JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2005, p. 6060–6063 Vol. 43, No. 12
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.12.6060–6063.2005

Distribution of *Streptococcus pneumoniae* Serotypes Responsible for Penicillin Resistance and the Potential Role of New Conjugate Vaccines in New Caledonia

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Received 24 March 2005/Returned for modification 28 April 2005/Accepted 8 September 2005

Invasive pneumococcal disease is a significant cause of morbidity and mortality worldwide. The aim of this study was to establish the serotypes responsible for pneumococcal disease and the serotypes responsible for penicillin resistance in Noumea, New Caledonia. Isolates of *Streptococcus pneumoniae* from all body sites referred to the Microbiology Department of the Pasteur Institute in New Caledonia between May 1999 and May 2001 had serotyping and susceptibility testing performed. Basic demographic data on patients were also collected. A total of 298 isolates were included in the study. The most common serotypes were types 1 (20%), 23F (10%), 12F (8%), 19F (8%), and 6B (5%). The serotype distribution differed significantly with age, site of collection, and ethnicity. Overall, 280 of 298 (94%) of the isolates had serogroups that are included in the 23-valent vaccine. However, only 14 of 20 (70%) of the isolates associated with invasive disease from children younger than 5 years of age and 26 of 113 (23%) of invasive isolates from patients more than 5 years of age were included in the new 7-valent conjugate vaccine. Overall, reduced susceptibility to penicillin was found in 43 of 298 (14.4%) of the isolates, with 13% falling into the intermediate resistance category and only 5 (2%) being high-level resistant. A higher prevalence of penicillin resistance occurred in younger age groups and in European patients and involved specifically certain serotypes. The 7-valent conjugate pneumococcal vaccine has potential benefit for the New Caledonian population under 5 years of age and should be considered for future vaccines schedules.

Streptococcus pneumoniae is a major cause of morbidity and mortality in children and adults worldwide (6–8, 11, 17). Among invasive illnesses, the treatment and outcome of resistant meningitis is of significant concern. France displays the highest rates of pneumococcal resistance in Europe (14), which prompted the present study in the French territory of New Caledonia. New Caledonia is an island located in the Pacific Ocean, 15,000 km from France, near Australia and New Zealand under the tropic of Capricorn. The population of New Caledonia is approximately 200,000 people (1996 census) and is split into different ethnic groups. The indigenous population is Melanesian and accounts for ca. 45% of the total population. Tahitians (5%) come from French Polynesia and Wallisians (10%) from Wallis and Futuna, both are French territories in the South Pacific region. Europeans and Asian populations settled in the country account, respectively, for 35 and 5%. Some Europeans were born in New Caledonia, and others are expatriates from Europe (the exact proportions are not known). Recently, a 7-valent conjugate vaccine (4, 6B, 9V, 14, 18C, 19F, and 23F) has been shown to be highly effective in the prevention of invasive pneumococcal disease (2) in the United States. Its role in the prevention of disease in indigenous populations is affected by the lower serotype coverage that is known to occur in these populations (10, 13). At the time of the present study, no specific recommendation has been made about the use of the 7-valent pneumococcal conjugate vaccine and, until now, only the 23-valent polysaccharide vaccine has been commonly used in the territory, mainly in the population over 65 years of age. The 11-valent conjugate vaccine has additional serotypes (1, 3, 5, and 7F) compared to the 7-valent but will not be available on the market for several years.

We sought here to examine the serotypes associated with pneumococcal disease in New Caledonia, including those responsible for penicillin resistance, to establish a baseline prior to introduction of a 7- or 11-valent conjugate pneumococcal vaccine. This is the first comprehensive description of the pneumococcal serotypes responsible for disease and penicillin resistance in New Caledonia and also in the South Pacific insular area.

MATERIALS AND METHODS

Study population and data collection. The Pasteur Institute is the laboratory for the only major hospital in Noumea. All isolates of *Streptococcus pneumoniae* referred to the Microbiology Laboratory of the Pasteur Institute between May 1999 and May 2001 were collected. Basic patient demographics including age, sex, ethnicity, date, and site of collection were also recorded. Ethnicity was defined through the patient's family name, which is thought to provide a reliable distinction between the different ethnic groups in New Caledonia. In New Caledonia it is standard practice to consider ethnicity in relation to population health issues.

Case definitions. Invasive pneumococcal disease was defined as isolation of *S. pneumoniae* from a normally sterile body site. It did not include patients with suspected pneumococcal infection or those with only a positive pneumococcal

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antigen test. Nonsterile-site isolates (noninvasive isolates) came from all other body sites.

Identification of *S. pneumoniae*. Identification of *S. pneumoniae* was based on Gram stain, catalase, colony morphology, and sensitivity to optochin. Confirmation was also made in some cases by positive slide agglutination (Slidex Pneumokit; bioMérieux, France). All strains were frozen at −80°C by using a cryosystem (AES, reference no. AEB 400100) for later testing.

Susceptibility testing. Susceptibility to penicillin, amoxicillin, and cefotaxime was determined by using the ATB Pneumo system (bioMérieux). All isolates identified as having reduced susceptibility to at least penicillin were then tested for the three antibiotics by using the E-test method (AB Biodisk, Sweden) according to the manufacturer's recommendations on Mueller-Hinton agar with 5% sheep blood. Interpretation criteria concerning susceptibility were as recommended by the Comité de l'Antibiogramme de la Société Française de Microbiologie (http://www.sfm.asso.fr/).

Pneumococcal serotyping. All isolates were referred to the New South Wales Pneumococcal Reference laboratory, which is located at the Children's Hospital at Westmead in Sydney, Australia. Isolates were serotyped by the Quellung method using initially pooled rabbit polyclonal antisera and later serogroup, serotype, and factor sera where indicated. Antisera were obtained from the Statens Serum Institute in Copenhagen, Denmark. The NSW Pneumococcal Reference Laboratory is a nationally accredited laboratory and participates in a national external quality assurance program.

Statistical methods. Statistical analysis was performed by using the Student *t* test to compare mean ages and the χ^2 test, odds ratios (ORs) with 95% confidence intervals (95%CI), and the Fisher exact test (for small numbers) to compare the distribution of categorical data (Epi-Info, version 6.0; Centers for Disease Control and Prevention, Atlanta, Ga.). In addition, ORs (SPSS, Chicago, Ill.), as estimates of the relative risks from multivariate logistic regression models, were used to define independent risk factors associated with penicillin resistance.

RESULTS

Number of episodes. During the study period, 298 isolates were collected, serotyped, and had susceptibility testing available.

Patient demographics. The median age of patients was 40 years, with a male/female ratio of 1.3:1. Overall, 47 of 298 (15.8%) of isolates came from children younger than 5 years of age, and 66 of 298 (22.1%) were from adults older than 65 years of age. A total of 169 strains (57%) were from Melanesian people, 60 (20%) were from Europeans, and 65 (22%) came from people from the Wallis Islands or from Tahitians, with only 4 (2%) isolates coming from people with an Asiatic background.

Clinical specimen distribution. Of 298 patients, 135 (45.3%) were considered invasive isolates from sterile sites, with the remainder coming from nonsterile body sites. Of the 298 isolates, blood cultures accounted for 109 (37%), spinal fluids accounted for 19 (6%), and respiratory specimens accounted for 121 (41%).

Serotype distribution. The 298 isolates represented 29 different serotypes. Fourteen strains were nontypeable. The serotype distribution differed according to age group, site of infection (invasive versus noninvasive), and also ethnicity (Table 1). Irrespective of the ethnicity of the patient, five prevalent serotypes account for over half of all isolates.

Factors influencing serotype distribution. (i) Ethnicity. Ethnicity had a significant impact on serotype distribution. In comparison to isolates from European patients over 5 years of age, serotype 1 was over-represented from sterile site isolates in Melanesian patients (OR = 11.59, 95%CI = 2.31 to 78.34) and patients with a Tahitian background or from the Wallis Islands (Tahitian/Wallisian patients) (OR = 5.25, 95%CI = 1.27 to 25.44). There was a trend for serotype 12F to also be over-represented in Melanesian and Tahitian/Wallisian patients older than 5 years of age compared to European patient's isolates, but this did not reach statistical significance. For invasive isolates from children younger than 5 years of age, there was no difference in serotype 1 or 12F distribution associated with ethnicity. No difference was seen in serotype distribution between males and females, and no seasonal impact on serotype distribution was noted.

TABLE 1. Serotype distribution according to ethnicity and age groups

Category	Serotypes (%)
Ethnicity	
Melanesians	1, 23F, 12F, 19F, 19A (55)
Tahitians/Wallisians	1, 12F, 6B, 19F, 23F (57)
Europeans	19F, 14, 4, 1, 23F (51)
Age group (invasive strains)	
Under 5 yr	6, 23, 18, 4 (52)
Over 5 yr	1, 12F, 23F, 4 (78)

(ii) Age. The serotype distribution did, however, differ between children and adults. For invasive isolates, serotype 1 was more common in Melanesians (OR = 11.59, 95%CI = 1.49 to 512.57), with a trend toward being more common in Tahitian/Wallisian patients (OR = 3.0, 95%CI = 0.24 to 159.84) over 5 years of age compared to children younger than 5 years of age. No age difference was seen for serotype 1 in European patients. Compared to invasive isolates from Melanesian or Tahitian/Wallisian patients over 5 years of age there was a trend for 12F to be less common from patients younger than 5 years of age (OR = 0.00, 95%CI = 0.00 to 1.57), although this did not reach statistical significance. The 7-valent vaccine serotypes such as 23F, 19F and 6B were more common in children.

(iii) Invasive versus noninvasive isolates. There was a significant difference in serotype distribution between sterile site isolates compared to nonsterile site isolates, suggesting a difference in invasive potential of some serotypes in specific patient populations (Table 2). In patients older than 5 years of age, serotype 1 was more commonly seen in Melanesian patients (OR = 12.87, 95%CI = 4.47 to 38.92) and Tahitian/Wallisian patients (OR = 5.50, 95% CI = 1.17 to 34.24) from sterile site isolates than from nonsterile site isolates. A similar trend was seen in European patients older than 5 years of age, although this did not reach statistical significance. No difference in frequency of serotype 1 from sterile sites compared to nonsterile sites was seen in children younger than 5 years of age. Interestingly, although serotype 1 was commonly found in

TABLE 2. Distribution of serotypes with all age and ethnic groups combined

Serotype	No. of serotypes (%)		
	Sterile	Nonsterile	Total
1	46 (75.4)	15	61
12F	15 (60)	10	25
23F	13 (44.8)	16	29
19F	1	24 (96)	25
14	4	11 (73.3)	15

TABLE 3. Vaccine serotype coverage for sterile-site isolates of 7- and 11-valent vaccines for children under 5 years of age according to ethnicity

Ethnicity	% Serotype coverage, % serogroup coverage		No. of isolates
	7-valent	11-valent	
Melanesians	75, 83.3	83.3, 91.6	12
Europeans	33, 33	67, 67	3
Tahitians/Wallisians	80, 80	100, 100	5
All ethnic groups	70, 75	85, 90	20

TABLE 5. Vaccine serotype coverage for nonsterile-site isolates of 7- and 11-valent vaccines for patients under 5 years of age according to ethnicity

Ethnicity	% Serotype coverage, % serogroup coverage		No. of isolates
	7-valent	11-valent	
Melanesians	50, 57.1	71.4, 78.9	14
Europeans	100, 100	100, 100	6
Tahitians/Wallisians	42.9, 42.9	57.1, 57.1	7
All ethnic groups	59.2, 62.9	74.1, 77.8	27

bacteremia, there were no cases of meningitis in the study period due to this serotype. There was a trend for serotype 12F to be over-represented in sterile-site isolates from Tahitian/Wallisian patients more than 5 years of age compared to nonsterile site isolates, although this did not reach statistical significance (OR = 2.19. 95%CI = 0.86 to 5.70). No significant difference in the prevalence of serotype 12F isolates from sterile sites versus nonsterile sites occurred in European or Melanesian patients. Serotype 19F was less common in sterile-site isolates compared to nonsterile-site isolates in European (OR = 0.00, 95%CI = 0.00 to 0.73) and Melanesian patients (OR = 0.00, 95%CI = 0.00 to 0.63) older than 5 years of age but not in Tahitian/Wallisian patients. No difference in serotype 19F distribution was seen between sterile-site and nonsterile-site isolates in children younger than 5 years of age. Serotype 23F was more commonly seen in sterile-site isolates compared to nonsterile-site isolate in Melanesian children less than 5 years of age (OR = 18.2, 95%CI = 1.48 to 881.51), but this difference was not seen for either European or Tahitian/Wallisian children.

Vaccine serotype coverage. In patients older than 65 years of age, 14 of 14 (100%) strains from European and 29 of 35 (82.8%) strains from Melanesian patients would be a serotype match for the 23-valent polysaccharide vaccine. This high coverage is similar for invasive and noninvasive strains. The serotype coverage of the 7- and 11-valent conjugate vaccines for invasive isolates for children younger than 5 years of age and patients older than 5 years of age are given in Tables 3 and 4, respectively. The serotype coverage for noninvasive isolates from children younger than 5 years of age is given in Table 5.

Seasonality. Despite the relatively mild seasonal climatic changes in New Caledonia, a significant season variation in the incidence of pneumococcal disease was noted. Isolates were more commonly recovered in the colder months of July and August, with 32.2% of all isolates being recovered in these 2 months of the year.

Penicillin resistance. During the 2-year study period, 43 of 298 (14.4%) isolates were found to have reduced susceptibility to penicillin, but only 5 (1.7%) of these had high-level resistance, with MICs to penicillin of >1 mg/liter. Of these 298 strains, 11 (3.7%) expressed intermediate sensitivity to amoxicillin, and 5 (1.7%) expressed intermediate sensitivity to both amoxicillin and cefotaxime. These 16 strains are included in the 43 previous strains. Of these 43 strains, 39 (90.7%) belonged to serotypes 6B, 9V, 14, 19F, and 23F, which are contained in the 7-valent conjugate vaccine.

Isolates with reduced susceptibility to penicillin were significantly more common in children younger than 5 years of age (20 of 47 [42.5%]) compared to patients older than 5 years of age (23 of 251 [9.2%]) (OR = 7.34, 95%CI = 3.37 to 16.06, $P < 0.00001$).

There was also a significant association between ethnicity and penicillin resistance, with 17 of 64 (26.6%) European patient isolates showing reduced susceptibility to penicillin compared to 17 of 169 (10%) Melanesian patient isolates and 9 of 65 (13.8%) isolates from the Wallisian or Tahitian patients. This difference was most marked in children younger than 5 years of age, including 9 of 26 (34.6%) isolates from Melanesian children, 5 of 12 (41.7%) isolates from Wallisian/Tahitian children, and 6 of 9 (66.7%) isolates from European children isolates with reduced susceptibility to penicillin. Regarding correlation between serotypes and susceptibility to penicillin, serotypes 6B, 9V, 14, 19F, and 23F are more likely to have reduced susceptibility compared to other serotypes, with an OR of 25 (95% CI = 8.7 to 71.7).

TABLE 4. Vaccine serotype coverage for sterile-site isolates of 7-and 11-valent vaccines for patients over 5 years of age according to ethnicity

Ethnicity	% Serotype coverage, % serogroup coverage		No. of isolates
	7-valent	11-valent	
Melanesians	15.8, 21	71.9, 77.2	57
Europeans	46.4, 57.1	71.4, 82.1	28
Tahitians/Wallisians	14.3, 21.4	64.3, 71.4	28
All ethnic groups	23, 30.1	69.9, 77	113

DISCUSSION

The new 7-valent conjugate vaccine has provided significant benefits directly to children (1, 2) and indirectly through herd immunity to adults (15) in the United States. The benefits are, however, dependent on the serotypes that are prevalent in the community. Marked differences in the serotype distribution were seen with ethnicity and age of patients in our survey. Much of this difference could be accounted for by the increased prevalence of serotypes 1 and 12F, particularly in adults and in Melanesian and Tahitian/Wallisian patients. Serotype 1 has previously been demonstrated to have a high invasive potential and has been known to be linked to outbreaks of invasive disease (12). Serotype 1 is rarely associated with penicillin resistance, possibly due to low nasopharyngeal

carriage rates with resultant reduced exposure to penicillin (3). Of interest is the fact that no cases of meningitis occurred in the study period due to serotype 1 despite its frequency as a cause of bacteremia.

Although not so easily demonstrated statistically, serotype 12F also appeared to have increased invasive potential, particularly in adults, but unlike serotype 1 it was a significant cause of meningitis accounting for 4 of 19 (21%) of all cases. It is also uncommon in children under 5 years of age and was not associated with penicillin resistance. Neither serotype 1 nor 12F are included in the 7-valent vaccine; however, serotype 1 is included in the new 11-valent conjugate vaccine.

The 11-valent conjugate vaccine has some advantage in serotype coverage over the 7-valent vaccine in children less than 5 years of age in our study, with an increase of 15% serotype coverage, irrespective of whether they were from sterile or nonsterile sites (Tables 3 and 5). However, this vaccine is unlikely to be commercially available in the near future. The vaccine serotype coverage for the 7-valent vaccine in children younger than 5 years of age would warrant its introduction since it will likely have significant benefit, particularly on the incidence of penicillin-resistant strains (15). If the degrees of herd immunity seen in New Caledonia are similar to those in the United States (15), with consequent benefits for the adult population, then the 11-valent vaccine could have marked social and economic advantages compared to the 7-valent vaccine.

Although there are relatively mild seasonal changes in ambient temperature throughout the year in New Caledonia, there is a clear seasonal increase in the incidence of pneumococcal disease in the colder months of the year. This is consistent with the findings of another study from the United States (9).

The population groups at risk of penicillin resistance have been underlined in the present study, with infants and Europeans being at significantly increased risk. During childhood, antibiotic pressure may be greater due to frequent use of antibiotics to treat otitis media (4, 5) and greater exposure to resistant strains through attendance at day care centers (16). It is likely that Europeans are more likely to receive antibiotics in New Caledonia than Melanesian or Tahitian/Wallisian patients. Both expatriate Europeans and those born in New Caledonia are more likely to use the available health care services compared to Melanesians or Tahitians/Wallisians. Despite this, the resistance rates in Europeans living in New Caledonia are still lower than in mainland France and Europe, which may be related to differences in the rate of antibiotic prescribing (14).

Resistant serotypes seen in New Caledonia are similar to those seen in other countries, with the assumption being that international resistant clones have spread to New Caledonia through immigration and tourism. The fact that a high percentage of serotypes are included in the 7- and 11-valent conjugate vaccines suggests that implementation of an infant vaccination schedule, even with the 7-valent form, should have a significant impact on the incidence of invasive illnesses, as well as on the rates of antibiotic resistance.

ACKNOWLEDGMENTS

We thank Marianne Gail Stewart for carrying out pneumococcal serotyping in the New South Wales Pneumococcal Reference Laboratory in Sydney.

This study was supported by the Aventis Pasteur Laboratory (Lyon, France).

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