Original Report

Trends in Antimicrobial Resistance and Serotype Distribution of Blood and Cerebrospinal Fluid Isolates of *Streptococcus pneumoniae* in South Africa, 1991–1998

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ABSTRACT

Objective: Since 1979, the South African Institute for Medical Research (SAIMR) has served as the national reference center for pneumococcal serotyping and monitoring of antibiotic resistance trends. This study documents trends in antimicrobial resistance in pneumococci isolated from blood or cerebrospinal fluid (CSF) between 1991 and 1998 in South Africa.

Methods: Pneumococcal isolates (n = 7406) from either blood or CSF were sent to the SAIMR reference laboratory for serotyping. The isolates were evaluated for resistance to penicillin, chloramphenicol, tetracycline, erythromycin, clindamycin, and rifampicin.

Results: Resistance to one or more antibiotics increased significantly from 19% in 1991 to 1994 to 25% in 1995 to 1998 in all ages, and in children from 32% to 38% ($P < 10^{-6}$). Although penicillin resistance did not increase in children (28.1% vs. 28.9%), penicillin resistance in all ages increased from 9.6% to 18.0%. Significant increases in resistance to chloramphenicol, tetracycline, erythromycin, and rifampicin also were seen in both groups. Multiple resistance increased significantly, from 2.2% to 3.8%. The proportion of isolates with intermediate or high-level penicillin resistance remained constant during the surveillance period. Erythromycin resistance, predominantly expressed as simultaneous resistance to erythromycin and clindamycin, increased from 1.6% to 2.6%. The percentage of erythromycin-resistant isolates that were resistance to erythromycin alone increased from 10.6% to 28.7%, suggesting the emergence of *mef*E-mediated resistance. In children 2 years of age and younger, although serogroup 6 remained the most common, there were significant increases in serogroups 19, 18, and 13. The percentage of the total invasive pneumococcal disease in this population that is caused by serogroups found in the nonavalent pneumococcal conjugate vaccine (serogroups 1, 4, 5, 6B, 9V, 14, 18C, 19F, 23F) increased from 72% to 91%.

Conclusions: Antibiotic resistance in the pneumococcus is increasing in South Africa, although the proportion of strains with high-level penicillin resistance has not increased. New conjugate vaccines may not only decrease the burden of all pneumococcal disease but, in addition, lower the incidence of antibiotic-resistant disease in South Africa.

Key Words: *antibiotic resistance, pneumococci*

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Streptococcus pneumoniae remains the leading cause of bacterial pneumonia and a significant cause of meningitis, bacteremia, and otitis media in young children, the elderly, and persons with underlying medical conditions, such as renal and liver failure, chronic pulmonary disease, and diabetes mellitus.[1] Pneumococcal disease also is a major cause of morbidity and mortality in persons infected with human immunodeficiency virus (HIV).[2] Treatment of pneumococcal disease is often empirical, with penicillin as the antibiotic of choice; however, the global spread of antibiotic-resistant pneumococci threatens to undermine current treatment recommendations.

Documentation of penicillin resistance first appeared in 1967,[3] and there has been a rapid expansion of resistance in the pneumococcus in the past 5 years.[1] In South Africa, multiply resistant pneumococci were first recognized in 1977, prompting the establishment of a national survey for antimicrobial resistance. The first report of this survey covered the years 1979 to 1986,[4] and the update of surveillance included 1979 to 1990.[5] The South African Institute for Medical Research (SAIMR) also serves as a national reference center for pneumococcal serotyping and monitoring of antibiotic resistance trends. This report, a follow-up to earlier reports, documents trends in antimicrobial resistance in pneumococci occurring between 1991 and 1998 in South Africa.

MATERIALS AND METHODS

Streptococcus pneumoniae isolates were sent to SAIMR either on 5% blood agar (plates or slopes) or, more

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recently, on Dorset egg medium (slopes).[6] The isolates were subcultured onto 5% blood agar plates and then confirmed to be pneumococci by colony morphology and susceptibility to optochin. Antimicrobial susceptibility was determined on Mueller-Hinton media containing 5% sheep blood, using Mastrings (Mast Diagnostics, Merseyside, England) with disks containing antibiotic concentrations specified by the National Committee for Laboratory Standards (NCCLS).[7] Susceptibility was determined to oxacillin to predict resistance to penicillin (P), chloramphenicol (C), tetracycline (T), erythromycin (E), clindamycin (Cd), and rifampicin (R).[7] The minimum inhibitory concentrations (MICs) were determined using the microtitre method and cation-adjusted Mueller-Hinton broth supplemented with 2.5% lysed horse blood. The NCCLs breakpoints for intermediate resistance were (μg/mL): penicillin (0.12-1.0), tetracycline (4), erythromycin (0.5), clindamycin (0.5), and rifampicin (2). There is no intermediate resistance level to chloramphenicol.[7] The breakpoints for high-level resistance were (μg/mL): penicillin (≥2), chloramphenicol (≥8), tetracycline (≥8), erythromycin (≥1), clindamycin (≥1), and rifampicin (≥4). Capsular typing was done by the Aquellung technique, using antisera obtained from the Statens Seruminstitut (Copenhagen).

Patient ages were not available for all of the specimens. In some instances, an age of less than 13 years was inferred by a hospital number indicating the patient had been admitted to a pediatric ward or if the hospital submitting the specimen admitted only children.

The data were entered into and analyzed using the Epi Info computer software package.[8]

RESULTS

Between 1991 and 1998, 7406 pneumococci isolated from either blood or CSF were sent to the SAIMR laboratory. The laboratory confirmed antibiotic susceptibility or resistance for 3712 blood or CSF isolates between 1991 and 1994 and for 3694 isolates from 1995 to 1998.

Antibiotic resistance increased significantly over the surveillance period, from 18.7% in 1991 to 1994 to 25% in 1995 to 1998 in all ages, and in children from 32.0% to 38.4% (Table 1). For all ages and for children, significant increases occurred in resistance to chloramphenicol, tetracycline, erythromycin, and rifampicin. In all ages penicillin resistance increased from 9.6% to 18.1%.

The patterns of antibiotic resistance are found in Table 2. Single resistance to penicillin rose from 10.4% to 12.7% of isolates and resistance to erythromycin alone, which was not seen prior to 1995, increased to 0.1%. Larger increases occurred in simultaneous resistance to two or more antibiotics (see Table 2). Combinations of P + C + T- and PTECd-resistance were most common. Concurrent resistance to all six antimicrobials decreased significantly from 1994. Minimum inhibitory concentrations were determined for 638 resistant pneumococci isolated from 1991 to 1994 and for 855 resistant isolates received from 1995 to 1998. In the 8-year sampling period, the proportion of both intermediate and high-level resistance to all of the antibiotics remained constant. High-level

Table 1. Antibiotic Resistance of Pneumococci Isolated from Blood and CSF from South Africa, 1991–1998

	Number of Isolates (%)			
	1991–1994		*1995–1998*	
Antibiotic	*Children (n = 765)*	*All Ages* (n = 3712)*	*Children (n = 1052)*	*All Ages* (n = 3694)*
Penicillin	215 (28.1)	356 (9.6)	304 (28.9)	667 (18.1)†
Chloramphenicol	18 (2.4)	44 (1.2)	53 (5.0)†	90 (2.4)†
Tetracycline	42 (5.5)	219 (5.9)	130 (12.4)†	345 (9.3)†
Erythromycin	14 (1.8)	58 (1.6)	44 (4.2)†	98 (2.6)†
Clindamycin	12 (1.6)	51 (1.4)	27 (2.6)	57 (1.5)
Rifampin	4 (0.5)	10 (0.3)	18 (1.7)†	31 (0.8)†
Any antibiotic	244 (32.0)	694 (18.7)	404 (38.4)†	924 (25.0)†

*All ages includes those known to be children; †significant difference between 1991–1994 and 1995–1998 ($P \leq 0.05$).

Table 2. Antibiotic Resistance Patterns of Pneumococcal Isolates from Blood or CSF in South Africa, 1991–1998

	Number of Isolates (%)*	
Antibiotic	*1991–1994 (n = 3712)*	*1995–1998 (n = 3694)*
Penicillin (P)	387 (10.40)	468 (12.70)†
Tetracyclin (T)	135 (3.60)	173 (4.70)†
Erythromycin (E)	–	3 (0.10)
Rifampin (R)	7 (0.20)	19 (0.50)†
P + T	19 (0.50)	31 (0.80)
P + R	16 (0.40)	13 (0.35)
P + C	2 (0.05)	4 (0.11)
P + E	2 (0.05)	7 (0.20)
P + clindamycin (Cd)	–	2 (0.05)
T + E	3 (0.10)	13 (0.35)†
T + R	–	3 (0.10)
C + T	–	9 (0.20)†
E + Cd	1 (0.03)	3 (0.10)
P + C + T	28 (0.75)	60 (1.60)†
P + E + Cd	5 (0.13)	11 (0.30)
P + C + R	–	1 (0.03)
P + T + E	4 (0.11)	5 (0.14)
P + T + R	2 (0.05)	3 (0.10)
T + E+ Cd	37 (1.00)	23 (0.60)
P + C + T + R	12 (0.32)	16 (0.40)
P + T + E + Cd	8 (0.10)	34 (0.90)†
P + C + T + E	–	7 (0.20)†
C+ T+ E + Cd	3 (0.01)	1 (0.03)
T + E + Cd + R	1 (0.03)	1 (0.03)
P + C + T + E + Cd	4 (0.11)	12 (0.30)†
P + C + T + E + R	1 (0.03)	–
P + T + E + Cd + R	–	2 (0.05)
P + C + T + E + Cd + R	17 (0.46)†	–
Pan-susceptible	3018 (81.30)†	2770 (75.00)
Multiply resistant‡	80 (2.20)	140 (3.80)

*Percentages may not total 100% due to rounding; †significant difference between 1991–1994 and 1995–1998 ($P \leq 0.05$); ‡resistant to three different classes of antibiotics.

resistance to penicillin was found in 8% and 10% of the isolates from 1991 to 1994 and 1995 to 1998, respectively. In contrast, more than 94% of the resistance to the other antibiotics was high-level resistance. A sample of 56 penicillin resistant isolates from 1991 to 1994 and 46 isolates from 1995 to 1998 also were tested for resistance to ceftriaxone; intermediate resistance was found in one isolate from each time interval (1.8% and 2.2%, respectively).

The distribution of serogroups from children 2 years of age and younger is found in Table 3. Serogroup 6 remained the most common, followed by group 19, which increased significantly since 1991, and then groups 1 and 14. Significant changes in prevalence also were seen for serogroups 18 and 13. The percentage of the total invasive pneumococcal disease in this population that is caused by serogroups found in the pneumococcal conjugate vaccine containing serogroups or types 1, 4, 5, 6B, 9V, 14, 18C, 19F, 23F increased from 72% to 91%.

DISCUSSION

The trend in antibiotic resistance from 1982 to 1998 is shown in Figure 1. Previous surveys of pneumococcal antibiotic resistance in South Africa documented increases in resistance to any antibiotic from 6% in 1979 to 1982 to 10% in the period 1983 to 1986.[5] Resistance to penicillin increased from 4.9% to 14.4% between 1979 and 1990.[6] Multiply resistant strains, resistant to three or more classes of antibiotics, accounted for 1.6% of the total number of isolates tested between 1979 and 1986.[5] In comparison, the present study found one-quarter of the isolates sent to the SAIMR between 1995 and 1998 to be resistant to at least one antimicrobial, and it found penicillin resistance in almost 20% of the isolates. The proportion of both intermediate and high-level resistance to penicillin did not change significantly, and high-level resistance remains less than 10% of the total penicillin resistance. Over 95% of the resistance to tetracycline is high level, whereas resistance to rifampicin, chloramphenicol, erythromycin, and clindamycin remains below 5%. The current study also found a rise in the number of multiply resistant pneumococci from 2% to 4%, although since 1995 there have been no isolates sent to the SAIMR that are resistant to all six antibiotics tested. Surveillance for resistance to the fluoroquinolones was begun in 1999.

Antibiotic resistance in children is usually higher than in the group of all ages with pneumococcal disease because the common serogroups causing disease in children (serogroups 6, 14, 19, and 23) are more likely to be antibiotic-resistant. In this study, resistance to any antibiotic in children increased significantly from 32% to 38.4%; penicillin resistance remained constant at approximately 29%. In contrast, a study conducted in 1998 in the private sector in northern Johannesburg found overall resistance rates of 69% and 45.4% of the isolates resistant to penicillin.[9] The discrepancy between antibiotic resistance in the public and private sector is likely a reflection of the availability and use of antibiotics in children attending private practitioners. The serogroup distribution in young children remained fairly stable over the 7 years of the study, although there was a statistically significant increase in isolation of serogroup 19, again a serogroup commonly associated with antibiotic resistance.

A limitation to this study was the lack of patient ages for all of the isolates, which would have allowed separate analyses of antibiotic resistance in adults and in children.

Table 3. Serotypes or Groups of Pneumococci Isolated from Blood or CSF of South African Children 2 Years of Age and Younger, 1991–1998

	1991–1994		*1995–1998*	
Serogroup or serotype	*Number (%) of Isolates (n = 506)*	*Rank*	*Number (%) of Isolates (n = 257)*	*Rank*
1	57 (11.3)	3	34 (13.2)*	3
2	4 (0.8)	13	–	–
3	–	–	2 (0.8)†	13
4	13 (2.6)	10	9 (3.5)*	6
5	14 (2.8)	7	7 (2.7)*	7
6	184 (36.4)	1	86 (33.5)*	1
7	19 (3.7)	5	5 (1.9)	9
8	7 (1.4)	12	5 (1.9)	9
9	10 (2.0)	1	7 (2.7)*	7
10	4 (0.8)	13	3 (1.2)	12
11	1 (0.2)	16	1 (0.4)	15
12	3 (0.6)	15	–	–
13	–	–	4 (1.5)†	11
14	85 (16.8)	2	31 (12.1)*	4
15	14 (2.8)	7	2 (0.8)	13
16	1 (0.2)	16	–	–
18	17 (3.3)	6	1 (0.4)*†	15
19	56 (11.1)	4	47 (18.3)*†	2
20	1 (0.2)	16	–	–
23	14 (2.8)	7	12 (4.7)*	5
28	1 (0.2)	16	–	–
29	1 (0.2)	16	–	–
34	–	–	1 (0.4)	–

*Serotypes found in the nonavalent conjugate vaccine; †significant difference between 1991–1994 and 1995–1998 ($P \leq 0.05$).

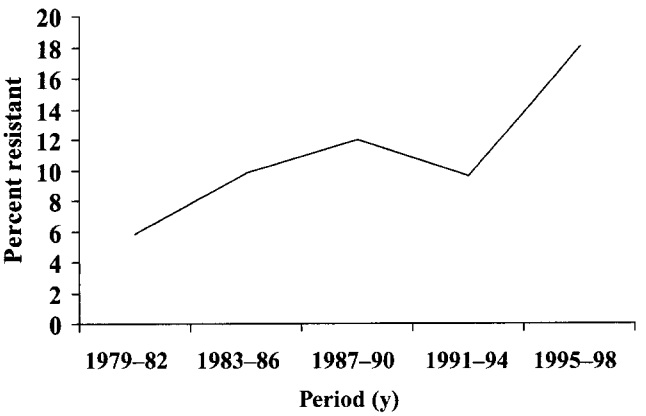

Figure 1 Trend in antibiotic resistance from 1982 to 1998 in South Africa.

Specimens are often submitted to hospital laboratories without complete patient demographic information; therefore, no ages were available when the resulting isolates were referred to the SAIMR.

Infection with HIV is increasing in South Africa, and as many as 23% of adults and 36% of children admitted to Chris Hani Baragwanath Hospital in Soweto are infected with the virus.[10] A retrospective study of pneumococci isolated from blood between 1993 and 1995 found that HIV-infected patients were more likely to have penicillin-resistant organisms than their HIV-uninfected counterparts (29.7% vs. 8.6%).[10] Serotype 14 and serogroup 6, pneumococcal strains more commonly found in children and frequently associated with antibiotic resistance, also were significantly increased in HIV-infected adults. The changing pattern of pneumococcal disease resulting from HIV infection also could mean an increase in antibiotic resistance in South Africa. Surprisingly, although tuberculosis also is increasing in South Africa, rifampicin resistance in the pneumococcus remains low.

This study describes one country experiencing increases in antibiotic resistance in the pneumococcus. In 1992 in an overview of pneumococcal resistance, Appelbaum described surveys of pneumococcal resistance from 25 countries on six continents.[11] Penicillin-resistant organisms were isolated in every survey, and resistance rates of 10% or less were found in 13 countries. Resistant pneumococci were prevalent in Europe, particularly Spain, France, and Hungary, as well as in Israel and the United States. In the United States, 33.5% of 9190 isolates collected over a 6-month period in 1996 to 1997 were resistant to penicillin.[12] In Spain, trends in penicillin resistance are similar to those in South Africa. One study from Spain examining pneumococci isolated from patients between 1979 and 1989 found an overall resistance rate of 65.5% and an increase in penicillin resistance from 6% at the start of the survey to 44% in 1989.[13] Pneumococcal penicillin resistance can be spread either through dissemination of resistant clones or by the transfer of resistance genes, those coding for penicillin-binding proteins (PBPs), between different pneumococci. Examination of the DNA sequences of PBPs from 27 pneumococcal isolates from South Africa identified three clones circulating within the country.[14] Two of the clones of serotypes 19A and 6B were penicillin-resistant and the third was a serotype 19A that was resistant to penicillin, chloramphenicol, tetracycline, erythromycin, clindamycin, and rifampicin. These clones were isolated from geographically distant areas of South Africa. Analysis of 328 isolates in the United States suggests that the explosion of penicillin resistance to almost 40% in that country is at least partially attributable to the importation and clonal expansion of two highly resistant international clones, the "Spanish" 23F clone and the "French" serogroup 9 or 14 clone.[15] Interestingly, a multiply resistant serotype 23F pneumococcus originating in Spain was isolated in 1991 from the throat of a 3-year old South African child with otitis media.[16] Epidemiologic investigations found that the strain likely was imported into South Africa by the child's aunt who had lived in Spain for a year. That particular strain also was isolated from the nasopharynx of six other persons, including the index child's mother and sibling, and two classmates and their siblings. Despite the early importation of that clone, it does not appear to have gained the stronghold it has gained in the United States. The background level of intermediate penicillin resistance in pneumococci in South Africa may be excluding the "foreign" clones from occupying the ecologic niche required for them to establish as the dominant resistant strains. It is, however, more likely that differences in antibiotic prescribing patterns may explain the lesser selective advantage of the multiresistant 23F clone in South Africa. Data from Sweden have shown that macrolides and broad-spectrum β-lactams are more effective selectors of penicillin-resistant pneumococci than are the oral penicillins.[17] The low level of macrolide selection in the public sector in South Africa may have been critical in the slow rate of emergence of this strain. In keeping with this explanation, erythromycin resistance remains low (< 5%). It was primarily expressed as simultaneous resistance to both erythromycin and clindamycin. Such a resistance pattern is associated with the *erm*AM gene, which reduces the affinity between the antibiotic and the ribosome.[18] The increasing percentage of erythromycin-resistant strains that do not show simultaneous resistance to clindamycin, however, suggests the emergence of the *mef*E gene in South Africa, a gene that affects the efflux of the antibiotic from the cell.[19] Most erythromycin resistance in the United States is attributable to the *mef* E gene.

What are the implications of the global emergence of antibiotic resistance in the pneumococcus? The use of penicillin for the empirical treatment of pneumonia may be unaffected by penicillin resistance up to a MIC of 4 g/mL. Several studies have shown that the mortality of children hospitalized with pneumococcal pneumonia and treated with β-lactam antibiotics does not differ depending on the resistance status of the organism.[20,21] The achievable blood and lung tissue levels of β-lactam antibiotics surpass the MICs of the pneumococci even at high levels of penicillin resistance. In contrast, antibiotic penetration into other tissues is less efficient, making penicillin resistance (intermediate or high-level) a major determinant in the choice of treatment for pneumococcal meningitis and, to a lesser extent, otitis media and sinusitis.[22,23] Data from the present study suggest that, in South Africa and possibly in many other African countries, access to the extended-spectrum cephalosporins, cefotaxime and ceftriaxone, is essential for the management of pneumococcal meningitis. Therefore, surveillance for antibiotic resistance is necessary, to ensure the successful management of pneumococcal meningitis, otitis media, and sinusitis. The antibiotic resistance patterns of *S. pneumoniae*

carried asymptomatically in the nasopharynx of children mirror the resistance rates of clinical isolates that cause invasive pneumococcal disease.[1,24,25] Carriage studies could serve as a useful alternative for monitoring antibiotic resistance in countries where isolation of clinical strains is not feasible.

All of the antibiotic-resistant pneumococcal isolates in this study were of serogroups found in a nonavalent conjugate vaccine. Vaccination may reduce the problem of antibiotic resistance in the pneumococcus. This vaccine, comprised of serogroups or types 1, 4, 5, 6B, 9V, 14, 18C, 19F, and 23F, has been shown to significantly reduce carriage of vaccine-serotypes by 50% and carriage of penicillin-resistant serotypes by 49%.[26] The success of this vaccine could not only substantially decrease the burden of all pneumococcal disease but also lower the incidence of antibiotic-resistant disease in South Africa.

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