Clonally Related Penicillin-Nonsusceptible *Streptococcus pneumoniae* Serotype 14 from Cases of Meningitis in Salvador, Brazil

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Active hospital-based surveillance in the city of Salvador, Brazil, from December 1995 through October 1998, identified 221 patients with confirmed pneumococcal meningitis. Of these 221 patients, 29 (13%) had isolates with intermediate-level resistance to penicillin. Infection with these penicillin-nonsusceptible isolates was significantly associated with age of <2 years ($P < .0019$), previous antibiotic use ($P < .0006$), and coresistance to trimethoprim-sulfamethoxazole ($P < .0000$). Serotype 14 was the most prevalent serotype (55.2%) of penicillin-nonsusceptible isolates. Strain typing by repetitive element BOX polymerase chain reaction (PCR) analysis showed that penicillin-nonsusceptible serotype 14 isolates had closely related BOX PCR patterns, whereas penicillin-susceptible serotype 14 isolates each had distinct, unrelated patterns. Penicillin-nonsusceptible serotype 14 isolates from Salvador and other Brazilian cities had similar BOX PCR patterns. These observations indicate that in Brazil a large proportion of cases of penicillin-nonsusceptible pneumococcal meningitis appear to be caused by a closely related group of serotype 14 strains that may have disseminated to widely separate geographic areas.

Pneumococcal disease is a leading worldwide cause of mortality and morbidity in patients with community-acquired infections. Each year, it accounts for >1 million deaths in children aged <5 years [1]. A major advance against pneumococcal disease has been the availability of inexpensive penicillin-based antimicrobial therapy. Pneumococcal meningitis was associated with mortality rates of 80%–100% in the preantibiotic era [2, 3]. With the introduction of penicillin, these rates dropped to 30% [4]. However, in the last 30 years increasing penicillin resistance in *Streptococcus pneumoniae* has been reported in many regions of the world [5] and threatens the advances achieved during the postantibiotic era.

Penicillin-resistant *S. pneumoniae* disease has significant repercussions for developing countries, where factors associated with poverty contribute to elevated rates of life-threatening pneumococcal disease such as meningitis [6]. In Brazil and many other countries, penicillin and chloramphenicol are commonly used as empirical therapy for bacterial meningitis. Both agents are considered to be ineffective for the treatment of pneumococcal meningitis due to strains with intermediate- and high-level resistance to penicillin [7, 8]. Third-generation cephalosporins alone or in combination with other antimicrobial agents are recommended for the treatment of penicillin-resistant pneumococcal meningitis [9]. However, these regimens cost >10 times more than penicillin and chloramphenicol [10], which limits or prohibits the use of these regimens in most developing countries.

In Brazil, penicillin resistance in *S. pneumoniae* has emerged rapidly since the first such clinical isolate was reported in 1988 [11]. Retrospective surveys of strain collections [12, 13] and national reference laboratory surveillance [14, 15] have demonstrated that up to 20% of the clinical isolates tested had decreased susceptibility to penicillin. Despite these findings, several obstacles hamper the formulation of a response to this emerging problem. Local ongoing laboratory-based surveil-

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Informed consent was obtained from the patients or guardians, and the guidelines of the Brazilian Ministry of Health and the US Department of Health and Human Services were followed in the conduct of the clinical research.

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lance is absent in Brazil. The most recent population-based study on invasive pneumococcal disease was performed in the 1970s [16], and information has not been available to assess the impact of disease due to penicillin-nonsusceptible isolates.

In 1995, surveillance for penicillin-nonsusceptible *S. pneumoniae* was established at an infectious disease referral hospital that identifies >95% of the cases of pneumococcal meningitis in the city of Salvador in northeast Brazil (unpublished case notification records of the Secretary of Health for the State of Bahia [Secretaria da Saúde do Estado da Bahia]). Using population-based data, we unertook a case-control investigation to assess risk factors for the acquisition of penicillin-nonsusceptible pneumococcal disease.

Repetitive element BOX PCR analysis was used to further stratify patient isolates. BOX PCR is an inexpensive and rapid method for typing penicillin-nonsusceptible *S. pneumoniae* [17, 18]; this method has been previously used in investigations of pneumococcal outbreaks and carriage [19–21]. We found that a closely related group of serotype 14 strains was responsible for >50% of the cases of penicillin-nonsusceptible pneumococcal meningitis identified during surveillance in Salvador and that these isolates may have spread to other regions, thus contributing to the recent emergence of penicillin-resistant *S. pneumoniae* in Brazil.

Methods

Surveillance and antimicrobial susceptibility testing. Active hospital-based surveillance for penicillin-nonsusceptible pneumococcal meningitis was established in Salvador, a city of >2 million inhabitants in northeast Brazil [22]. As part of the state health department protocol for suspected cases of meningitis in the metropolitan region of Salvador, initial diagnostic evaluation, including lumbar puncture and CSF analysis, is performed at the emergency department of a single state infectious disease hospital (Hospital Couto Maia). More than 95% of the cases of pneumococcal meningitis in the region are reported from this hospital []. After the initial evaluation, patients are admitted to this hospital or transferred to another one. From 1 December 1995 to 31 October 1998, the surveillance team reviewed the daily clinical laboratory record at the infectious disease hospital to prospectively identify all patients for whom CSF cultures yielded *S. pneumoniae.*

Patient isolates were screened for reduced susceptibility to penicillin with use of a 1-μg oxacillin disk according to guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) [23]. For isolates that demonstrated reduced susceptibility (zone diameter of growth inhibition, <20 mm), the Etest [24, 25] was used to determine the MICs of penicillin and cefotaxime. Plates with Mueller-Hinton sheep blood (5%) agar were inoculated with a bacterial suspension in 0.9% saline that was equivalent to a 0.5 McFarland turbidity standard. After application of Etest strips (AB BIODISK, Solna, Sweden), the plates were incubated at 35°C in 5% CO_2 for 24 h. The MIC was identified at the point where the growth margin intersected the Etest strip. Etest MICs that fell between 2 standard $\log_2$ dilution concentrations were determined to be equivalent to the higher concentration [24].

S. pneumoniae ATCC 49619 was used as a quality control strain in all disk diffusion and Etest assays. NCCLS interpretative criteria for MICs [26] were used to define susceptibility categories. A case of penicillin-nonsusceptible pneumococcal meningitis was defined as recovery of an isolate with intermediate-level (MIC, 0.12–1 μg/mL) or high-level (MIC, >1 μg/mL) resistance to penicillin.

The disk diffusion test was performed according to NCCLS guidelines [23] to determine patients' susceptibilities to trimethoprim-sulfamethoxazole (TMP-SMZ), tetracycline, erythromycin, clindamycin, chloramphenicol, rifampin, and vancomycin. NCCLS breakpoint values were used to define susceptibility and resistance to antimicrobial agents. A multidrug-nonsusceptible isolate was defined as being intermediately or highly resistant to $\geqslant$2 of 6 major classes of antimicrobial agents: β-lactam agents, TMP-SMZ, tetracyclines, macrolides, chloramphenicol, or rifampin.

Clinical and epidemiological data collection. For all identified patients with pneumococcal meningitis who were admitted to the surveillance hospital, a standardized data entry form was used to extract demographic and clinical information from the medical records. Immediately after their cases were identified, patients were interviewed to obtain information on potential risk factors for acquiring penicillin-nonsusceptible pneumococci, such as recent hospitalizations and outpatient antibiotic use. Population estimates used to calculate incidence were obtained from the 1991 census report of the Brazilian Institute for Geography and Statistics [22].

Serotyping and BOX PCR strain typing. The quellung reaction was used to determine the capsular serotypes of the pneumococcal isolates with use of antisera obtained from Statens Seruminstitut (Copenhagen, Denmark). For the purpose of BOX PCR strain typing of the isolates, colonies from overnight cultures on tryptic soy agare with sheep blood (5%) were harvested, washed with PBS (pH 7.4), and reconstituted to make a suspension equivalent to a 1.0 McFarland turbidity standard in 50 mM potassium chloride, 10 mM Tris-HCl, 1.5 mM magnesium chloride, 0.01% gelatin, 0.01% polysorbate 20, and 0.5 mg of proteinase K/mL (Boehringer Mannheim, Mannheim, Germany), pH 8.3. The suspension was incubated at 55°C for 20 min, boiled for 15 min, and centrifuged. A 50-μL PCR reaction mixture was prepared; the reaction mixture contained 5 μL of the supernatant, 1 μM BOX A primer [18], 200 μM each dNTP (Pharmacia, Piscataway, NJ), 10% dimethyl sulfoxide (Sigma, St. Louis), and 2 U of Taq polymerase (GIBCO Laboratories, Gaithersburg, MD) in its appropriate buffer. The mixture was heated to 94°C for 7 min followed by 35 cycles each consisting of denaturation at 94°C for 1 min, annealing at 53°C for 1 min, and extension at 65°C for 8 min. A final extension step was performed at 65°C for 16 min. Amplified products were subjected to 1.5% agarose gel electrophoresis in standard Tris acetate–EDTA buffer at 80 V for 3 h.

Ethidium bromide–stained gels were photographed under ultraviolet transillumination, and DNA banding patterns were analyzed visually. The number of bands with identical and nonidentical electrophoretic mobilities was compared. For strains with patterns containing >7 bands, those that differed by $\leqslant$3 bands were defined as being closely related and assigned the same letter code. Within a group of related patterns, distinct, nonidentical patterns were assigned a numerical index. A pattern was defined to be identical if

BOX PCR analysis of ⩾2 isolates produced bands with identical electrophoretic mobilities.

Statistical analyses. A clinical and epidemiological database was created and analyzed with use of Epi-Info Version 6.04 (Centers for Disease Control and Prevention, Atlanta, GA). Fisher's exact test or χ^2 test was used to compare differences between proportions for dichotomous variables, and ORs and their 95% CIs were calculated. The two-sample Wilcoxon rank-sum test was used to compare differences in means for continuous variables. All *P* values were based on two-sided tests; $P < .05$ was considered statistically significant.

Results

Surveillance for penicillin-nonsusceptible meningitis. A total of 230 patients with meningitis and CSF cultures positive for *S. pneumoniae* were consecutively identified at the surveillance hospital from 1 December 1995 to 31 October 1998 (figure 1). Increased numbers of monthly cases were identified during the winters, from May through September. Of 221 patients for whom demographic information was obtained, 103 (47%) were children aged <5 years and 80 (36%) aged <1 year, and 146 (66%) were males. All patients were residents of the metropolitan region that includes the city of Salvador. On the basis of the 102 cases that occurred in residents of the municipal boundaries of Salvador, the annual incidence of pneumococcal meningitis was estimated to be 1.7 cases per 100,000 population for all age groups, 8 cases per 100,000 population for children aged <5 years, and 31.7 cases per 100,000 population for infants aged <1 year.

In 221 (96%) of the 230 cases, penicillin susceptibility testing demonstrated that 29 isolates (13%) had intermediate-level resistance (table 1), with an MIC range of 0.125–1 μg/mL. In 9 (4%) of the 230 cases, isolates were not tested because they failed to grow after primary culture or were contaminated. Isolates with high-level resistance were not identified. All isolates with intermediate-level resistance to penicillin were susceptible to cefotaxime: the range of MICs for these isolates was 0.064–0.380 μg/mL. On the basis of 17 cases that occurred in patients who resided in Salvador and had isolates with intermediate-level resistance, the annual incidence for penicillin-nonsusceptible meningitis was estimated to be 0.3 case per 100,000 population for all age groups, 2 cases per 100,000 population for children aged <5 years, and 7.9 cases per 100,000 population for infants aged <1 year.

Of the 29 penicillin-nonsusceptible isolates, 22 (76%) were also nonsusceptible to TMP-SMZ, 4 (14%) were also nonsusceptible to tetracycline, and 2 (7%) were nonsusceptible to all 3 antimicrobial agents. One isolate (3%) was nonsusceptible to rifampin, and all isolates were susceptible to erythromycin, clindamycin, and chloramphenicol. Of the 192 penicillin-suscep-

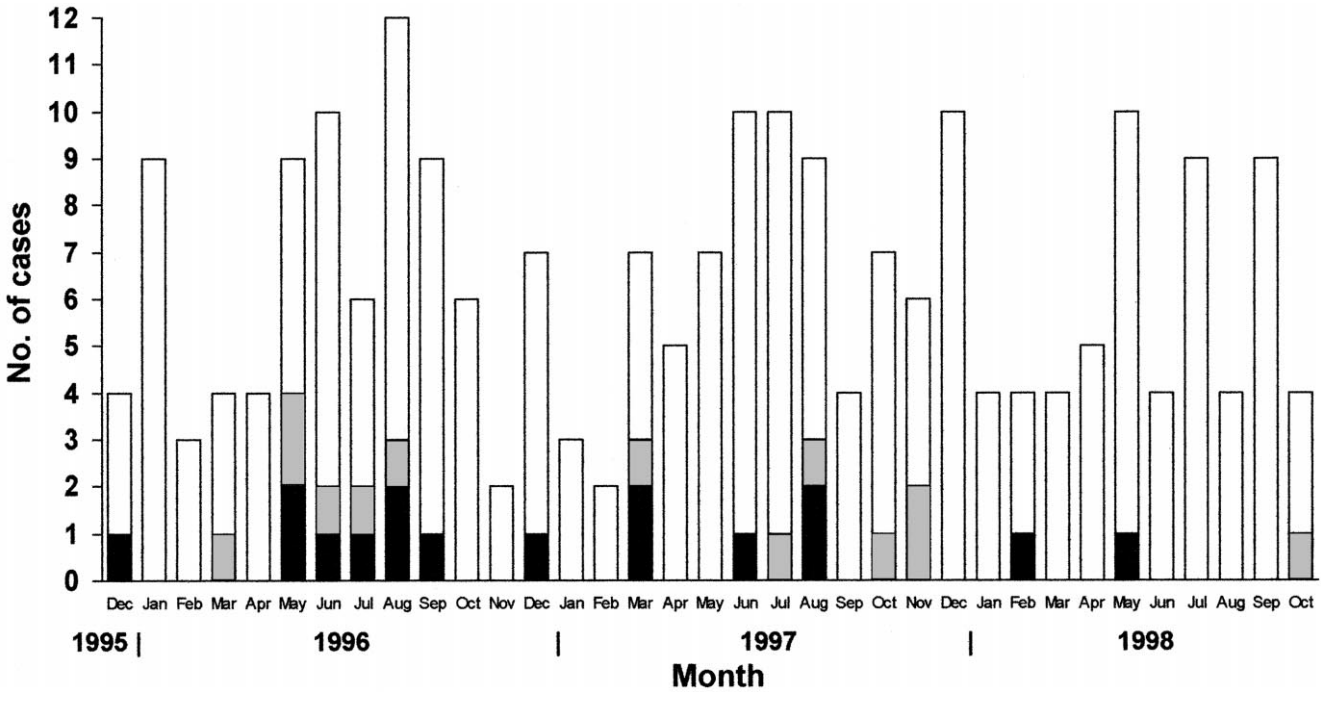

Figure 1. Distribution of 221 cases of *Streptococcus pneumoniae* meningitis according to month of identification during active surveillance for penicillin-nonsusceptible pneumococcal meningitis in Salvador, Brazil, December 1995 through October 1998. *Open bars*, penicillin-susceptible pneumococcal meningitis; *black bars,* penicillin-nonsusceptible serotype 14 meningitis; *gray bars,* penicillin-nonsusceptible nonserotype-14 meningitis; the latter two were defined as recovery of an isolate for which the penicillin MIC was ⩾0.12 μg/mL.

Table 1. Antimicrobial susceptibility of 221 strains of *Streptococcus pneumoniae* isolated from patients with pneumococcal meningitis during active surveillance in Salvador, Brazil.

	Cefotaxime			No. (%) of isolates nonsusceptible to					
Penicillin susceptibility	No. (%) of susceptible isolates	MIC range (μg/mL)	MIC_{90} (μg/mL)	TMP-SMZ	Tetracycline	Erythromycin	Clindamycin	Chloramphenicol	Rifampin
Susceptible	192 (87)	ND	ND	53 (28)	65 (34)	5 (3)	3 (2)	1 (1)	0
Intermediate-level resistance	29 (13)	0.064–0.380	0.250	22 (76)	4 (14)	0	0	0	1 (3)
High-level resistance	0	NA	NA	NA	NA	NA	NA	NA	NA

NOTE. MICs of penicillin and cefotaxime were determined for all isolates that demonstrated reduced susceptibility in the oxacillin (1 μg) disk diffusion assay. NCCLS interpretive criteria for MICs [26] were used to define susceptibility to penicillin (MIC, $\leq$0.06 μg/mL) and intermediate-level (0.12–1 μg/mL) or high-level (>1 μg/mL) resistance. Susceptibility to TMP-SMZ, tetracycline, erythromycin, clindamycin, chloramphenicol, and rifampin was determined by the disk diffusion test. Breakpoint values used to define susceptibility are those recommended by the NCCLS. NA, not applicable; NCCLS, National Committee for Clinical Laboratory Standards; ND, not determined; TMP-SMZ, trimethoprim-sulfamethoxazole.

tible isolates, 53 (28%) were nonsusceptible to TMP-SMZ, 65 (34%) to tetracycline, 5 (3%) to erythromycin, 3 (2%) to clindamycin, 1 (1%) to chloramphenicol; all isolates were susceptible to rifampin. Multidrug nonsusceptibility was identified in 23 (12%) of 192 penicillin-susceptible isolates. Of these isolates, 18 (78%) were nonsusceptible to TMP-SMZ and tetracycline, and 4 (17%) were nonsusceptible to TMP-SMZ, tetracycline, and erythromycin.

Patient characteristics. Of the 221 patients for whom penicillin susceptibility testing for isolates was performed, 211 (96%) were admitted to the surveillance hospital, and 10 (4%) were transferred to another hospital after initial evaluation in the emergency department. Of the patients admitted to the surveillance hospital, 28 (13%) had penicillin-nonsusceptible and 183 (87%) had penicillin-susceptible meningitis. The proportion of males and females was not significantly different between patients with penicillin-nonsusceptible (21 [75%] of 28) and those with penicillin-susceptible pneumococcal meningitis (121 [66%] of 183). Patients with penicillin-nonsusceptible meningitis were significantly younger than patients with penicillin-susceptible meningitis (mean age $\pm$ SD, 6 $\pm$ 12.4 vs. 13.9 $\pm$ 17.3 years, respectively; $P < .005$; table 2). Twenty (71%) of the 28 cases of penicillin-nonsusceptible meningitis occurred in infants aged <2 years, whereas 72 (39%) of the 183 cases of penicillin-susceptible meningitis occurred in these infants (OR, 3.85; 95% CI, 1.52–10.61).

Clinical histories were obtained from 199 (94%) of 211 patients who were not transferred to another hospital or did not die before an evaluation by the surveillance team could be performed. Of the 199 patients, 22 (11%) had at least 1 chronic underlying disease before the development of pneumococcal meningitis (alcohol abuse [6 patients], sickle cell anemia [2], diabetes mellitus [2], chronic liver disease [2], chronic obstructive pulmonary disease [2], and other illnesses [10]). A significant association was not found between the presence of a chronic illness and the acquisition of penicillin-nonsusceptible disease. In 70% of the cases, antecedent pneumonia (19 [10%]), acute otitis media (45 [23%]), non–otitis media upper respiratory tract infection (55 [28%]), cranial trauma and/or skull fracture (14 [7%]), or another type of infection (7 [4%]) was identified before the onset of meningitis. A clinical presentation of meningitis preceded by pneumonia was significantly associated with the acquisition of penicillin-nonsusceptible disease (OR, 4.67; 95% CI, 1.37–14.57), whereas significant associations with other antecedent acute processes were not identified (table 2).

The overall mortality rate for the 211 patients admitted to the surveillance hospital was 47% (100 deaths). Seizure as a complication during hospitalization occurred in 92 (46%) of 199 patients for whom clinical histories were reviewed. Between the patients with penicillin-nonsusceptible those with penicillin-susceptible meningitis, there was no significant difference in the mortality rate (57% vs. 43%, respectively; $P = .17$) or in the frequency of seizures during hospitalization (52% vs. 45%, respectively; $P = .53$). However, treatment regimens were modified as a result of the newly instituted surveillance. During the initial phase, penicillin with or without chloramphenicol was used as routine empirical treatment of suspected bacterial meningitis at the surveillance hospital. When a pneumococcal isolate with reduced susceptibility to penicillin was identified, the results were provided within the first 48 h of the patient's hospitalization, and treatment was changed to ceftriaxone. After a cluster of 12 cases of penicillin-nonsusceptible pneumococcal meningitis (41% of 29 cases) was identified during the period from May through September 1996 (figure 1), empirical therapy with ceftriaxone was initiated for suspected cases of bacterial meningitis.

Risk factors for penicillin-nonsusceptible meningitis. Outpatient use of antimicrobial agents 1 month before case identification was significantly associated with the acquisition of penicillin-nonsusceptible disease (OR, 4.56; 95% CI, 1.8–11.6; table 2). Of 24 patients who recalled having received a specific antimicrobial agent, 16 (67%) identified TMP-SMZ and 6 (25%) identified penicillin G benzathine. A multidrug-nonsusceptible phenotype of the isolate was significantly associated with acquisition of penicillin-nonsusceptible disease (OR, 35.27; 95% CI, 12.4–109.82). Cosegregation of phenotype nonsusceptible to TMP-SMZ and to penicillin was the contributing factor in this association: nonsusceptibility to penicillin was positively associated with nonsusceptibility to TMP-SMZ (OR, 8.24; 95%

Table 2. Comparison of the characteristics of patients with penicillin-nonsusceptible pneumococcal meningitis with those of patients with penicillin-susceptible pneumococcal meningitis and risk factors for the acquisition of penicillin-nonsusceptible strains during active surveillance in Salvador, Brazil.

Characteristic	Penicillin-nonsusceptible pneumococcal meningitis (n = 28)	Penicillin-susceptible pneumococcal meningitis (n = 183)	OR (95% CI)	P[a]
Age (y), mean ± SD	6.0 ± 12.4	13.9 ± 17.3		<.005
<2	20 (71)	72 (39)	3.85 (1.52–10.61)	<.0019
2–14	3 (10)	47 (26)	NS	
15–49	5 (18)	52 (28)	NS	
>50	0	12 (7)	NS	
Male	21 (75)	121 (66)	NS	
Chronic underlying illness	2 (7)	22 (13)	NS	
Acute illness preceding meningitis				
Pneumonia	7 (26)	12 (7)	4.67 (1.37–14.57)	<.0063
AOM	2 (7)	43 (25)	NS	
Non-AOM URTI	5 (19)	50 (30)	NS	
Cranial trauma and/or skull fracture	2 (7)	12 (7)	NS	
Other infections	2 (7)	5 (3)	NS	
Not identified	9 (33)	50 (30)	NS	
Seizures during hospitalization	14 (52)	78 (45)	NS	
Death	16 (57)	79 (43)	1.33 (0.85–2.07)[b]	
Previous hospitalization <6 mo PTI	8 (30)	22 (13)	NS	
History of antibiotic use <1 mo PTI	15 (56)	37 (22)	4.56 (1.80–11.60)	<.0006
TMP-SMZ–nonsusceptible isolate	22 (76)	53 (28)	8.24 (3.14–23.97)	<.0000
Tetracycline-nonsusceptible isolate	4 (14)	65 (34)	0.31 (0.08–0.96)	<.0483
Serotype 14 isolate	16 (55)	10 (5)	22.4 (7.63–66.16)	<.0000
Serotype 6B isolate	8 (28)	15 (8)	4.5 (1.46–12.87)	.0042

NOTE. Data are no. (%) of patients for whom responses were obtained, except as noted. Antimicrobial susceptibility testing and serotyping were performed for isolates from 221 culture-positive cases identified at the emergency department of the study hospital. Demographic characteristics and mortality data are shown for the 211 patients admitted to the study hospital. Information on risk factors for penicillin-nonsusceptible disease is shown for the 199 patients who were not transferred or did not die before evaluation by the study team. AOM, acute otitis media; NS, not significant; PTI, prior to case identification; TMP-SMZ, trimethoprim-sulfamethoxazole; URTI, upper respiratory tract infection.

[a] P values were calculated by Fisher's exact test, except for age, which was calculated by the two-sample Wilcoxon rank-sum test.

[b] Relative risk. and 95% CI. The difference between the rates was NS.

CI, 3.14–23.97), and negatively associated with nonsusceptibility to tetracycline (OR, 0.31; 95% CI, 0.08–0.96).

A higher proportion of patients with penicillin-nonsusceptible than patients with penicillin-susceptible meningitis had been hospitalized within the 6 months before the disease was identified (30% [8 of 27] vs. 13% [22 of 172], respectively), but this difference was not significant (P = .059). For all patients interviewed during surveillance in Salvador, attendance at a day care center (2 cases), chronic care facility (0), or nursing home (0) was infrequent or not observed. Geographic clustering of cases of penicillin-nonsusceptible pneumococcal meningitis was not identified according to the location of residence.

Isolate serotyping. Forty capsular serotypes were found among the pneumococcal isolates identified during surveillance (table 3). Prevalent serotypes included 14 (26 [11.8%] of 221), 3 (25 [11.3%]), 6B (23 [10.4%]), 19F (20 [9%]), 4 (11 [5%]), 6A (11 [5%]), 8 (11 [5%]), 18C (11 [5%]), and 23F (10 [4.5%]). Together, these 9 serotypes represented 148 (67%) of the isolates. Three formulations of protein-polysaccharide conjugate vaccines (Vac7, Vac12, and Vac15), which have a reduced number of capsular types, have been proposed for use in Latin America [15]. Based on the serotype profile of isolates from cases of pneumococcal meningitis in Salvador, the estimated rates of coverage would be 45% for Vac7, 69% for Vac12, 73% for Vac15, and 83% for the 23-valent pneumococcal vaccine (Merck, Sharp and Dohme: West Point, PA).

Six serotypes were found among the penicillin-nonsusceptible isolates: 14 (16 isolates), 6B (8), 19A (2), 19F (1), 23B (1), and 23F (1). Infection with 2 serotypes, 14 and 6B, was responsible for 83% (16 [55.2%] and 8 [27.6%], respectively) of the 29 cases of penicillin-nonsusceptible pneumococcal meningitis. The presence of these serotypes was significantly associated with the acquisition of penicillin-nonsusceptible disease (serotype 14: OR, 22.4; 95% CI, 7.63–66.16; serotype 6B: OR, 4.5; 95% CI, 1.46–12.87; table 2). Of the 12 patients with penicillin-nonsusceptible pneumococcal meningitis who were identified between May and September 1996, 7 (58%) had a serotype 14 isolate (figure 1).

BOX PCR strain typing of serotype 14 isolates. BOX PCR analysis of 21 isolates yielded a total of 13 patterns (figure 2; table 4). The 15 penicillin-nonsusceptible isolates tested had 7 related patterns of 7–10 electrophoretic bands (patterns A1–A7), which differed from each other by ⩽3 bands. Seventy-three percent of the penicillin-nonsusceptible serotype 14 iso-

Table 3. Capsular serotypes of *Streptococcus pneumoniae* isolates from cases of pneumococcal meningitis identified during active surveillance in Salvador, Brazil.

Serotype	No. (%) of isolates		
	Penicillin-nonsusceptible (n = 29)	Penicillin-susceptible (n = 192)	Total (n = 221)
14	16 (55.2)	10 (5.2)	26 (11.8)
3	0	25 (13.0)	25 (11.3)
6B	8 (27.6)	15 (7.8)	23 (10.4)
19F	1 (3.4)	19 (9.9)	20 (9.0)
4	0	11 (5.7)	11 (5.0)
6A	0	11 (5.7)	11 (5.0)
18C	0	11 (5.7)	11 (5.0)
8	0	11 (5.7)	11 (5.0)
23F	1 (3.4)	9 (4.7)	10 (4.5)
10A	0	6 (3.1)	6 (2.7)
7F	0	5 (2.6)	5 (2.3)
9N	0	4 (2.1)	4 (1.8)
5	0	4 (2.1)	4 (1.8)
19A	2 (6.9)	2 (1.0)	4 (1.8)
23B	1 (3.4)	3 (1.6)	4 (1.8)
7C	0	3 (1.6)	3 (1.4)
13	0	3 (1.6)	3 (1.4)
15B	0	3 (1.6)	3 (1.4)
17F	0	3 (1.6)	3 (1.4)
18B	0	3 (1.6)	3 (1.4)
28A	0	3 (1.6)	3 (1.4)
7B	0	2 (1.0)	2 (0.9)
9V	0	2 (1.0)	2 (0.9)
11A	0	2 (1.0)	2 (0.9)
16	0	2 (1.0)	2 (0.9)
34	0	2 (1.0)	2 (0.9)
1	0	1 (0.5)	1 (0.5)
10F	0	1 (0.5)	1 (0.5)
12F	0	1 (0.5)	1 (0.5)
18A	0	1 (0.5)	1 (0.5)
20	0	1 (0.5)	1 (0.5)
21	0	1 (0.5)	1 (0.5)
22F	0	1 (0.5)	1 (0.5)
24F	0	1 (0.5)	1 (0.5)
27	0	1 (0.5)	1 (0.5)
28B	0	1 (0.5)	1 (0.5)
35F	0	1 (0.5)	1 (0.5)
38	0	1 (0.5)	1 (0.5)
48	0	1 (0.5)	1 (0.5)

NOTE. Serotypes are reported according to the Danish system of nomenclature.

lates had 1 of 3 identical patterns: A1, A2, and A3 (6, 2, and 3 isolates, respectively). All isolates with pattern A had penicillin- and TMP-SMZ–nonsusceptible phenotypes.

In contrast, the 6 penicillin-susceptible serotype 14 isolates had unrelated BOX PCR patterns of 4–11 electrophoretic bands (table 4). Patterns differed by 4–10 bands when those for penicillin-susceptible isolates were compared with each other and by 6–14 bands when those for penicillin-susceptible isolates were compared with pattern A. All patterns for the serotype 14 isolates were distinct and unrelated to those for penicillin-nonsusceptible or -susceptible isolates of other serotypes (data not shown). The proportion of TMP-SMZ nonsusceptibility among the penicillin-susceptible isolates with patterns other than A (4 [67%] of 6) was not significantly different from that observed for penicillin-nonsusceptible isolates with pattern A (15 [100%] of 15).

Penicillin-nonsusceptible serotype 14 isolates identified during surveillance in Salvador had BOX PCR patterns identical (4 of 15) or related (11 of 15) to those for penicillin-nonsusceptible serotype 14 isolates from the Brazilian cities of São Paulo, Brasília, and Recife (figure 2; table 4). These patterns were unrelated to those observed for penicillin-susceptible serotype 14 isolates from other Brazilian cities and Atlanta, Georgia. In contrast to penicillin-nonsusceptible isolates, penicillin-susceptible isolates had patterns that were distinct from and unrelated to those for serotype 14 isolates from other cities. BOX PCR patterns for penicillin-nonsusceptible serotype 14 isolates from Salvador were unrelated to those obtained for previously identified penicillin-nonsusceptible serotype 14 clones, SPAIN[14]-5 [27] and SLOVAKIA[14]-10 [28] (results not shown).

Discussion

This active hospital-based surveillance investigation shows that pneumococcal meningitis remains a major cause of bacterial meningitis in urban settings in Brazil. Because >95% of cases in Salvador are referred to the surveillance hospital, the data collected in this study may be considered population-based. The annual incidence of disease in the city was 31.7 cases per 100,000 population for children aged <1 year; this is similar to the incidence reported in the most recent population-based study, which was performed from 1960 to 1977 in the largest city in Brazil, São Paulo [16]. The mortality rate associated with pneumococcal meningitis was 47% in Salvador.

The major findings of this surveillance investigation are as follows: a significant proportion (13%) of the isolates were intermediately resistant to penicillin; infection with these strains was associated with age of <2 years, previous antibiotic use, coresistance to TMP-SMZ, and infection with serotypes 14 and 6B; penicillin-nonsusceptible serotype 14 isolates belonged to a closely related group (pattern A) of *S. pneumoniae* strains as revealed by BOX PCR strain typing; and the penicillin-nonsusceptible serotype 14 strains from Salvador appeared to be related to penicillin-nonsusceptible serotype 14 strains isolated from patients in other cities of Brazil, while penicillin-susceptible serotype 14 strains from Salvador or other cities in Brazil had distinct BOX PCR patterns.

Although *S. pneumoniae* with high-level resistance to penicillin was not identified, intermediately-resistant *S. pneumoniae* has an important impact on treatment and outcome of pneumococcal meningitis in Brazil. In contrast to pneumococcal pneumonia, there is increasing evidence that penicillin and chloramphenicol may not be adequate to treat meningitis caused by *S. pneumoniae* with intermediate-level resistance to penicillin [7, 9]. Treatment failure has been reported in cases when high

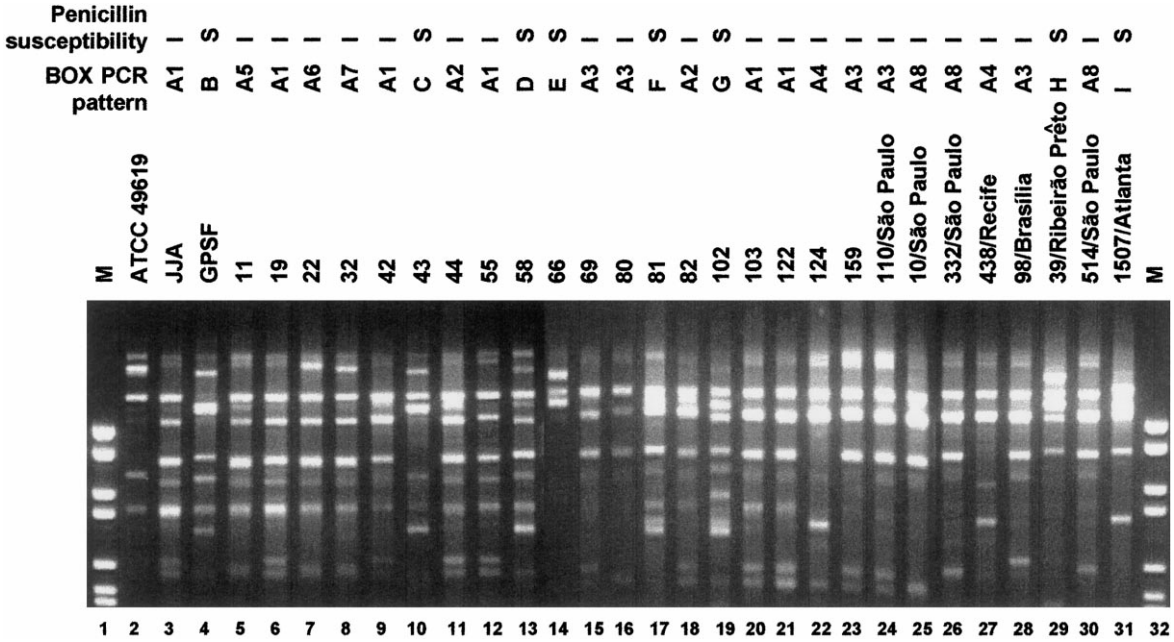

Figure 2. BOX PCR DNA fingerprints for *Streptococcus pneumoniae* serotype 14 strains from Salvador, Brazil; other Brazilian cities; and Atlanta. *Lanes 1 and 32*, DNA VI markers with band sizes of 2176, 1666, 1230, 1033, 653, 517, and 453 bp; *lanes 2–31*, fingerprint patterns for the ATCC 49619 reference strain (*lane 2*), strains isolated during active surveillance in Salvador (*lanes 3–23*), and clinical isolates from the Brazilian cities of São Paulo (*lanes 24, 25, 26, and 30*), Recife (*lane 27*), Brasília (*lane 28*), and Ribeirão Prêto (*lane 29*) and Atlanta (*lane 31*). BOX PCR patterns are identified by a letter code and numerical index: related patterns were assigned the same letter code; distinct, nonidentical patterns within a group of related patterns were given a numerical index. Penicillin susceptibilities for isolates are shown above the BOX PCR patterns: I, intermediately resistant; S, susceptible.

doses of penicillin and chloramphenicol were used [8, 29]. Data from experimental models indicate that antibiotic concentrations in CSF need to be 8-fold higher than the MBC to eradicate organisms [30]; these concentrations are not achieved with iv administration.

Findings from the initial year of surveillance enabled the hospital to identify penicillin-nonsusceptible *S. pneumoniae* as a new problem and replace penicillin with ceftriaxone as the empirical treatment of bacterial meningitis. All isolates were susceptible to ceftriaxone in vitro, and this intervention may have obviated the association between penicillin resistance and mortality. However, the economic consequences of these treatment decisions are substantial. Antimicrobial therapy in Salvador is 38 times more expensive when ceftriaxone is used in place of penicillin (US$550.00 vs. US$14.25, respectively, for a 10-day treatment course for an adult patient). The use of third-generation cephalosporins in the empirical treatment of bacterial meningitis and therapy for pneumococcal meningitis is a critical burden for the Brazilian public sector, whose per capita annual health expenditure in Salvador is US$20 [31]. A response to this emerging problem in Brazil will require interventions focused on preventing transmission of penicillin-nonsusceptible *S. pneumoniae.*

Outpatient antibiotic use was significantly associated with the acquisition of penicillin-nonsusceptible pneumococcal meningitis. Exposure to antibiotics is believed to provide the selective pressure contributing to the emergence of penicillin-nonsusceptible strains [32]. Prior use of β-lactam agents [33–35] and TMP-SMZ [36, 37] has been shown to be a risk factor for penicillin-nonsusceptible pneumococcal carriage and disease. Because national programs controlling the use of macrolide antibiotics have reduced resistance in group A streptococci to these agents in Finland [38], similar interventions focused on controlling the use of antibiotics that are selecting for penicillin-nonsusceptible *S. pneumoniae* will be an important step against the increasing problem in Brazil.

In Salvador during 1995–1998, the proportion of cases of penicillin-nonsusceptible pneumococcal meningitis that were attributable to serotype 14 strains with pattern A was 52%. We can conclude that during this period in Salvador much of the increase in incidence of penicillin-nonsusceptible pneumococcal meningitis was due to this single related group of *S. pneumoniae.* It is interesting that all strains with pattern A were coresistant to TMP-SMZ. In Brazil, oral suspensions of TMP-SMZ are widely used for treatment of respiratory tract infections in the outpatient pediatric population because they are relatively low cost and widely available. During interviews, it was identified as the most common antibiotic used in the outpatient setting.

Table 4. *Streptococcus pneumoniae* serotype 14 isolates recovered during active surveillance in Salvador, Brazil, according to BOX PCR fingerprinting pattern and the relationship between these patterns, antimicrobial susceptibility, and BOX PCR fingerprinting patterns of serotype 14 (S14) strains isolated from other Brazilian cities.

Pattern[a]	No. (%) of isolates (n = 21)	Antimicrobial susceptibility (no. of isolates)			Relationship to patterns for other S14 Brazilian strains[b]
		Penicillin	TMP-SMZ	Tetracycline	
A	15 (71)	I	R (14), I (1)	R (1), I (1), S (13)	
A1	6 (29)	I	R	S	Related
A2	2 (10)	I	R (1), I (1)	R (1), S (1)	Related
A3	3 (14)	I	R	S	Identical
A4	1 (5)	I	R	I	Identical
A5	1 (5)	I	R	S	Related
A6	1 (5)	I	R	S	Related
A7	1 (5)	I	R	S	Related
B	1 (5)	S	R	R	Unrelated
C	1 (5)	S	R	R	Unrelated
D	1 (5)	S	R	R	Unrelated
E	1 (5)	S	S	S	Unrelated
F	1 (5)	S	S	S	Unrelated
G	1 (5)	S	R	R	Unrelated

NOTE. I, intermediately resistant; R, resistant; S, susceptible; TMP-SMZ, trimethoprim-sulfamethoxazole.

[a] BOX PCR patterns are represented by a letter code with or without a numerical index. Strains with ⩽3 band differences in their BOX PCR fingerprints were assigned a unique letter code. For pattern A, a unique numerical index was assigned to strains that had identical fingerprints. Characteristics for pattern A were those observed among the related strains assigned to this group.

[b] We defined the relationships between BOX PCR patterns of serotype 14 isolates identified in Salvador and those of strains from other Brazilian cities (patterns A3, A4, A8, H and I in figure 2) as follows: identical, no band differences; related, ⩽3 band differences; unrelated, >3 band differences.

Together, these observations suggest that TMP-SMZ may be the antibiotic that is providing selective pressure for the emergence of penicillin-nonsusceptible serotype 14 clones in Brazilian communities.

BOX PCR analysis demonstrated that serotype 14 isolates from Salvador with intermediate-level resistance to penicillin were identical or related to those from several other Brazilian cities. Clonally related *S. pneumoniae* strains implicated in the geographic spread of penicillin have generally been found to be highly resistant [39–41]. Studies from several countries have shown that *S. pneumoniae* strains with intermediate-level resistance to penicillin demonstrate large variations in their genetic background [42–44]. However, in Brazil, analysis of strains from reference laboratory collections by pulsed-field gel electrophoresis has identified clusters of isolates with intermediate-level resistance, including those belonging to serotype 14, that were obtained from geographically distinct regions [45]. The evidence from population-based surveillance in Salvador supports the assertion that geographic spread of clonally related serotype 14 strains with intermediate-level resistance may be a major contributory factor in the emergence of penicillin-resistant *S. pneumoniae* in Brazil.

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