CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Nov. 2002, p. 1328–1331 Vol. 9, No. 6
1071-412X/02/$04.00+0 DOI: 10.1128/CDLI.9.6.1328–1331.2002

Antimicrobial Susceptibilities and Serotype Distribution of *Streptococcus pneumoniae* Isolates from a Low Socioeconomic Area in Lima, Peru

Anna R. Cullotta,[1] H. D. Kalter,[2] Jose Delgado,[3] Robert H. Gilman,[2,3]* Richard R. Facklam,[4] Billie Velapatino,[5] Jorge Coronel,[6] Lilia Cabrera,[3] and M. Urbina[7]

Emory University Hospital[1] and Respiratory Diseases Branch, Centers for Disease Control and Prevention,[4] Atlanta, Georgia; Department of International Health, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland[2]; and Asociación Benéfica Proyectos en Informática, Salud, Medicina, y Agricultura (AB PRISMA),[3] and Infectious Disease Laboratory, Department of Pathology,[5] and Department of Pathology,[6] Universidad Peruana Cayetano Heredia, and Hospital María Auxiliadora,[7] Lima, Perú

Received 29 April 2002/Returned for modification 12 June 2002/Accepted 22 July 2002

Streptococcus pneumoniae **isolates were obtained from nasopharyngeal swabs taken from children living in a low socioeconomic area of Lima, Peru, to determine the rates of antimicrobial resistance and serotype distribution. A total of 146 nasopharyngeal isolates were collected from children from 3 to 38 months of age. Twenty-one clinical laboratory isolates from both sterile and nonsterile sites were obtained from a local hospital. Isolates with reduced susceptibilities to penicillin represented 15.1 and 42.9% of the nasopharyngeal and clinical isolates, respectively. For neither group of isolates did penicillin MICs exceed 1.5 μg/ml, indicating only intermediate resistance. Thirty-two different serotypes were identified from the 146 nasopharyngeal isolates. The serotypes of the clinical isolates were represented among those 32 types. Isolates with reduced susceptibility to multiple antimicrobial agents were present in both settings. These findings indicate some of the highest rates of antimicrobial resistance in the region as well as a slightly different serotype distribution pattern from those of other South American countries. The 7-valent conjugate pneumococcal vaccines would only have a limited effect, providing coverage for about half of all isolates. Increasing rates of resistance in Peru necessitate an awareness of antimicrobial treatment practices and vaccination strategies.**

Streptococcus pneumoniae is an etiologic agent responsible for a variety of clinical illnesses (11). It is one of the leading causes of meningitis, sepsis, pneumonia, and otitis media worldwide. Illnesses caused by *S. pneumoniae* represent a substantial contribution to morbidity and mortality, especially in infants and children (4). In developing countries, where rates of infant mortality are high and respiratory disease is a leading cause of death, *S. pneumoniae* is a significant pathogen (2).

Recent studies aimed at characterizing *S. pneumoniae* in Latin America have revealed various degrees of antimicrobial resistance. Chile, Colombia, Brazil, and Argentina have reported penicillin resistance rates of 9.0 (9), 12.0 (2), 21.4 (1), and 24.4% (14), respectively. Rates of penicillin resistance as high as 48.1% in Mexico and 40% in Uruguay have been reported and are consistent with the worldwide pattern of increasing resistance among *S. pneumoniae* isolates (5, 8). Resistance to other classes of antimicrobial agents and resistance to multiple agents have also been reported (17). Serotype distribution patterns in Latin America, however, differ slightly from those in other regions of the world, with some patterns unique to the region (7). Some of these serotypes identified in both clinical and community isolates collected in various South American countries exhibit particular antimicrobial resistance patterns and represent clones that pose serious treatment challenges for physicians in the clinical setting (9, 17).

There is an urgent need to further identify and monitor patterns of pneumococcal disease and antimicrobial resistance in areas of the world in which the organism is poorly characterized. Treatment and vaccination strategies are critical as resistance rates increase and resistant clones spread. With the advent of new pneumococcal vaccines, there is an opportunity to stem the threat of this infectious organism and improve the state of public health.

The community we sampled in Lima, Peru, is representative of most of the impoverished communities throughout Latin America. These communities traditionally have high rates of morbidity and mortality due to infectious diseases, including pneumonia, which is the leading cause of infant mortality in Peru according to the Pan American Health Organization (http://www.paho.org). *S. pneumoniae* is carried by a high percentage of young children and is a major etiologic agent of both respiratory and invasive disease in these settings. For these reasons, we have chosen to study isolates in both community and clinical settings in Peru.

MATERIALS AND METHODS

Study population. We selected a population of children from Pampas de San Juan de Miraflores (total population, ~40,000), a typical impoverished shanty-

* Corresponding author. Mailing address: The Johns Hopkins University, School of Hygiene and Public Health, Department of International Health, 615 North Wolfe St., Room W5039, Baltimore, MD 21205. Phone: (410) 614-3639. Fax: (410) 614-6060. E-mail: rgilman@jhsph.edu.

town community (pueblo joven) in the southern sector of the capital city of Lima, Peru. Most homes are constructed of cement or woven thatch, approximately half of which are connected to sewer lines or have latrines. Water is supplied by municipal trucks and stored in cisterns. Children from households throughout the community were randomly selected for nasopharyngeal sampling from 6 June 2000 through 21 August 2000. Written consent for each child was obtained from either a parent or guardian. Clinical isolates were obtained from the clinical microbiology laboratory of Maria Auxiliadora Hospital, the major medical facility serving the community of Pampas de San Juan de Miraflores. Strains had been isolated from patients of all age groups and a variety of body sites between 23 May 2000 and 23 January 2001.

Specimen collection. Nasopharyngeal swabs were collected and placed into Amies transport medium and transported to the infectious disease research laboratory of the Department of Pathology, Universidad Peruana Cayetano Heredia, Lima, Peru. Clinical isolates were saved on Trypticase soy agar supplemented with 5% sheep blood (TSA-SB) and transported to the laboratory mentioned above within 24 h of the initial isolation.

Microbiology. Nasopharyngeal swabs were inoculated onto TSA-SB and incubated at 35 to 37°C in 5 to 10% CO_2 for 24 to 48 h. Cultures were observed at 24 and 48 h for typical colonial growth of *S. pneumoniae*. Suspect colonies were subcultured to TSA-SB, and an optochin disk was placed on the inoculated plate. The following day, optochin-positive (growth inhibition zones of 14 mm or greater) isolates were tested for bile solubility with 10% sodium deoxycholate. Cultures with positive optochin and bile solubility tests were considered *S. pneumoniae*. The clinical isolates were also confirmed by optochin susceptibility and bile solubility testing.

Susceptibility testing. Susceptibility testing was performed by the E-test strip method (AB Biodisk) on Mueller-Hinton agar supplemented with 5% sheep blood according to the performance standards of the National Committee for Clinical Laboratory Standards (12). Cultures were tested for susceptibility to chloramphenicol, erythromycin, penicillin, and trimethoprim-sulfamethoxazole, the four agents used most frequently to treat pneumococcal infection in Peru.

Serotyping. Serotyping was performed at the Centers for Disease Control and Prevention (CDC), Atlanta, Ga., by using CDC antisera to capsular polysaccharides and the Quellung reaction.

RESULTS

A total of 302 children from the ages of 3 to 38 months (mean = 11.45, median = 12) were selected for nasopharyngeal sampling. *S. pneumoniae* was isolated from 146 (48.3%) of the children. Among the nasopharyngeal isolates, 15.1% showed antimicrobial resistance to penicillin, and 2.1, 6.8, and 56.9% showed resistance to chloramphenicol, erythromycin, and trimethoprim-sulfamethoxazole, respectively. For the purposes of this article, resistance refers to isolates exhibiting an intermediate or high level of resistance. Sixty-one (41.8%) isolates were completely sensitive to all four antimicrobial agents tested, 41.8% were resistant to only one agent, 10.3% were resistant to two agents, and 6.2% were resistant to three agents. None of the isolates exhibited resistance to all four antimicrobials tested.

The nasopharyngeal isolates comprised 32 different serotypes (Table 1). The serotype most frequently isolated was 23F, but it accounted for only 9.1% of penicillin-resistant isolates, while type 14 and the nontypeable isolates accounted for 54.5% of penicillin-resistant isolates and were the 3rd and 5th most frequently isolated types. In all, eight serotypes (6A, 6B, 10F, 14, 15B, 19F, 23B, and 23F) and the nontypeable strains accounted for all of the penicillin- and multiple-drug-resistant isolates.

Among the 21 clinical isolates collected, 8 were obtained from sterile body sites (cerebrospinal fluid [CSF], pleural fluid, ascitic fluid, and lymph node), and the remaining isolates were obtained from respiratory secretions (sputum, endotracheal aspirates, etc.) (Table 2). Reduced susceptibility to penicillin (MIC of >0.12 μg/ml) was demonstrated in nine (42.9%) of the isolates. Resistance rates to other antimicrobials were as follows: chloramphenicol, 23.8%; erythromycin, 14.3%; and trimethoprim-sulfamethoxazole, 61.9%. Of the antimicrobials tested, high levels of resistance were detected to all agents except penicillin. Nine (42.9%) of the 21 isolates exhibited resistance to multiple antimicrobial agents: 14.3, 19.0, and 9.5% to two, three, and four agents, respectively. Of the penicillin-resistant isolates, 23.8% were resistant to chloramphenicol, and 14.3% were resistant to erythromycin, and 100% of isolates with reduced susceptibility to penicillin were resistant to trimethoprim-sulfamethoxazole. Twenty-five percent of sterile isolates and 53.8% ($P = 0.063$, Fisher's exact test) of nonsterile isolates were resistant to penicillin. The two sterile site isolates resistant to penicillin were also resistant to other antimicrobial agents.

A total of 11 different serotypes were identified among the clinical isolates. Sterile site isolates were associated with five serotypes: 9A, 9L, 23F, 6A, and 18F. Nonsterile site isolates were associated with eight serotypes: 23F, 19F, 6A, 5, 11A, 13, 14, and 35F. Four serotypes (6A, 14, 19F, and 23F) accounted for all of the penicillin-resistant isolates and all of the multiple-

TABLE 1. Serotype distribution and antimicrobial resistance among nasopharyngeal isolates

Serotype ($n = 32$)[a]	Distribution ($n = 146$)		No. of isolates resistant to[b]:			
	No.	%	Chlor	Ery	Pen	SXT
23F (V)	20	13.7	2		2	15
6A	15	10.3		3	1	6
6B (V)	15	10.3		1	3	10
15B	13	8.9			1	8
NT	13	8.9		5	6	9
19F	12	8.2	1		1	9
14 (V)	11	7.5			6	10
34	5	3.4				1
23B	4	2.7			1	2
9V (V)	3	2.1				1
19A (V)	3	2.1				2
21	3	2.1				
4 (V)	2	1.4				
10A	2	1.4				
11A	2	1.4				1
16F	2	1.4				1
17F	2	1.4				
24F	2	1.4				
35F	2	1.4				
38	2	1.4				2
5	1	0.7				1
9A	1	0.7				
10F	1	0.7		1	1	1
12B	1	0.7				1
13	1	0.7				
15A	1	0.7				1
15C	1	0.7				1
15F	1	0.7				1
18C (V)	1	0.7				
18F	1	0.7				
19B	1	0.7				
24B	1	0.7				
35C	1	0.7				

[a] V, vaccine type; NT, not typeable.
[b] Chlor, chloramphenicol; Ery, erythromycin; Pen, penicillin; SXT, trimethoprim-sulfamethoxazole.

TABLE 2. Characteristics of *S. pneumoniae* isolates from Maria Auxiliadora Hospital

Serotype	Level of resistance to[a]:				Age(s)[b]
	Chlor	Ery	Pen	SXT	
Sterile site isolates ($n = 8$)[c]					
6A	R	R	I	R	6 mo
9A	S	S	S	R	4 mo, 38 yr
9L	S	S	S	S	Unknown, 28 yr
18F	S	S	S	S	3 mo
23F	S	S	S	R	3 mo
	R	S	I	R	3 mo
Nonsterile site isolates ($n = 13$)[d]					
5	S	S	S	I	62 yr
6A	S	S	S	S	7 mo
	S	S	I	R	3 mo
11A	S	S	S	S	33 yr
13	S	S	S	S	57 yr
14	S	R	I	R	Unknown
19F	S	S	S	S	10 yr
	S	S	I	R	38 yr
	R	R	I	R	3 mo
23F	S	S	I	R	8 yr
	R	S	I	R	25 yr, 87 yr
35F	S	S	S	S	72 yr

[a] Chlor, chloramphenicol; Ery, erythromycin; Pen, penicillin; SXT, trimethoprim-sulfamethoxazole. R, resistant; I, intermediate; S, susceptible.
[b] Ages were available for 19 of 21 isolates.
[c] CSF, pleural fluid, lymph node, and ascitic fluid isolates.
[d] Endotracheal aspirate, sputum, pharyngeal, and nasal isolates.

drug-resistant isolates. Serotype 23F was the most common serotype among all of the clinical isolates. The MICs for four of the five 23F isolates were >0.12 μg/ml (range, 0.23 to 1.0 μg/ml). Serotypes 6A and 23F were the only two types common to both sterile and nonsterile site isolates.

Among the clinical isolates, we found that penicillin-resistant strains were unevenly distributed among subjects <5 years of age (50%) and >5 years of age (38%). The serotype distributions differed slightly, with both populations sharing only 4 of 11 types: 6A, 9A, 19F, and 23F.

The serotypes that were common to both clinical and nasopharyngeal groups of isolates were 6A, 14, 19F, and 23F.

DISCUSSION

There have been few studies and little data available regarding the characterization of *S. pneumoniae* in Peru. To our knowledge, this is the first study to identify antimicrobial resistance rates as well as serotypes of both clinical and community isolates. A study of sterile site infections in children in Lima from 1983 to 1993 revealed that *S. pneumoniae* was the most common organism isolated from pleural fluid, and from 1981 to 1993, it was the 2nd most common etiologic agent of bacterial meningitis, indicating that this organism is a substantial contributor to childhood invasive disease (E. N. Janoff, G. Castellares, R. L. Zerpa, J. Moran, J. Moody, C. W. Gray, and D. N. Taylor, Abstr. 35 Intersci. Conf. Antimicrob. Agents Chemother., abstr. K6, p. 288, 1995). Other studies (published within Peru) have indicated rates of reduced susceptibility to penicillin of 3.3% in 1993 to 1994 and 5.3% in 1996 to 1997 (13, 16). From the data of these studies and our findings of 42.9% and 15.1% reduced susceptibility to penicillin in clinical and community nasopharyngeal isolates, respectively, we conclude that resistance rates not only have increased, but are now among some of the highest in the South American region. None of our isolates demonstrated a high level of resistance to penicillin. The highest MIC of penicillin found in both groups of isolates was 1.5 μg/ml. This finding is consistent with surveillance done in previous years in Peru. In neither of the studies by Shirazawa et al. (16) nor Ochoa (13) was the penicillin MIC for isolates ≥2 μg/ml. Because all studies thus far have been focused on Lima and because there is no national reporting mechanism or surveillance system for *S. pneumoniae* resistance in place in Peru, it is impossible to determine if a high level of resistance may be present in other areas of the country. Increasing rates of intermediate resistance to penicillin indicate a trend toward the development of high resistance and are of particular concern when considering increasing rates of resistance to other antimicrobial agents.

In Pampas de San Juan de Miraflores, living conditions are crowded and respiratory and other health problems are common. In the year 2000, there were 24 reported cases of pneumonia and 107 reported cases of otitis media in children 0 to 4years old in the community (A. Gaffo, personal communication). Unfortunately, there are no data available to indicate the actual number of cases or the proportion of these infections or other infections that were due to *S. pneumoniae*. The recently developed 7-valent pneumococcal polysaccharide-protein conjugate vaccine for infants (Prevnar Wyeth Lederle Vaccines) contains serotypes 6B, 9V, 14, 19F, 23F, 4, and 18C. It would provide coverage for half of the penicillin-resistant and multidrug-resistant nasopharyngeal isolates and half of all clinical isolates obtained from children 5 years and younger. The 23-valent capsular polysaccharide vaccines recommended for use in adults and children older than 5 years would provide coverage for approximately two-thirds of the clinical isolates for those age groups (3). This does not take into account serogroup- or vaccine-related type cross-reacting immunity.

It is interesting to note some of the differences between serotype distributions in Peru and other South American countries. While serotype 23F predominated in our isolates, 14F was the predominant serotype in isolates from Argentina, Brazil, Chile, Colombia, and Uruguay (17). Serogroups 6, 9, and 19 were also frequently isolated in our samples, but except for serogroup 6, ranked much lower in the other countries. Serotypes 1 and 7 ranked among the top 10 isolates of the other five countries, but were not present in any of our isolates.

Capsular type 23F is a consistent commonality among the most pertinent associations made with our data. It is the one serotype implicated in invasive and noninvasive infections as

well as penicillin and multiple-antimicrobial resistance. This serotype has emerged as one of the leading causes of inter- and intracontinental spread of penicillin resistance (10, 17). Of particular importance is the 23F "Spanish" clone, which is resistant to penicillin as well as other classes of antimicrobial agents. A comprehensive study revealed that these clones are now present throughout South America (17). Closely related clones have been found in Peru's neighbor, Chile (6). Three of the five 23F strains among our clinical isolates and two of our nasopharyngeal isolates had antimicrobial patterns corresponding to that of the Spanish clone. Molecular typing was unavailable, but similar susceptibility patterns may indicate the spread of resistant clones into Peru.

Conclusions. The extent of pneumococcal carriage and disease, serotypes, and antimicrobial susceptibility patterns are poorly characterized in Peru. The lack of a childhood vaccine coupled with high rates of antimicrobial resistance in neighboring countries has the potential to seriously exacerbate rates of childhood mortality associated with *S. pneumoniae*. As has been indicated in many other studies, antimicrobial resistance and serotype distribution can differ from city to city and region to region within a country (1, 9, 15). A comprehensive, large-scale surveillance of both clinical and community isolates is necessary to identify serotypes and the extent of drug-resistant strains of *S. pneumoniae* in Peru to allow treatment and prevention strategies to be established before options become severely limited.

ACKNOWLEDGMENTS

This study was partially funded by USAID through the Johns Hopkins Family Health and Child Survival Cooperative Agreement.

We thank Richard B. Thomson, Jr., for microbiology and laboratory advice and the *Streptococcus* Laboratory at the CDC for assistance with serotyping of the *S. pneumoniae* isolates. We also thank J. B. Phu and D. Sara for assistance.

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