Distribution of Capsular Types and Antibiotic Susceptibility of Invasive *Streptococcus pneumoniae* Isolated from Aborigines in Central Australia

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Streptococcus pneumoniae strains isolated from 203 episodes of invasive disease in central Australian Aborigines were studied. Capsular types from children aged 0 to 4 years ($n = 89$) belonged most commonly to types 14, 6B, 9V, 4, 18C, and 19F, which together accounted for 67% of the pediatric strains. In adults ($n = 98$), types 1, 7F, 3, 4, 12F, and 8 contributed 68% of the isolates. Of 114 pneumococci from patients 5 years and older, 102 (89.5%) were types represented in the 23-valent pneumococcal polysaccharide vaccine. The MICs of five antibiotics were determined for 201 strains by using the E-Test (AB Biodisk). No chloramphenicol or ceftriaxone resistance was found, but 46 strains (22.9%) showed diminished susceptibility to one or more of the drugs penicillin, erythromycin, and trimethoprim-sulfamethoxazole. Penicillin resistance occurred in 15.4% of all isolates tested but only within the intermediate range (0.1 to 1.0 µg/ml). Resistance to trimethoprim-sulfamethoxazole affected 13.9% of the pneumococci tested. All type 23F and most type 19F organisms were resistant to one or more antibiotics. Resistance was significantly more common in pediatric isolates than in those from adults ($\chi^2_1 = 14.1; P < 0.001$).

Despite antibiotic usage, serious pneumococcal infections such as pneumonia, bacteremia, meningitis, and otitis media remain major causes of morbidity and mortality in persons of all ages worldwide. While attack rates for pneumococcal disease are extremely high in developing countries (3), indigenous and disadvantaged minorities in developed countries are also at particular risk (7), as are splenectomized children (21). Since the first report of decreased susceptibility to penicillin in 1967 (13), resistance of *Streptococcus pneumoniae* to this drug, as well as other antibiotics, has been reported in many countries (1). Multiple drug resistance has been identified (15) and is increasing (1). Therefore, a regional knowledge of the distribution of capsular types and antibiotic susceptibility of pneumococci is essential for the development of effective vaccine strategies and treatment protocols.

In central Australian Aboriginal communities, incidence rates for invasive pneumococcal disease of 178 episodes per 100,000 adults aged 20 to 59 years and 2,053 episodes per 100,000 children less than 2 years old have been recently reported (30). This paper describes the type distribution and antibiotic susceptibility of pneumococci isolated from Aboriginal people hospitalized with invasive disease in central Australia from December 1989 to October 1994.

MATERIALS AND METHODS

Study area. The region described as central Australia has an area of 1.2 million km², occupies about two-thirds of the Northern Territory, and includes adjacent parts of Western Australia and South Australia. Much of the region is semiarid with low and unreliable rainfall. The Aboriginal population of about 18,000 live mostly in isolated communities of 10 to several hundred people. Housing is of poor standard and varies from shelters constructed of tin and bush materials to more conventional structures which are overcrowded and poorly maintained. Small clinics staffed by community nurses and indigenous health workers serve most of the larger settlements. Emergency cases in remote communities are evacuated by air to either of two hospitals in the region which have diagnostic laboratory facilities.

Bacterial isolates. A total of 203 invasive *S. pneumoniae* strains cultured from Aborigines admitted to Alice Springs Hospital and Katherine District Hospital from December 1989 to October 1994 were studied. Most strains were consecutive isolates cultured and presumptively identified at each of the two hospitals and referred immediately to one of us (M.G.) for capsular typing. Strains were stored in skim milk-glycerol broth at $-75°C$ while awaiting antimicrobial susceptibility testing. The clinical sites of isolation included blood and/or cerebrospinal fluid ($n = 199$), blood and aspirate ($n = 1$), aspirate ($n = 2$), and a swab of brain tissue taken postmortem ($n = 1$). The patient population comprised children aged 0 to 4 years ($n = 89$) and 5 to 14 years ($n = 16$) and adults ($n = 98$). Two children had three episodes, and five adults had two episodes. A number of episodes of invasive disease in the current investigation have been included in a clinical study reported elsewhere (31).

Identification. Pneumococci were identified by Gram stain, colonial morphology on blood agar, and optochin susceptibility and were typed by the quellung reaction with antisera raised at Statens Seruminstitut as described previously (17).

Antimicrobial susceptibility testing. The E-Test (AB Biodisk, Solna, Sweden) was used to determine the MICs for 201 isolates of benzylpenicillin (gradient range of 0.002 to 32 µg/ml), ceftriaxone (gradient range of 0.002 to 32 µg/ml), chloramphenicol (gradient range of 0.016 to 256 µg/ml), erythromycin (gradient range of 0.016 to 256 µg/ml), and trimethoprim-sulfamethoxazole (TMP-SXT) (gradient range of 0.002-0.038 [where the first value is that for trimethoprim and the second value is that for sulfamethoxazole] to 32-608 µg/ml). The manufacturer's instructions were followed. Briefly, predried Mueller-Hinton agar plates (Oxoid Pty Ltd) supplemented with 5% defibrinated horse blood were inoculated with test suspensions prepared in Mueller-Hinton broth (Oxoid Pty Ltd) and adjusted to the density of a 0.5 McFarland standard. E-Test strips were applied, and the plates were incubated at 36°C for 20 h in 5% carbon dioxide. MICs were read at the point of intersection of the growth ellipse with the scale on the strip. When required, the results were rounded up to the next highest dilution value. *S. pneumoniae* ATCC 49619 was used as a control for each batch of test isolates. MICs were interpreted according to the guidelines of the National Committee for Clinical Laboratory Standards (18).

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TABLE 1. Distribution of invasive *S. pneumoniae* capsular types from Aborigines in central Australia and relationship to types in the 23-valent pneumococcal polysaccharide vaccine

Type	No. of isolates among subjects aged:				Total no. (%) of isolates
	0–23 mo	24–59 mo	60–179 mo	≥15 yr	
Vaccine types					
1	3	1	2	17	23 (11.3)
3				13	13 (6.4)
4	5	2		8	15 (7.4)
6B	6	4	1	1	12 (5.9)
7F	3		2	14	19 (9.4)
8	2			7	9 (4.4)
9N	1	1	1	2	5 (2.5)
9V	8	1	1	2	12 (5.9)
10A			1	2	3 (1.5)
12F	3		7	8	18 (8.9)
14	9	2		2	13 (6.4)
18C	5	2	1	1	9 (4.4)
19F	5	1			6 (2.9)
23F	9	1		1	11 (5.4)
33F	3	1		4	8 (3.9)
Other[a]	1			4	5 (2.5)
Total	63	16	16	86	181 (89.2)
Vaccine-related types					
6A	3	1			4 (2.0)
7C				2	2 (1.0)
18A	1			4	5 (2.5)
22A	1			1	2 (1.0)
23B	1			1	2 (1.0)
Other[b]	1	1		1	3 (1.5)
Total	7	2		9	18 (9.0)
Non-vaccine-related types[c]	1			3	4 (2.0)

[a] One isolate each of types 17F (subjects 0 to 23 months) and 11A, 15B, 19A, and 20 (subjects ≥15 years).

[b] One isolate each of types 15C (subjects 0 to 23 months), 10B (subjects 24 to 59 months), and 15A (subjects ≥15 years).

[c] One isolate each of types 16 (subjects 0 to 23 months) and 13, 34, and 45 (subjects ≥15 years).

RESULTS

The type distribution of 203 pneumococci is given in Table 1. Thirty-two capsular types were identified, including 22 from children and 26 from adults. Among children under 5 years old, the most common types, in order of increasing frequency, were 14, 6B, 23F, 9V, 4, 18C, and 19F, which together comprised 67% of the pediatric strains. In adults, types 1, 7F, 3, 4, 12F, and 8 accounted for 77.9% of the isolates. Of 114 pneumococci from subjects 5 years and older, 102 (89.5%) were represented in the 23-valent pneumococcal polysaccharide vaccine, while an additional 9 isolates (8%) were vaccine related.

The MICs for 201 pneumococci were determined. The resistance patterns and relation to type for children aged to 0 to 14 years (0 to 179 months) and for adults are detailed in Table 2. No chloramphenicol or ceftriaxone resistance was found. Forty-six strains (22.9%) were resistant to one or more of the drugs penicillin, erythromycin, and TMP-SXT. Penicillin resistance was present in 15.4% of all strains but only in the intermediate range (0.1 to 1.0 μg/ml). Resistance to TMP-SXT occurred at both the resistant (≥4-76 μg/ml) and intermediate (1-19 to 2-38 μg/ml) levels and affected 13.9% of all pneumococci tested. Strains showing complete resistance belonged to types 14 (four strains) and 15A, 18C, and 34 (one strain each). All type 23F organisms and six of seven type 19F organisms were resistant to one or more antibiotics.

During the first 3 years of the study (1990 to 1992), resistance to penicillin, TMP-SXT, and erythromycin was present in 6.8, 7.8, and 1.9% of 103 isolates, respectively. Of 98 pneumococci cultured in 1993 and 1994, resistance to penicillin and TMP-SXT increased to 25.5 and 20.4% of strains, respectively, while susceptibility to erythromycin remained constant. During the two periods, the combined penicillin and TMP-SXT resistance rose from 2.9% in 1990 to 1992 to 14.3% in 1993 to 1994.

Resistance was significantly more common in isolates from subjects ≤14 years old than in those from adults ($\chi_1^2 = 14.1$; $P < 0.001$). A marked difference in type diversity between the two groups was also apparent. While 11 resistant adult strains included nine types, 33 of 35 resistant pediatric isolates belonged to six types (6B, 9V, 14, 18C, 19F, and 23F). Of 17 pneumococci resistant to more than one antibiotic, 16 were cultured from children.

DISCUSSION

This study is the first comprehensive bacteriological study of invasive pneumococcal disease in Australian Aborigines. The serotype distribution is similar to that in indigenous and other populations elsewhere (5, 19, 20). In subjects older than 5 years, 89.5% of the isolates are represented in the 23-valent pneumococcal polysaccharide vaccine. It has been shown that in children less than 5 years old, the immunogenicity of polysaccharide antigens varies widely. While those of types 1, 3, and 5 (29); 2, 5, and 7F (23); 3 (24); and 2, 3, 4, 7F, 8, and 9N (8, 22) are reported to produce good to excellent responses in

TABLE 2. Antibiotic resistance by capsular type in 201 invasive *S. pneumoniae* strains isolated from Aboriginal children (*n* = 104) and adults (*n* = 97) in central Australia, December 1989 to October 1994

Type (no. of isolates)	No. of isolates resistant to[a]:							Total no. of resistant isolates (%)
	PEN (MIC ≥ 0.1 µg/ml)		PEN (MIC ≥ 0.1 µg/ml) plus TMP-SXT (MIC ≥ 1-19 µg/ml)		TMP-SXT (MIC ≥ 1-19 µg/ml)		EM (MIC ≥ 4 µg/ml) (adults)	
	Children[b]	Adults	Children	Adults	Children	Adults		
6B (15)			6	1				7 (47)
8 (9)							2	2 (22)
9V (12)	4							4 (33)
14 (14)			3		1			4 (28)
18C (9)					3			3 (33)
19F (7)	6							6 (86)
23F (11)	1		7		2	1		11 (100)
23B (2)	1	1						2 (100)
33F (8)							2	2 (25)
Others[c] (5)	1					4		5
Total (% of total no. of isolates)	13 (6.5)	1 (0.5)	16 (8.0)	1 (0.5)	6 (3.0)	5 (2.5)	4 (2.0)	46

[a] PEN, penicillin; EM, erythromycin.
[b] Children 0 to 179 months old.
[c] One isolate each of types 15C (penicillin-resistant isolate in a child) and 9N, 11A, 15A, and 34 (TMP–SXT-resistant isolates in adults).

all age groups, those of types 6A, 6B, 14, 18C, 19F, and 23F are poorly immunogenic in children until the age of 4.5 years or more (2, 8). In our study, the poorly immunogenic types contributed 53.9% of the pneumococci isolated from children less than 5 years old and 52.1% of those from children less than 2 years old.

During the surveillance period, a type 10B pneumococcus was recovered from a child with bacteremia. Type 10B pneumococci have been identified only recently. The only other type 10B isolations have been from adults with invasive disease in Belgium and Denmark (two cases each) and Holland and Sri Lanka (one case each) (14).

The increasing resistance of pneumococci to penicillin and other antibiotics is a worldwide phenomenon (1). In Australia in 1989, 1.7% of 1,822 pneumococci tested in 15 participating centers were recorded as penicillin resistant (6). In 1994, susceptibility data for 467 invasive pneumococcal isolates from 30 Australian laboratories showed that 3.2, 10, 4.9, and 40.7% of strains had complete or intermediate resistance to penicillin, erythromycin, chloramphenicol, and TMP-SXT, respectively (32).

Although no isolates from central Australia were included in the earlier study (32), the current investigation has identified a marked increase in resistance to penicillin and TMP-SXT in pneumococci isolated during 1990 to 1992 (6.8 and 7.8%, respectively) and 1993 to 1994 (25.5 and 20.4%, respectively). Overall, resistant pneumococci were identified in 23% of 201 invasive isolates. Reduced susceptibility to penicillin occurred in 15.5% of the strains, 14% were resistant to TMP-SXT, and 8.5% were resistant to both penicillin and TMP-SXT. Resistance was most common in types 6B, 9V, 14, 18C, 19F, and 23F from children. Resistance to two antibiotics occurred in 37% of these strains and was confined to types 6B, 14, and 23F. Studies elsewhere have found resistance in similar types (4, 16, 27). Children are more frequently infected with pneumococci resistant to commonly used antibiotics than adults (4).

There is a close association between upper respiratory tract carriage of pneumococci and the acquisition of drug resistance (11). The upper respiratory tracts of 80% of young Aboriginal children hospitalized with acute lower respiratory infections in Alice Springs are colonized with *S. pneumoniae*. Among carriage-positive children, 92% were densely colonized and 20% harbored multiple pneumococcal populations. Types 19F, 23F, 9V, and 14 together contributed 47.5% of the carriage pneumococci (10). In young children with dense, persistent pneumococcal carriage, the emergence of resistant mutants is favored by the perfusion of sublethal concentrations of antibiotics to colonized mucosal surfaces. Horizontal transfer of altered penicillin-binding protein genes from a penicillin-resistant pneumococcus to penicillin-susceptible strains has been reported (9). Coexisting penicillin-susceptible and -resistant types, as components of multiple carriage populations, in children in Papua New Guinea have been reported (12).

Several pneumococcal protein conjugate vaccines are under development for use in young children (33). Polysaccharide antigens linked to protein carriers have been used successfully to protect children against *Haemophilus influenzae* type b disease (26). A pneumococcal conjugate vaccine containing polysaccharide type antigens 4, 6B, 9V, 14, 18C, 19F, and 23F for use in young children in industrialized countries has been described (28). As types 6A and 6B are cross-protective (25), this formulation would provide a level of coverage for 72% of children aged under 5 years in the current study.

Because of the increasing resistance of pneumococci to antibiotics worldwide, the need for an effective vaccine to decrease disease in young children is of paramount importance. A polysaccharide-protein conjugate vaccine which is under development (28) may provide protection against 72% of invasive pneumococci isolated from children under 5 years old in our study. In patients 5 years and over, 89.5% coverage would be given by the present 23-valent polysaccharide vaccine.

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