. 1). Together, they contributed 42.5% of all colonizing isolates compared to 21.4% of all invasive isolates ($P < 0.001$).

When the logistic regression analysis of the association of individual serotypes/serogroups with invasive and colonizing isolates was repeated using serotype 14 as a reference in analogy to the study reported previously by Brueggemann et al. (2), similar results were obtained. Serotypes 1, 4, 7F, 8, 9V, and 19A were at least as invasive as serotype 14, whereas all other serotypes tested were significantly more often associated with colonization than serotype 14.

Small numbers did not allow for an in-depth analysis of serotype-specific invasiveness among different age groups. However, the observed trends confirmed the findings of the overall analysis (data not shown).

Serotype/serogroup distribution by age and geographic region. The serotype distribution did not differ significantly between the age groups 0 to 1 and 2 to 4 years and between the age groups 5 to 16 and 17 to 64 years (data not shown), which were therefore pooled for further analysis. Figure 2 illustrates the distribution of serotypes/serogroups by age group and geographic region. The following associations were confirmed by multivariate analysis, adjusting for geographic region or age group (as appropriate) and colonizing versus invasive isolates (data not shown): (i) serotypes/serogroups 6 ($P = 0.009$), 6B ($P < 0.001$), 14 ($P = 0.008$), 15 ($P = 0.003$), 19F ($P < 0.001$), and 23F ($P < 0.001$) were most common among young children <5 years of age; (ii) serotypes/serogroups 1 ($P < 0.001$), 3 ($P < 0.001$), 7 ($P = 0.01$), 7F ($P = 0.05$), 8 ($P = 0.001$), 9 ($P = 0.04$), and 22 ($P = 0.001$) were more frequent among older children and adults; and (iii) serotypes/serogroups 9 ($P = 0.05$) and 19A ($P = 0.02$) prevailed in the western part of Switzerland, while serotypes 3 ($P = 0.04$) and 7F ($P = 0.03$) were more frequent in other areas.

Serotype/serogroup and antibiotic resistance. The overall proportions of PNSP (MIC > 0.06 mg/liter) and high-level penicillin (MIC ≥ 2 mg/liter), erythromycin, cotrimoxazole, and levofloxacin resistance were 12.1%, 1.0%, 13.2%, 18.9%, and 0.7%, respectively.

PNSP isolates were significantly more frequent among colonizing isolates, while high-level resistance to levofloxacin and penicillin was more frequent among invasive isolates. These differences remained significant for high-level penicillin resistance and levofloxacin resistance after adjustment for age and geographic region ($P = 0.04$ for each antibiotic). Half (48.8%) of the isolates with high-level penicillin resistance belonged to serotype 14. Serotype distribution among levofloxacin-resistant isolates was more diverse (data not shown).

Serotype-specific PNSP proportions (serotype-specific resistance [SSR]) differed between serotypes/serogroups (Table 2). PNSP proportions were higher in serotypes/serogroups 5, 6A, 6B, 9, 9V, 14, 15, 19A, 19F, and 23F and nontypeable isolates.

In the univariate analysis, SSR differed significantly between colonizing and invasive *S. pneumoniae* isolates for serotypes/serogroups 1 (11.5 versus 0%; $P = 0.004$), 9 (without 9V, 28.8 versus 7.5%; $P = 0.001$), and 23F (9.8 versus 20.4%; $P = 0.02$). After controlling for age and geographic region, 23F remained the only serotype for which SSR and invasiveness were significantly associated (adjusted OR, 3.38; 95% CI, 1.34 to 8.55; $P = 0.009$). Interestingly, four serotypes with high odds ratios for the association with invasiveness (serotypes 1, 4, 7F, and 8) belonged to the serotypes with the lowest SSR (Table 2 and Fig. 1). However, there was no significant linear correlation between the OR for invasiveness and SSR. As illustrated in Fig. 1, serotype 3 and serogroup 7, which were more prevalent among colonizing serotypes, had low SSR, and serotypes 14 and 9V, which belong to the "invasive" serotypes, had high SSR.

SSR did not vary significantly by age group (data not shown). However, SSR proportions for serotypes 6B, 9V, 14, and 19F and nontypeable isolates were significantly higher in the West than in other geographic regions (Table 2). Interestingly, serotypes 6B, 9V, 14, and 19F belong to the five so-called pediatric serotypes.

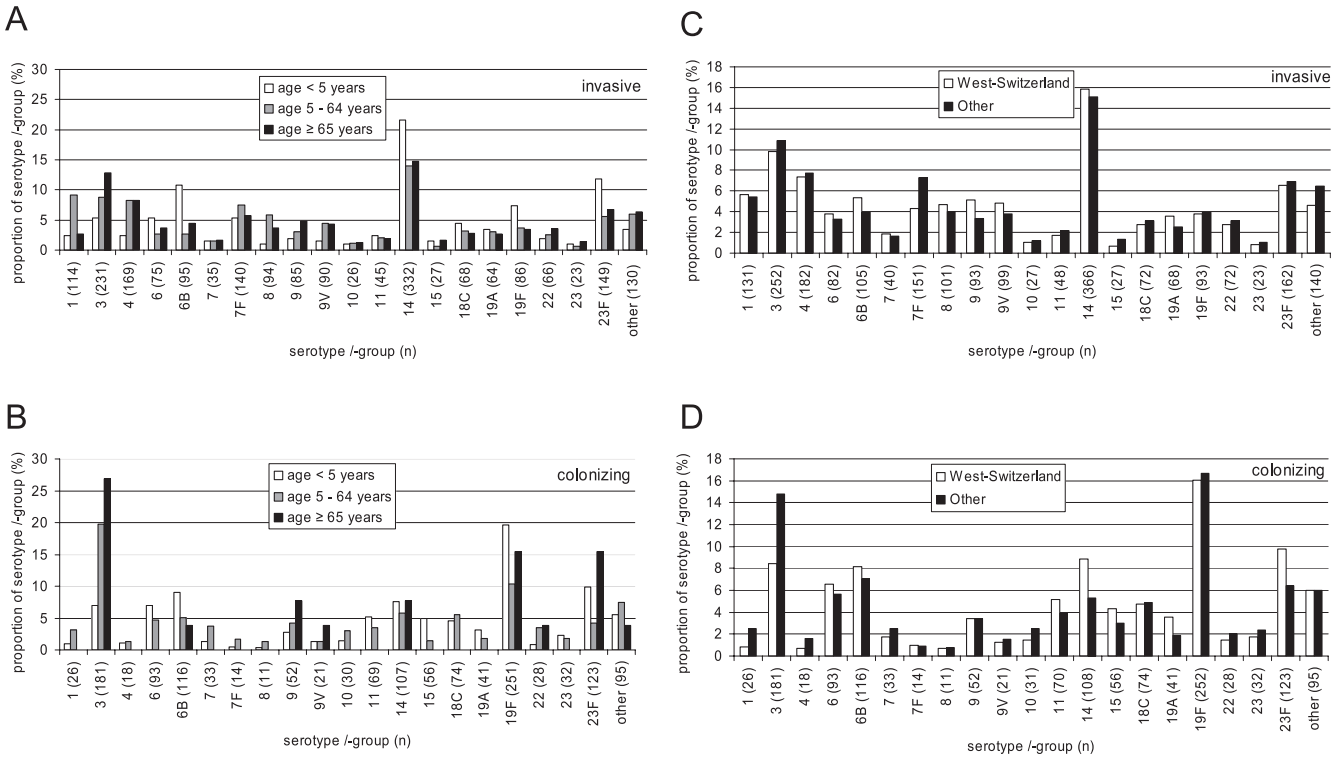

FIG. 2. Serotype/serogroup distribution of invasive (A and C) and colonizing (B and D) *S. pneumoniae* isolates by age group and geographic region. The numbers in parentheses next to the serotypes/serogroups on the *x* axis indicate the number of isolates.

TABLE 2. Serotype/serogroup-specific penicillin resistance of invasive and colonizing *S. pneumoniae* isolates by geographic region

Serotype/serogroup	No. of isolates	% SSR			P^a
		All	West	Other	
1	157	1.9	0.0	2.7	NS
3	433	0.7	1.4	0.3	NS
4	200	1.0	1.6	0.7	NS
5	9	66.7	50	80	NS
6	175	10.3	6.5	13.2	NS
6A	6	16.7	0	20	NS
6B	221	14.5	21.8	8.3	0.01
7	73	1.4	3.7	0.0	NS
7F	165	1.2	0.0	1.6	NS
8	112	0.0	0	0	NS
9	145	15.2	23.4	8.6	NS
9V	120	28.3	50.0	14.9	<0.001
10	58	5.2	0.0	7.7	NS
11	118	5.9	9.8	3.0	NS
14	474	19.6	32.3	11.8	<0.001
15	83	15.7	8.1	21.7	NS
18C	146	3.4	3.6	3.3	NS
19A	109	39.4	49.1	30.4	NS
19F	345	25.5	32.7	19.8	0.02
22	100	8.0	3.1	10.4	NS
23	55	9.1	5.3	11.1	NS
23F	285	15.8	15.6	16.0	NS
Not typeable	119	15.1	24.1	7.7	0.02

[a] *P* value was adjusted for age group and the proportion of colonizing and invasive isolates. NS, not significant ($P > 0.05$).

Pediatric serotypes and vaccine coverage. The pediatric serotypes 6B, 9V, 14, 19F, and 23F were found more frequently among children <5 years of age (48.5%) and elderly patients (34.1%) than among patients between 5 and 64 years old (29.1%) ($P < 0.001$ for children <5 years old; $P = 0.007$ for elderly patients >64 years old) (Table 3). The proportions of pediatric serotypes did not differ significantly between the age groups 0 to 6 months (43.0%), 7 to 24 months (49.8%), and 25 to 48 months (46.8%). Also, pediatric serotypes did not occur more frequently among women of childbearing age (17 to <45 years old) than among men of the same age group (27.1% versus 28.9%, respectively). There was a trend towards a higher proportion of pediatric serotypes in the Western region (Table 3).

The stratified analysis for penicillin-susceptible and PNSP isolates showed that the variation of the proportion of pediatric serotypes by age group was seen only among penicillin-susceptible isolates (Table 3). In the Western region, the predominance of pediatric serotypes occurred only among PNSP isolates.

The proportions of invasive (46.2%) and colonizing (48.3%) isolates covered by PCV-7 (excluding potentially cross-reacting serotypes) were relatively low but comparable. The proportions were higher among infants <2 years of age (65.1% for invasive and 56.1% for colonizing isolates) and children 2 to 4 years old (51.3% for invasive and 49.9% for colonizing isolates). Similar, albeit smaller, variations were found for the proportion of vaccine serotypes in different age groups and geographic regions. This is not unexpected, since five of the seven serotypes included in the vaccine belong to the pediatric serotypes.

TABLE 3. Risk factors for pneumococcal carriage or invasive disease with pediatric serotypes (6B, 9V, 14, 19F, and 23F) among 3,928 children and adults in Switzerland from 2001 to 2004

Isolate group and characteristic	No. of subjects	% Pediatric serotypes	OR[a]	95% CI
All				
Geographical region				
Other	2,422	34.7	Reference	Reference
West	1,498	40.2	1.12	0.97–1.29
Age groups (yr)				
<5	1,176	48.5	2.42	2.01–2.91
5–64	1,407	29.1	Reference	Reference
>64	1,147	34.1	1.19	1.00–1.42
Penicillin susceptible				
Geographical region				
Other	2,205	32.7	Reference	Reference
West	1,241	34.6	0.97	0.83–1.14
Age groups (yr)				
<5	977	46.4	2.67	2.19–3.26
5–64	1,272	25.5	Reference	Reference
>64	1,036	31.3	1.27	1.04–1.55
Penicillin nonsusceptible				
Geographical region				
Other	217	55.3	Reference	Reference
West	257	66.9	1.50	1.02–2.21
Age groups (yr)				
<5	117	58.8	0.96	0.55–1.67
5–64	135	62.2	Reference	Reference
>64	111	60.4	0.84	0.49–1.45

[a] Odds ratio was adjusted for age or geographic region (as appropriate) and anatomical site (colonization versus invasive infection).

Outpatient antibiotic consumption. Antibiotic consumption was higher in West Switzerland (12.4 to 13.0 DDD per 1,000 inhabitants daily) than in the rest of Switzerland (7.9 to 8.5 DDD per 1,000 inhabitants daily). In addition, cephalosporins were used more often in West Switzerland than in the rest of Switzerland (10.7 to 11.6% versus 6.8 to 7.1% of total antibiotic consumption). Total consumption rates as well as the relative distribution of different antibiotic classes were stable between 2002 and 2004 (Fig. 3).

DISCUSSION

The particular strength of this study was the availability of large strain collections from two simultaneous, ongoing, prospective, nationwide, and representative pneumococcal surveillance programs for invasive and colonizing *S. pneumoniae* isolates in combination with national data on antibiotic consumption. Previous studies, which compared invasive and colonizing pneumococcal isolates, were based on smaller strain collections and were restricted to children or children attending day care centers (1, 2, 14, 28). In Switzerland, PCV-7 was licensed in 2000. Official recommendations were restricted to children at risk, and vaccine use has been low (Swiss Federal Office of Public Health recommendations). Therefore, PCV-7 had no influence on the study results.

The present study also has some limitations. Epidemiological data related to study isolates were scarce, i.e., there was no information about comorbidities. Colonizing isolates were obtained from the nasopharynx of patients with acute respiratory tract infection and not from healthy persons. This may have influenced the serotype distribution. However, previous studies have shown no significant differences in the serotype distributions between children with acute respiratory tract infection and healthy children (1, 14, 30). Due to the design of the two surveillance programs that provided the study isolates, invasive and colonizing isolates differed significantly in their distribution by age and geographical location. However, the large number of strains included allowed us to control for potential confounders by multivariate analysis. Previous studies have analyzed the characteristics of individual clones within prevalent serotypes (1, 14, 28). In this study, the genetic population structure was obtained for only a small fraction of the study isolates (data not shown), which did not allow for a detailed analysis. However, the serotype/serogroup can still serve as a valid unit for analysis of the invasive potential of *S. pneumoniae* (16).

This study found a significant association of serotypes/serogroups 1, 4, 7F, 8, 9V, 12F, and 14 with invasive disease and an association of serotypes/serogroups 3, 6A, 7 (other than 7F), 10, 11, 15, 19F, 23 (other than 23F), and 38 with colonization. This corresponds with recent reports from other geographic regions (1, 2, 14, 28). There were some exceptions. Serotype 18C was associated with a higher invasive potential in studies from the United Kingdom, Sweden, and Finland (1, 14, 28), but this association could not be confirmed in this study despite a sufficient number of serotype 18C isolates. Similar to the findings by Brueggemann et al. (1, 2), the rare serotype 38 did

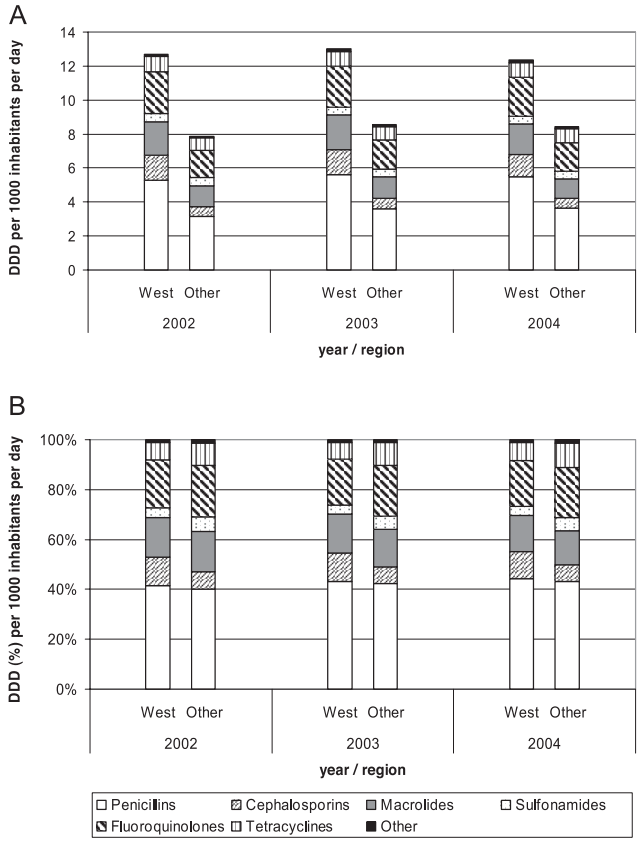

FIG. 3. Absolute (A) and relative (B) outpatient antibiotic consumption in DDD per 1,000 inhabitants per day in West Switzerland compared to the rest of Switzerland from 2002 to 2004.

not appear to exhibit an increased invasive potential as suggested previously by Hanage et al. (14). One likely explanation for this discrepancy could be that distinct clones of serotypes 18C and 38 circulate in Northern Europe. The characterization of serotypes/serogroups with high invasive potential is of epidemiological and public health importance. Pneumococcal serotypes/serogroups with a propensity for colonization are also relevant since they can cause high morbidity through upper respiratory tract infections such as acute otitis media. Surveillance should therefore include both colonizing and invasive pneumococcal strains.

Serotypes 1, 4, 7F, and 8, which have a high risk for invasiveness, had very low proportions of serotype-specific penicillin resistance. Low exposure to antibiotic selection pressure and reduced probability for the acquisition of resistance genes due to a short duration of colonization may explain this association. Serotype 3 had little resistance despite a propensity for colonization. A constitutively high level of expression of the polysaccharide capsule in serotype 3 may impede invasiveness and the acquisition of resistance genes during colonization (13, 31). Serotypes 14 and 9V have been associated with both invasive disease and antibiotic resistance. However, both serotypes are probably good invaders and colonizers, as described in this study and previous studies (1, 14, 28).

The relative serotype/serogroup distribution differed between invasive and colonizing *S. pneumoniae* isolates. Variations were due mostly to different rank orders of serotypes/serogroups rather than a vastly different composition. The differences in rank orders reflected the invasive or colonizing potential of individual serotypes/serogroups as described above. This is in accordance with findings of previous studies (20, 21, 26). The major discrepancies between colonizing and invasive pneumococci were related to serotypes 14, which predominated among invasive isolates, and 19F, which was the most frequent colonizing serotype. Since both serotypes were associated with penicillin resistance and since both serotypes are included in the 7-valent conjugated vaccine, neither the overall penicillin resistance nor the proportion of serotypes covered by the vaccine differed significantly between colonizing and invasive isolates. However, this balance could easily be disturbed by serotype redistribution, for instance, due to vaccine selection pressure. Therefore, colonizing isolates should only be used with caution to predict serotype distribution and resistance among invasive isolates.

Age-specific serotype/serogroup distribution of invasive *S. pneumoniae* isolates in this study corresponded well with findings in other European countries. The pediatric serotypes 6B, 9V, 14, 19F, and 23F predominated among the youngest age group, and serotypes 1, 3, 4, 7, 7F, 8, and 22 were relatively more prevalent after infancy (15). Serotype 3 accounted for a larger percentage of invasive disease among young children in Switzerland (13%) than in other European countries (maximum of 5 to 6%) (15). Serotype 3 has rarely been associated with antibiotic resistance. The high prevalence of serotype 3 may therefore reflect the relatively low antibiotic selection pressure in Switzerland, as discussed below.

The proportion of pediatric serotypes was higher among elderly persons than among middle-aged adults. Others have also observed this association. However, in this study, the proportion of pediatric serotypes among the elderly (34%) was considerably lower than that reported in previous reports from the United States (>44%), Scotland (58%), and Sweden (48%) (8, 10, 19, 23). Waning immunity with increasing age, antibiotic selection pressure, and increased exposure to pediatric serotypes have been discussed as possible explanations for the predominance of pediatric serotypes among the elderly population (10). All three factors probably play a role and act in concert. Here, as in the United States (10), the association of pediatric serotypes with old age was also preserved when the analysis was restricted to penicillin-susceptible isolates. This supports the suggested role of waning immunity in the selection of pediatric serotypes. However, antibiotic selection pressure is also likely to be an important factor. In Switzerland, the rate of antibiotic consumption in outpatients is among the lowest observed in Europe, with an average of 8.97 DDD per 1,000 inhabitants per day (5, 11, 12). Only in The Netherlands has antibiotic consumption been equally low. Low antibiotic selection pressure may explain the relatively low proportion of pediatric serotypes among the elderly population observed in this study. These findings are relevant for the expected indirect effect (herd immunity) of PCV-7 on older age groups and for the potential use of this vaccine in the elderly (10, 32). The proportion of serotypes covered by PCV-7 among infants was lower in Switzerland than in other European countries (16, 17). Again, low antibiotic selection pressure seems to be an important reason for this observation. In contrast with previous studies from the United States and South Africa, no association was found between pediatric serotypes and women of childbearing age (3, 10). This suggests that sociodemographic factors associated with low crowding may have also influenced the study results.

A higher proportion of pediatric serotypes and significantly higher serotype-specific proportions of penicillin resistance for the pediatric serotypes 6B, 9V, 14, and 19F were observed in West Switzerland. Antibiotic consumption among outpatients in this region is significantly higher than in other regions of Switzerland (Fig. 3) (11, 25). In addition, cephalosporins, which exert a higher selection pressure for penicillin-resistant *S. pneumoniae* isolates than amoxicillin (4), are used more frequently in West Switzerland (Fig. 3). Also, day care attendance has been shown to be significantly higher in West Switzerland (20.7 versus 11.9%), although it was not an independent risk factor for the carriage of PNSP (25). The spread of penicillin resistance in *S. pneumoniae* isolates is probably due to the geographical spread of a small number of resistant clones rather than a frequent de novo development of resistance in individual isolates (22). Therefore, antibiotic selection pressure as well as easier dissemination in day care centers were probably responsible for the selection of resistant clones in West Switzerland, leading to higher proportions of SSR and resistant serotypes in this geographic region. However, there is some evidence that pneumococcal strains with recently acquired penicillin resistance may have also been selected (18).

In conclusion, the epidemiology of invasive and colonizing *S. pneumoniae* isolates is influenced by the serotype-specific potential for invasiveness and colonization. Surveillance programs should therefore include both colonizing and invasive *S. pneumoniae* isolates. In addition, antibiotic selection pressure determines the serotype distribution in different age groups and in

different geographic regions. Therefore, it has a significant influence on the expected direct and indirect effects of PCV-7.

ACKNOWLEDGMENTS

This study was financially supported by the Federal Office of Public Health and Wyeth. We also acknowledge the support from the Swiss Sentinel Network (http://www.bag.admin.ch/sentinella/system/e/info.htm).

No author has a commercial or other association that might pose a conflict of interest.

REFERENCES

1. **Brueggemann, A. B., D. T. Griffiths, E. Meats, T. Peto, D. W. Crook, and B. G. Spratt.** 2003. Clonal relationships between invasive and carriage *Streptococcus pneumoniae* and serotype- and clone-specific differences in invasive disease potential. J. Infect. Dis. **187:**1424–1432.
2. **Brueggemann, A. B., T. E. A. Peto, D. W. Crook, J. C. Butler, K. G. Kristinsson, and B. G. Spratt.** 2004. Temporal and geographic stability of the serogroup-specific invasive disease potential of *Streptococcus pneumoniae* in children. J. Infect. Dis. **190:**1203–1211.
3. **Buie, K. A., K. P. Klugman, A. von Gottberg, O. Perovic, A. Karstaedt, H. H. Crewe-Brown, S. A. Madhi, and C. Feldman.** 2004. Gender as a risk factor for both antibiotic resistance and infection with pediatric serogroups/serotypes, in HIV-infected and -uninfected adults with pneumococcal bacteremia. J. Infect. Dis. **189:**1996–2000.
4. **Canet, J. J., and J. Garau.** 2002. Importance of dose and duration of beta-lactam therapy in nasopharyngeal colonization with resistant pneumococci. J. Antimicrob. Chemother. **50**(Suppl. S2)**:**39–43.
5. **Cars, O., S. Molstad, and A. Melander.** 2001. Variation in antibiotic use in the European Union. Lancet **357:**1851–1853.
6. **Centers for Disease Control and Prevention.** 1996. Defining the public health impact of drug-resistant *Streptococcus pneumoniae*: report of a working group. Morb. Mortal. Wkly. Rep. Recomm. Rep. **45**(RR-1 Suppl.)**:**1–20.
7. **Clinical and Laboratory Standards Institute.** 2005. Performance standards for antimicrobial susceptibility testing, fifteenth informational supplement. Document M100-S14. CLSI, Wayne, Pa.
8. **Colman, G., E. M. Cooke, B. D. Cookson, P. G. Cooper, A. Efstratiou, and R. C. George.** 1998. Pneumococci causing invasive disease in Britain 1982–1990. J. Med. Microbiol. **47:**17–27.
9. **Ess, S. M., U. B. Schaad, A. Gervaix, S. Pinösch, and T. D. Szucs.** 2003. Cost-effectiveness of a pneumococcal conjugate immunisation program for infants in Switzerland. Vaccine **21:**3273–3281.
10. **Feikin, D. R., K. P. Klugman, R. R. Facklam, E. R. Zell, A. Schuchat, C. G. Whitney, et al.** 2005. Increased prevalence of pediatric pneumococcal serotypes in elderly adults. Clin. Infect. Dis. **41:**481–487.
11. **Filippini, M., G. Masiero, and K. Moschetti.** Socioeconomic determinants of regional differences in outpatient antibiotic consumption: evidence from Switzerland. Health Policy, in press.
12. **Goossens, H., M. Ferech, R. Vander Stichele, M. Elseviers, et al.** 2005. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. Lancet **365:**579–587.
13. **Hammerschmidt, S., S. Wolff, A. Hocke, S. Rosseau, E. Muller, and M. Rohde.** 2005. Illustration of pneumococcal polysaccharide capsule during adherence and invasion of epithelial cells. Infect. Immun. **73:**4653–4667.
14. **Hanage, W. P., T. H. Kaijalainen, R. K. Syrjänen, K. Auranen, M. Leinonen, P. H. Mäkelä, and B. G. Spratt.** 2005. Invasiveness of serotypes and clones of *Streptococcus pneumoniae* among children in Finland. Infect. Immun. **73:**431–435.
15. **Hausdorff, W. P., J. Bryant, P. R. Paradiso, and G. R. Siber.** 2000. Which pneumococcal serogroups cause the most invasive disease: implications for conjugate vaccine formulation and use, part I. Clin. Infect. Dis. **30:**100–121.
16. **Hausdorff, W. P., D. R. Feikin, and K. P. Klugman.** 2005. Epidemiological differences among pneumococcal serotypes. Lancet Infect. Dis. **5:**83–93.
17. **Hausdorff, W. P.** 2002. Invasive pneumococcal disease in children: geographic and temporal variations in incidence and serotype distribution. Eur. J. Pediatr. **161**(Suppl. 2)**:**S135–S139.
18. **Hauser, C., S. Aebi, and K. Muhlemann.** 2004. An internationally spread clone of *Streptococcus pneumoniae* evolves from low-level to higher-level penicillin resistance by uptake of penicillin-binding protein gene fragments from nonencapsulated pneumococci. Antimicrob. Agents Chemother. **48:**3563–3566.
19. **Hedlund, J., S. B. Svenson, M. Kalin, J. Henrichsen, B. Olsson-Liljequist, G. Mollerberg, and G. Kallenius.** 1995. Incidence, capsular types, and antibiotic susceptibility of invasive *Streptococcus pneumoniae* in Sweden. Clin. Infect. Dis. **21:**948–953.
20. **Ho, P. L., K. F. Lam, F. K. H. Chow, Y. L. Lau, S. S. Y. Wong, S. L. E. Cheng, and S. S. Chiu.** 2004. Serotype distribution and antimicrobial resistance patterns of nasopharyngeal and invasive *Streptococcus pneumoniae* isolates in Hong Kong children. Vaccine **22:**3334–3339.
21. **Kellner, J. D., A. McGeer, M. S. Cetron, D. E. Low, J. C. Butler, A. Matlow, J. Talbot, and E. L. Ford-Jones.** 1998. The use of *Streptococcus pneumoniae* nasopharyngeal isolates from healthy children to predict features of invasive disease. Pediatr. Infect. Dis. J. **17:**279–286.
22. **Klugman, K. P.** 2002. The successful clone: the vector of dissemination of resistance in *Streptococcus pneumoniae*. J. Antimicrob. Chemother. **50**(Suppl. S2)**:**1–5.
23. **Kyaw, M. H., P. Christie, S. C. Clarke, J. D. Mooney, S. Ahmed, I. G. Jones, and H. Campbell.** 2003. Invasive pneumococcal disease in Scotland, 1999-2001: use of record linkage to explore associations between patients and disease in relation to future vaccination policy. Clin. Infect. Dis. **37:**1283–1291.
24. **Mastro, T. D., N. K. Nomani, Z. Ishaq, A. Ghafoor, N. F. Shaukat, E. Esko, M. Leinonen, J. Henrichsen, R. F. Breiman, B. Schwartz, et al.** 1993. Use of nasopharyngeal isolates of Streptococcus pneumoniae and Haemophilus influenzae from children in Pakistan for surveillance for antimicrobial resistance. Pediatr. Infect. Dis. J. **12:**824–830.
25. **Mühlemann, K., H. C. Matter, M. G. Täuber, and T. Bodmer.** 2003. Nationwide surveillance of nasopharyngeal *Streptococcus pneumoniae* isolates from children with respiratory infection, Switzerland, 1998-1999. J. Infect. Dis. **187:**589–596.
26. **Robinson, D. A., K. M. Edwards, K. B. Waites, D. E. Briles, M. J. Crain, and S. K. Hollingshead.** 2001. Clones of *Streptococcus pneumoniae* isolated from nasopharyngeal carriage and invasive disease in young children in central Tennessee. J. Infect. Dis. **183:**1501–1507. (Erratum, **184:**1228.)
27. **Saha, S. K., A. H. Baqui, G. L. Darmstadt, M. Ruhulamin, M. Hanif, S. El Arifeen, M. Santosham, K. Oishi, T. Nagatake, and R. E. Black.** 2003. Comparison of antibiotic resistance and serotype composition of carriage and invasive pneumococci among Bangladeshi children: implications for treatment policy and vaccine formulation. J. Clin. Microbiol. **41:**5582–5587.
28. **Sandgren, A., K. Sjöström, B. Olsson-Liljequist, B. Christensson, A. Samuelsson, G. Kronvall, and B. H. Normark.** 2004. Effect of clonal and serotype-specific properties on the invasive capacity of *Streptococcus pneumoniae*. J. Infect. Dis. **189:**785–796.
29. **Scott, J. A., A. J. Hall, R. Dagan, J. M. Dixon, S. J. Eykyn, A. Fenoll, M. Hortal, L. P. Jette, J. H. Jorgensen, F. Lamothe, C. Latorre, J. T. Macfarlane, D. M. Shlaes, L. E. Smart, and A. Taunay.** 1996. Serogroup-specific epidemiology of *Streptococcus pneumoniae*: associations with age, sex, and geography in 7,000 episodes of invasive disease. Clin. Infect. Dis. **22:**973–981.
30. **Syrjanen, R. K., T. M. Kilpi, T. H. Kaijalainen, E. E. Herva, and A. K. Takala.** 2001. Nasopharyngeal carriage of *Streptococcus pneumoniae* in Finnish children younger than 2 years old. J. Infect. Dis. **184:**451–459.
31. **Weiser, J. N., and M. Kapoor.** 1999. Effect of intrastrain variation in the amount of capsular polysaccharide on genetic transformation of *Streptococcus pneumoniae*: implications for virulence studies of encapsulated strains. Infect. Immun. **67:**3690–3692.
32. **Whitney, C. G., M. M. Farley, and J. Hadler.** 2003. Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. N. Engl. J. Med. **348:**1737–1746.