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TESE DE DOUTORADO

EMERGÊNCIA DE *STREPTOCOCCUS PNEUMONIAE* RESISTENTE À PENICILINA EM SALVADOR, BAHIA: UM ESTUDO EPIDEMIOLÓGICO E MOLECULAR

JOICE NEVES REIS PEDREIRA

Salvador - Bahia - Brasil
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Emergência de *Streptococcus pneumonie* resistente à penicilina em Salvador, Bahia: Um Estudo Epidemiológico e Molecular

JOICE NEVES REIS PEDREIRA

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Sonhe com aquilo que você quiser
Seja o que você quer ser,
porque você possui apenas uma vida
e nela só se tem uma chance
de fazer aquilo que se quer.

Clarice Lispector

A Deus pelo dom da vida e por tudo que tenho.

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SUMÁRIO

LISTA DE ABREVIATURAS

ATCC	do inglês, *American Typing Culture Collection*
Box-A	reação da polimerase em cadeia de regiões repetitivas
CO_2	dióxido de carbono
cols.	colaboradores
DNA	ácido desoxiribonucléico
HCM	Hospital Couto Maia
MLST	do inglês, *multilocus sequence typing*
NT	não sorotipável
PCR	reação da polimerase em cadeia
PFGE	do inglês, *pulse field gel electrophoresis*
Taq DNA	DNA polimerase do organismo *Thermus aquaticus*
UPGMA	do inglês, *unweighted pair group method arithmetic averages*

LISTA DE ANEXOS

RESUMO

Emergência de *Streptococcus pneumoniae* resistente à penicilina em Salvador, Bahia: Um Estudo Epidemiológico e Molecular. **Joice Neves Reis** – As infecções pelo *S. pneumoniae* persistem como uma das principais causas de morbi-mortalidade, apesar da disponibilidade de antibioticoterapia apropriada. Em 1995, foi implantado uma rede de vigilância para identificar pneumococos não susceptível à penicilina em dois diferentes grupos populacionais: 1) pacientes com meningite pneumocócica e 2) indivíduos saudáveis na comunidade. No período de dezembro de 1995 a agosto de 2002, o Hospital Couto Maia atendeu 477 pacientes com meningite pneumocócica identificados através da cultura de líquor positiva para *S. pneumoniae*, 453 (95%) tiveram os isolados caracterizados quanto à susceptibilidade e sorotipagem. Cepas não susceptíveis à penicilina (CIM 0,1 – 1,0 μg/ml) foram isoladas em 59 (13%) indivíduos com meningite pneumocócica. Os sorotipos mais prevalentes foram 14 (14%), 3 (10%), 19F (8%), 6B (8%), 6A (7%), 23F (6%), 4 (5%), 18C (5%) e 8 (4%), sendo que reduzida sensibilidade à penicilina foi associada aos sorogrupos 14 (38), 6 (9), 23 (7) e 19 (5). A provável taxa de cobertura da vacina heptavalente conjugada foi de 70% entre pacientes < 5 anos e 91% entre aqueles com isolados não susceptíveis à penicilina. Tipagem de cepas pela reação de polimerase em cadeia (PCR) do elemento repetitivo – BOX A, demonstou que os isolados de sorotipo 14 não susceptíveis à penicilina tinham um padrão clonal relacionado e quando comparados com isolados de outras cidades brasileiras tiveram um padrão similar. O segundo grupo populacional foram residentes de 39 domicílios selecionados randomicamente em uma comunidade urbana. Neste grupo, a colonização nasofaringeana foi investigada em 262 indivíduos, 95 (36%) estavam colonizados por pneumococos. Destes, 9 (9,5%) tiveram isolados não susceptíveis à penicilina. Os sorotipos mais prevalentes foram 19F (13%), e 6A (11%), seguidos do sorotipo 14 (7%), 23F (7%), 18C (6%) e 19A (5%). A cobertura teórica da vacina conjugada heptavalente nesta comunidade seria de 49% para todos os indivíduos e 57% para as crianças < 5 anos de idade. Tipagem molecular utilizando a reação de polimerase em cadeia pelo BOX A e eletroforese de campo pulsado demonstrou que as cepas não sensíveis à penicilina tinham um padrão distinto, não relacionado. Em contraste com o estudo de cepas causadoras de meningite, pneumococos não susceptíveis à penicilina associados com colonização não parecem ser clonais. Além do mais, uma limitada proporção destas cepas é de sorotipos representados na vacina heptavalente conjugada.

[Palavras-Chave] *Streptococcus pneumoniae*; Resistência à penicilina; Colonização

ABSTRACT

Emergence of penicillin-resistant *Streptococcus pneumoniae* in Salvador, Bahia: A molecular epidemiology study. **Joice Neves Reis** – Infections due to *S. pneumoniae* remains a major cause of mortality and morbidity, despite the availability of effective antibiotic therapy. In 1995, a network surveillance was implemented to identify penicillin-nonsusceptible pneumococcus in two different population groups: 1) patients with pneumococcal meningitis and 2) healthy individuals in a community. From December 1995 to August 2002, the Couto Maia Hospital attended to 477 patients with pneumococcal meningitis identified through positive CSF cultures for *S. pneumoniae*, 453 (95%) had isolates with complete susceptibility and serotype information. Penicillin non-susceptible strains (MIC 0.1 – 1.0 μg/ml) were isolated from 59 (13%) patients. The most prevalent serotypes were 14 (14%), 3 (10%), 19F (8%), 6B (8%), 6A (7%), 23F (6%), 4 (5%), 18C (5%) and 8 (4%), being that those which were penicillin non-susceptible were associated with serogroups 14 (38), 6 (9), 23 (7) e 19 (5). The estimated rate of coverage of the seven-valent conjugate vaccine was 70% among patients < 5 years of age and 91% among those infected with penicillin non-susceptible isolates. Strain typing by repetitive element BOX A polymerase chain reaction (PCR) analysis showed that penicilin non-susceptible isolates had closely related patterns and similar pattern with isolates from other Brazilian cities. The second population group was formed by residents of 39 randomly selected households in an urban community. In this group, the nasopharygeal carriage was investigated among 262 subjects, of which 95 (36%) had pneumococcal carriage. Of these, 9(10%) had penicillin non-susceptible isolates. The most prevalent serotypes were 19F (13%), and 6A (11%), followed by serotype 14 (7%), 23F (7%), 18C (6%) and 19A (5%). The theoretical vaccine coverage of the seven-valent conjugate vaccine is 49% for all individuals and 57% for children less than 5 years of age. BOX A PCR and PFGE typing showed that penicillin non-susceptible strains had unrelated fingerprinting patterns. In contrast to studies with strains from meningitis, penicillin non-susceptible pneumococci associated with carriage do not appear to be clonally related. Furthermore, a limited proportion of these strains have serotypes represented in the conjugate vaccine.

[Key-Words] *Streptococcus pneumoniae*; penicillin-resistance; colonization

1 INTRODUÇÃO

O *Streptococcus pneumoniae* foi descoberto por acaso e quase que simultaneamente, em 1881, por dois microbiologistas: George Sternberg nos EUA e Louis Pasteur na França. Ambos pesquisadores injetaram saliva humana em coelhos e subsequentemente recuperaram diplococos no sangue destes animais (AUSTRIAN, 1985). Pneumococos são cocos Gram-positivos, encapsulados ou não, anaeróbios facultativo, medindo 0,5 a 1,2 μm e que se apresentam formando pequenas cadeias ou aos pares quando em cultura. Distinguem-se de outros estreptococos pela sensibilidade ao disco de 30 μg de optoquina (etil-hidrocupreína) e pela solubilidade em sais biliares. A sensibilidade à optoquina é verificada pelo diâmetro do halo de inibição de crescimento maior que 14 mm. Porém, uma pequena proporção deste microrganismo apresenta halos menores de crescimento em torno do disco de optoquina. Por isso, as colônias de aspecto típico, alfa-hemolíticas mas com halos inferiores a 14 mm frente à optoquina, precisam ser testadas quanto à solubilidade em bile para confirmação da espécie (RUOFF et al., 1999).

Posteriormente, Friedlander e col. estabeleceram a associação desta bactéria com a pneumonia lobar descrevendo características de sua cápsula e achados morfológicos de suas colônias (AUSTRIAN, 1985). Atualmente, este patógeno é considerado a causa mais frequente de pneumonia aguda, assim como a principal causa de outras infecções como otite média e meningite (HAUSDORFF et al., 2002).

As infecções pelo *S. pneumoniae* persistem como importante causa de morbidade e mortalidade no mundo, sendo responsável por um terço dos cinco milhões de óbitos anuais por pneumonia nos países em desenvolvimento. A cada ano, 150.000 óbitos em menores de cinco anos ocorrem nas Américas devido a infecções respiratórias agudas causadas por *S. pneumoniae* (DEBBIA et al., 2001).

A manifestação mais comun da infecção pneumocócica é a otite média aguda, especialmente em crianças pequenas, correspondendo de 30% a 50% de todas as infecções de ouvido médio. Pelo menos 20% das crianças menores de dois anos de idade experimentarão um episódio de otite média aguda causada pelo pneumococo (JACOBS, 2002).

A meningite pelo *S. pneumoniae* tem se tornado a infecção bacteriana mais comum do sistema nervoso central, sendo o agente etiológico mais frequentemente associado com morte e com seqüelas graves na infância (TAN, 2002). A taxa de letalidade pode alcançar 30% a 50%, e 29% a 50% de seqüelas neurológicas podem aparecer como deficiência auditiva, hidrocefalia e retardo mental (GREENE & DEMASI, 1996). A penetração do pneumococo no espaço subaracnóide se dá, provavelmente, pelos vasos do plexo coróide, após invasão da mucosa do trato respiratório alto e bacteremia. Outra forma de acesso liquórico, por contiguidade, são as fraturas fechadas de crânio, as válvulas de deivação ventrículo-peritoneal e malformações com fístula liquórica (TUNKEL et al., 1990).

A colonização nasofaringeana é a principal etapa na patogêneses do *S. pneumoniae.* O pneumococo integra a microflora da nasofaringe, com a colonização começando logo após o nascimento, usualmente é assintomática e subseqüentemente serve como reservatório para infecções pneumocócicas em crianças, idosos, imunocomprometidos e indivíduos que sofrem de doença crônica. Evidências dos estudos mostram que a colonização da nasofaringe por *S. pneumoniae* ocorre logo no início da vida com idade de quatro dias a 18 meses, particularmente com os sorogrupos 6, 19, 23 e 14, em ordem de freqüência, os quais representam 65% das amostras isoladas de crianças menores de três anos de idade. Reaquisição e persistência de colonização ocorre em 30% das crianças. Embora a significância do estado de colonizado não seja completamente entendida, a colonização do trato respiratório superior pode ser o primeiro passo para o desenvolvimento de uma infecção local ou sistêmica (GRAY et al., 1980; GHAFFAR et al., 1999).

Normalmente a mucosa é resistente à invasão por *S. pneumoniae* funcionando como uma barreira, entretanto se há alguma lesão de mucosa por uma infecção viral ou agentes físicos e químicos, suas funções são alteradas e a invasão bacteriana pode ocorrer. A ocorrência de infecções e sua gravidade dependem de fatores relacionados à patogenicidade e virulência da cepa, as quais são definidos por particularidades da cápsula e das condições do hospedeiro (GHAFFAR et al., 1999).

Os estudos de colonização nasofaringeana oferecem-nos o perfil das cepas que circulam na comunidade, e estas não necessariamente serão causadoras diretas das doenças invasivas. Pouco se conhece sobre a dinâmica da colonização nasofaringeana pelo *S. pneumoniae* e o significado do estado de portador e, ainda que ela seja detectada já nas primeiras semanas de vida e perdure durante a infância, estima-se que apenas 1% das crianças colonizadas desenvolverá doença invasiva (GREENWOOD, 1999). Isso porque o caráter invasivo de um determinado sorotipo não depende de sua resistência aos antimicrobianos, mas da sua virulência e do estado imunológico do hospedeiro.

O Centro de Prevenção e Controle de Doenças Infecciosas (EUA) e a Organização Mundial de Saúde (Programa de Controle de Infecção Respiratória Aguda) estabeleceram medidas preventivas para reduzir o impacto da resistência á penicilina pelo pneumococo, sendo um dos objetivos, investigar a transmissão dos pneumococos através de estudos de colonização nasofaringeana, e determinar se os isolados desta região são representativos de isolados de doença invasiva, sendo portanto um instrumento útil na monitorização da resitência na comunidade (CENTER..., 1996).

1.1. Resistência à penicilina pelo pneumococos e fatores associados

A atual emergência global da multi-resistência e resistência à penicilina pelo *S.*

pneumoniae é um problema grave para o tratamento de infecções pneumocócicas. Embora a resistência à penicilina *in vitro* tenha sido descrita com a introdução dos antibióticos (AUSTRIAN, 1994), cepas resistentes à penicilina não foram isoladas de pacientes até os anos 60 (HANSMAN, 1967). A propagação de resistência pelo *S. pneumoniae* tem sido agora descrita em várias localidades geográficas (APPELBAUM, 1992; COHEN, 1992) com uma crescente freqüência. Muitas destas cepas são multi-resistentes e demonstram resistência a cefalosporinas de terceira geração. Resistência a outras classes de antibióticos não β-lactâmicos como o cloranfenicol, tetraciclina, eritromicina, clindamicina, rifampicina e trimetoprim-sulfametoxazol também tem sido reportada (APPELBAUM, 1996; KLUGMAN, 1996) e às vezes está associada com o decréscimo de susceptibilidade à penicilina (KLUGMAN, 1996). Pneumococos resistentes à penicilina tem sido reportado com uma freqüência particularmente elevada na Espanha (FENOLL et al., 1998), África do Sul (FRIEDLAND & KLUGMAN, 1992) e Hungria (MARTON et al., 1991) desde os anos 70, na França, Irlanda e EUA desde os anos 80. No Brasil, a resistência à penicilina por *S. pneumoniae* é um problema em ascensão, onde a taxa de resistência intermediária e plena saltou de zero (TEIXEIRA et al., 1988) no início dos anos 80, para 20% em 1998 (SESSEGOLO et al., 1994; BRANDILEONE et al., 1995; BRANDILEONE et al., 1997; TEIXEIRA et al., 1997), logo após a identificação da primeira amostra clínica com resistência intermediária à penicilina isolada de uma criança com meningite (DE SOUSA MARQUES et al., 1988). Em 1997, o projeto multicêntrico SIREVA estudou pneumococos a partir de fluídos estéreis em pacientes com infecções sistêmicas, identificando no Brasil 20% de pneumococos com resistência intermediária à penicilina e 1,4% com resistência plena (BRANDILEONE et al., 1997).

Embora o primeiro isolamento de pneumococos resistentes á penicilina tenha sido em um adulto (HANSMAN, 1967), as primeiras cepas multi-resistentes foram isoladas a partir de

crianças (*apud* APPELBAUM, 1992), e em qualquer lugar em que seja estudada a resistência à penicilina tem sido muito mais comum em crianças. Pneumococos multi-resistentes e resistentes à penicilina têm sido restritos a poucos sorogrupos, particularmente 23, 6, 19, 9 e sorotipo 14, os quais são associados com colonização e doença em crianças (KAPLAN et al., 1998; DEEKS et al., 1999). A aplicação de técnicas de tipagem molecular também revelou que as infecções causadas por pneumococos resistentes a penicilina é restrita a poucos grupos clonais, que se disseminaram localmente e até mundialmente (DAVIES et al., 1999).

1.2.Aplicação de métodos moleculares em estudos epidemiológicos das infecções pneumocócicas

Em 1997, a união internacional das sociedades de microbiologia estabeleceu uma rede sobre Epidemiologia Molecular de Pneumococos - "The Pneumococcal Molecular Epidemiology Network (PMEN)" com o objetivo de padronizar a nomenclatura e classificar através de métodos de tipagem molecular os clones de pneumococos resistentes aos antimicrobianos de importância para infecção e colonização. Em 2001, McGee e col descreveram a nomenclatura para 16 clones de pneumococos definidos por eletroforese de campo pulsado (PFGE), Box-PCR e MLST(multilocus sequence typing). Atualmente recomenda-se que para determinar novos genótipos de pneumococos, as cepas sejam cuidadosamente submetidas às técnicas de PFGE e Box-PCR ou análises de MLST. Os seguintes critérios foram estabelecidos para inclusão de clones na rede de epidemiologia molecular (PMEN): (i) o clone deve ter uma ampla disseminação geográfica através do país ou internacionalmente; (ii) o clone deve ser bem estabelecido durante vários anos; (iii) o clone deve ser resistente a um ou mais antibióticos de amplo uso clinico; (iv) os dados referentes ao clone precisam ser publicados antes da ratificação pela PMEN; e (v) um isolado clinico representativo do clone deve ser submetido a tipagem molecular para confirmar que

ele difere dos clones previamente descritos e uma permissão deve ser garantida para depositá-lo na ATCC (American Typing Culture Collection) (MCGEE et al., 2001)

Uma vez, cumprido os critérios acima descritos os clones serão nomeados de acordo com a seguinte regra: para países geograficamente pequenos, utiliza-se o nome do país seguido pelo sorotipo sobrescrito, e pelo número do clone, de acordo com o seguinte exemplo: Spain^{23F} – 1, é um clone de pneumococos de sorotipo 23F resistente à penicilina identificado na Espanha no inicio dos anos 80, e que posteriormente se disseminou pelo mundo (MUNOZ et al., 1991). Em países geograficamente extensos, como EUA e Rússia, os clones podem ter o nome de acordo com o estado responsável pelo primeiro isolamento como, por exemplo: Tennessee^{23F} – 4 (MCGEE et al., 2001).

2 OBJETIVOS

Nesta tese, os estudos aqui desenvolvidos procuraram avançar no entendimento da emergência de pneumococos resistentes à penicilina e do impacto que possa ocorrer na saúde pública do Brasil; existem sérios problemas que impedem o desenvolvimento de medidas de intervenções e controle. Devido à extensão territorial e às diferenças regionais, é essencial a implantação de um sistema de vigilância epidemiológica e laboratorial ativa que possam guiar investigações de controle e prevenção. Embora ferramentas moleculares desenvolvidas recentemente sejam úteis para identificar fontes comuns de transmissão para cepas resistentes à penicilina (MUSHER, 1992; ERTUGRUL et al., 1997; RODRIGUEZ-BARRADAS et al., 1997), estes métodos não são utilizados de maneira continua e específica, ou mesmo não foram integrados à vigilância local. Este trabalho teve como objetivo identificar pneumococos resistentes à penicilina e desenvolver estudos populacionais utilizando ferramentas de epidemiologia molecular, através da criação de uma rede ativa de vigilância epidemiológica e laboratorial em Salvador, Bahia.

Como objetivos específicos em cada um dos estudos, destacamos:

3 PUBLICAÇÕES E MANUSCRITOS

1. *Clonally Related Penicillin-Nonsusceptible* Streptococcus pnumoniae *serotype 14 from Cases of Meningitis in Salvador, Brazil.*

Objetivo: Desenvolver um estudo de base populacional utilizando ferramentas de epidemiologia molecular através de um sistema de vigilância hospitalar ativa para os casos de meningite pneumocócica. Os objetivos especificos foram:

a. Determinar a prevalência de *S. pneumoniae* não sensível à penicilina nos casos de meningite no Hospital Couto Maia.
b. Identificar fatores de risco para a aquisição de meningite por *S. pneumoniae* não sensível à penicilina.
c. Determinar o impacto no tratamento da meningite causada por pneumococos não sensíveis à penicilina.
d. Avaliar o padrão molecular de isolados não sensíveis à pencilina utilizando métodos baseados em PCR e PFGE.

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 ≥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 ≤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-

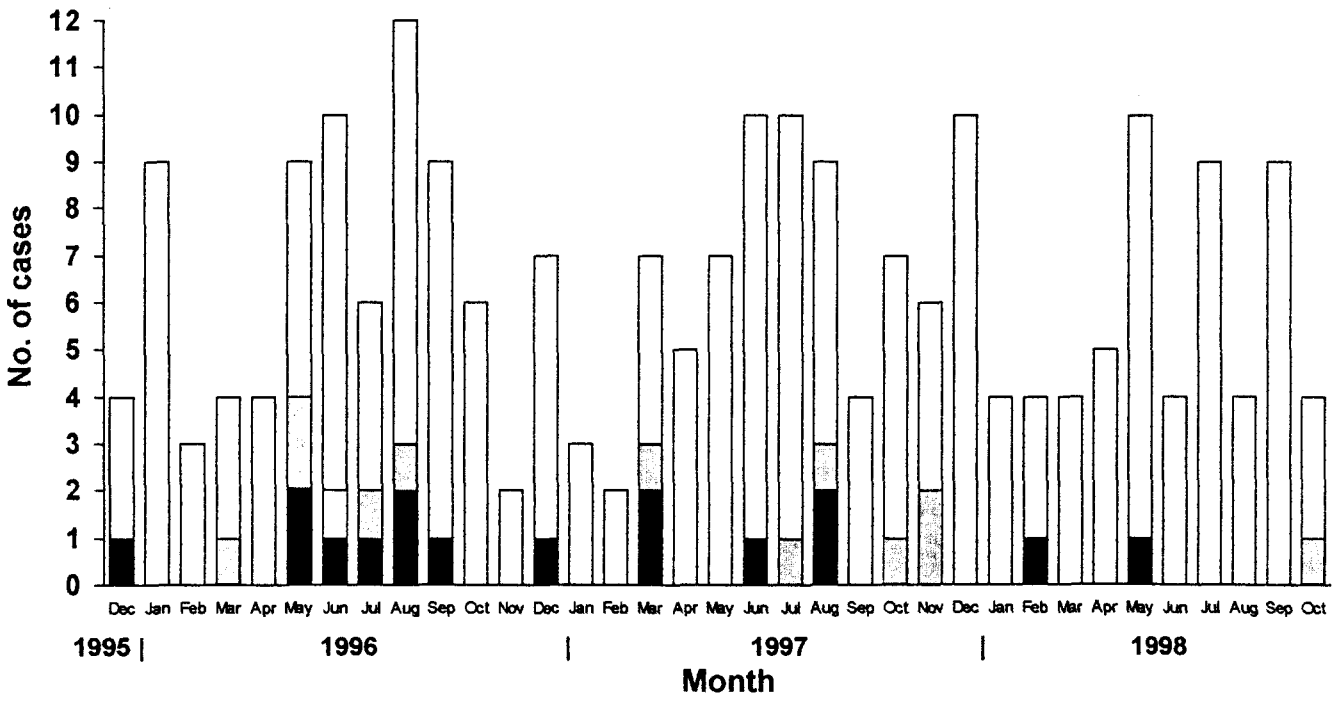

Figure 1. Distribution of 221 cases of *Streptococcus pneumoniae* meningitis according to month of identification during active surveillance for penicillin-nonsusceptible pneumococcal meningitis in Salvador, Brazil, December 1995 through October 1998. *Open bars*, penicillin-susceptible pneumococcal 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.

Penicillin susceptibility	Cefotaxime			No. (%) of isolates nonsusceptible to					
	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, ≤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 ± SD, 6 ± 12.4 vs. 13.9 ± 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, Brasilia, 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

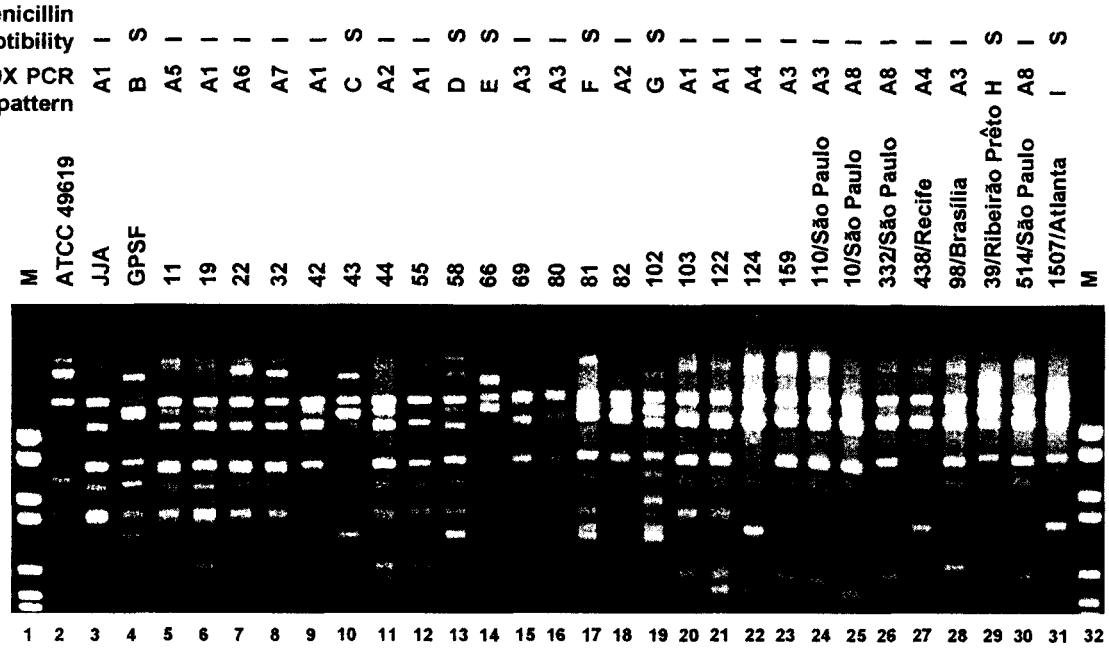

Figure 2. BOX PCR DNA fingerprints for *Streptococcus pneumoniae* serotype 14 strains from Salvador, Brazil; other Brazilian cities; and Atlanta. *Lanes 1 and 32*, DNA VI markers with band sizes of 2176, 1666, 1230, 1033, 653, 517, and 453 bp; *lanes 2–31*, fingerprint patterns for the ATCC 49619 reference 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*), Brasilia (*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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2. *Population-Based Survey of Antimicrobial Susceptibility and Serotype Distribution of* Streptococcus pneumoniae *from Meningitis Patients in Salvador, Brazil.*

Objetivo: O prinicpal objetivo deste trabalho foi avaliar a susceptibilidade antimicrobiana e a distribuição de sorotipos de *S. pneumoniae* em casos de meningite identificados no Hospital Couto Maia no período de dezembro de 1995 a novembro de 1999.

a. Mensurar a prevalência da resistência à penicilina e a outros agentes antimicrobianos em cepas de *S. pneumoniae* isoladas de pacientes com meningite.

b. Determinar a prevalência dos sorotipos causadores de meningite e avaliar a provável cobertura vacinal para esta população

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Population-Based Survey of Antimicrobial Susceptibility and Serotype Distribution of *Streptococcus pneumoniae* from Meningitis Patients in Salvador, Brazil

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Penicillin-nonsusceptible strains were isolated from 15% of 303 individuals with pneumococcal meningitis identified during a 4-year surveillance study in Salvador, Brazil. The estimated rate of coverage of the seven-valent conjugate vaccine was 74% among patients <5 years of age and 94% among those infected with nonsusceptible isolates, indicating that the use of conjugate vaccines may be an approach to the control of emerging penicillin resistance in Brazil.

Pneumococcal disease is responsible for over 1 million deaths each year in children under 5 years of age (22). Its public health impact has been further compounded by the global emergence of penicillin-resistant *Streptococcus pneumoniae* (1). Resistant strains have been isolated in all continents (1) and in several countries; over 50% of the clinical isolates demonstrate high-level resistance to penicillin (7, 19).

With the emergence of penicillin resistance, it is ever more urgent that surveillance be implemented in developing countries, where conditions of poverty contribute to an already large burden of pneumococcal disease (25, 26). In Latin America, surveys of reference collections of isolates (13, 24) and laboratory-based surveillance (2, 3, 5, 15) have documented penicillin resistance in up to 20% of clinical isolates. However, significant variabilities in antimicrobial susceptibility and serotype patterns have been observed between and within countries (2, 3, 15). These findings may be due, in part, to regional differences in case ascertainment and isolation procedures (12) and emphasize the importance of population-based information in guiding antibiotic control strategies and national vaccine policies.

Active surveillance for pneumococcal meningitis was established at the state infectious disease hospital in the city of Salvador, Brazil. The state health secretary requires that suspected cases of meningitis from the metropolitan region be referred to that hospital, and more than 95% of the reports of meningitis from the region are reported from that site (secretary of health for the state of Bahia, unpublished case notification records). Therefore, patients referred to that hospital represent patients with population-based cases of meningitis occurring in this city. Between December 1995 and November 1999, 317 patients were consecutively identified with cerebrospinal fluid cultures positive for *S. pneumoniae*, as determined by morphology on Gram staining of smears, susceptibility to optochin (Difco Laboratories, Detroit, Mich.), and bile solubility (BBL Microbiology Systems, Cockeysville, Md.). After enrollment of patients by protocols approved by the institutional review boards of the Brazilian Ministry of Health and Weill Medical College of Cornell University, information on the demographic characteristics and the clinical presentations of the patients was obtained for 305 patients (97% of 317 patients) during interviews or medical chart review. On the basis of the fact that 140 patients who resided within the municipal borders of Salvador (population in 1996, 2,211,467) (14) had pneumococcal meningitis, the annual incidences of pneumococcal meningitis were 1.6 and 24.7 per 100,000 person-years for all age groups and children <5 years of age, respectively. The overall mortality rates were 42% (127 of 305) for all patients and 60% (92 of 153) for those <5 years of age.

Among 303 isolates tested by the broth microdilution method (21), 46 (15%) were penicillin nonsusceptible (MICs, 0.125 to 1.0 μg/ml) and all were susceptible to cefotaxime (Table 1). Patients <5 years of age had a greater chance of being infected with penicillin-nonsusceptible isolates than those ≥5 years of age (35 versus 9%, respectively; odd's ratio [OR], 4.8; 95% confidence interval [CI], 2.2 to 11.4). In addition, greater proportions of isolates from patients <5 years of age were not susceptible to co-trimoxazole (41% [60 of 145]) or tetracycline (27% [39 of 145]). The drugs involved in the most frequently identified patterns of multidrug nonsusceptibility, defined as being intermediate or resistant to two or more classes of antibiotics, were penicillin and co-trimoxazole (33 of 303 isolates [11%]) and tetracycline and co-trimoxazole (33 of 303 isolates [11%]). Children <5 years of age had a greater

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TABLE 1. Antimicrobial susceptibilities of 303 *S. pneumoniae* isolates from patients with meningitis identified during 4 years of active surveillance in Salvador, Brazil

Antimicrobial agent	MIC (μg/ml)[a]			No. (%) of strains that were[b]:		
	Range	MIC_{50}	MIC_{90}	S	I	R
Cefotaxime	0.008–0.5	0.016	0.125	303 (100)	—	—
Chloramphenicol	0.062–8	2.0	4.0	297 (98.0)	NA	6 (2.0)
Clindamycin	0.008–0.5	0.032	0.125	303 (100)	—	—
Erythromycin	0.008–1	0.032	0.0625	301 (99.3)	2 (0.7)	—
Ofloxacin	0.031–4	1.0	2.0	299 (99.0)	4 (1.0)	—
Penicillin	0.008–1	0.016	0.25	257 (85.0)	46 (15.0)	—
Rifampin	0.008–4	0.016	0.0625	302 (99.7)	—	1 (0.3)
Tetracycline	0.031–32	0.25	32.0	219 (72.0)	16 (5.0)	68 (23.0)
Co-trimoxazole	0.062–8	0.5	4.0	198 (65.0)	75 (25.0)	30 (10.0)
Vancomycin	0.016–1	0.5	0.5	303 (100)	NA	—

[a] MICs were determined by the broth microdilution method (21). MIC_{50} and MIC_{90}, concentrations at which the growth of 50 and 90%, respectively, of the isolates is inhibited.

[b] S, susceptible; I, intermediate; R, resistant; NA, not applicable. The breakpoints used to define susceptibility categories were those recommended by the National Committee for Clinical Laboratory Standards (21). —, no isolates were identified.

chance of being infected with penicillin- and co-trimoxazole-nonsusceptible isolates (OR, 4.3; 95% CI, 1.7 to 11.4).

The 303 isolates were distributed among 29 serogroups and 43 serotypes, as determined by the capsular swelling method with type-specific antisera (Centers for Disease Control and Prevention, Atlanta, Ga.) (Table 2). Among isolates from 145 patients <5 years of age, none were serotype 1 and 2.7% (4 of 145) were serotype 5, whereas serotype 3 was isolated from 2.1% (3 of 145) of the patients. In patients <5 years of age, 74, 77, and 82% were infected with isolates with serotypes that are represented in the 7-, 9-, and 11-valent conjugate vaccines, respectively (Table 2).

Penicillin-nonsusceptible isolates were restricted to six serotypes: 14 (n = 26 [56%]), 6B (n = 10 [22%]), 23F (n = 5 [11%]), 19F (n = 2 [4%]), 19A (n = 2 [4%]), and 23B (n = 1 [2%]). Serotypes 14 (OR, 18.3; 95% CI, 8.5 to 39.3) and 6B (OR, 3.7; 95% CI, 1.6 to 8.6) were associated with decreased sensitivity to penicillin ($P < 0.05$). Among the 35 penicillin-nonsusceptible isolates from patients <5 years of age, 94% (33 of 35) had serotypes represented in the three conjugate vaccines.

The finding that a significant (15%) proportion of cases of pneumococcal infection identified during population-based surveillance were due to penicillin-nonsusceptible pneumococci has important implications for the treatment of pneumococcal meningitis in Brazil. Penicillin, often in combination with chloramphenicol, is the recommended empirical treatment regimen for meningitis (9). The current consensus is that penicillin and chloramphenicol are not adequate for the treatment of meningitis caused by resistant pneumococci, even when strains demonstrate intermediate-level resistance (4, 8, 16). In the present study, all penicillin-nonsusceptible isolates were susceptible to cefotaxime, indicating that an extended-spectrum cephalosporin alone may be an appropriate alternative treatment for penicillin-resistant pneumococcal meningitis and empirical, initial therapy for suspected bacterial meningitis.

Our findings on serotype distributions support those from laboratory-based surveys in Brazil (2, 5, 24), indicating that a limited spectrum of serotypes is responsible for invasive pneumococcal disease (10, 15, 17). However, the pattern of predominant serotypes observed in the present study differs from those reported in other regions in Brazil (2, 24) and other

TABLE 2. Serotypes of pneumococcal isolates from meningitis patients in Salvador, Brazil, by age group[a]

Capsular serotype	No. (% of total) of isolates from patients:		
	Ages <5 yr	Ages ≥5 yr	Total
Total	145	146	291
14	40 (27.4)	1 (0.7)	41 (14.0)
3	3 (2.1)	27 (18.5)	30 (10.0)
6B	17 (11.6)	9 (6.2)	26 (9.0)
19F	10 (6.8)	15 (10.0)	25 (8.6)
6A	12 (8.2)	5 (3.4)	17 (5.8)
23F	7 (4.8)	9 (6.2)	16 (5.5)
18C	9 (6.2)	5 (3.4)	14 (4.8)
4	6 (4.1)	7 (4.8)	13 (4.5)
8	6 (4.1)	7 (4.8)	13 (4.5)
10A	2 (1.4)	8 (5.5)	10 (3.4)
9N	3 (2.1)	5 (3.4)	8 (2.7)
7F	4 (2.7)	2 (1.4)	6 (2.1)
5	4 (2.7)	1 (0.7)	5 (1.7)
23B	—[b]	5 (3.4)	5 (1.7)
13	1 (0.7)	4 (2.8)	5 (1.7)
11A	—	4 (2.8)	4 (1.4)
15B	—	4 (2.8)	4 (1.4)
17F	3 (2.1)	1 (0.7)	4 (1.4)
18F	3 (2.1)	1 (0.7)	4 (1.4)
19A	2 (1.4)	2 (1.4)	4 (1.4)
Other types[c]	13 (8.9)	24 (16.4)	37 (12.7)
23-valent polysaccharide vaccine types[d]	131 (90)	115 (79)	246 (85)
7-valent conjugate vaccine types[e]	108 (74)	54 (37)	162 (56)
9-valent conjugate vaccine types[f]	112 (77)	56 (39)	168 (58)
11-valent conjugate vaccine type[g]	119 (82)	85 (58)	204 (70)

[a] Results are shown for the 291 isolates for which information on patient's age was recorded.

[b] —, no isolates were identified.

[c] Other types include 18B (n = 3), 28A (n = 3), 7B (n = 3), 7C (n = 3), 9V (n = 3), 10F (n = 2), 16 (n = 2), 21 (n = 2), 34 (n = 2), 35B (n = 2), 1 (n = 1), 12F (n = 1), 15C (n = 1), 18A (n = 1), 22F (n = 1), 24F (n = 1), 27 (n = 1), 28B (n = 1), 35A (n = 1), 35F (n = 1), 38 (n = 1), and 48 (n = 1).

[d] Includes capsular serotypes 14, 3, 6, 19F, 23F, 4, 18C, 8, 10A, 9N, 5, 7F, 11A, 15B, 17F, 19A, 9V, 1, 12F, 20, 22F, 2, and 33F (Merck Sharp & Dohme).

[e] Includes capsular serotypes 4, 6, 9V, 14, 18C, 19F, and 23F (White Lerdele).

[f] Includes capsular serotypes 1, 4, 5, 6, 9V, 14, 18C, 19F, and 23F (Merck Sharp & Dohme).

[g] Includes capsular serotypes 1, 3, 4, 5, 6, 7F, 9V, 14, 18C, 19F, and 23F (Pasteur Merriex).

countries in Latin America (3, 13). Whereas serotypes 1 and 5 account for 8 to 12% of isolates from pediatric patients in other Brazilian cities (2), Chile (18), Colombia (3), and Uruguay (13), these serotypes were found in less than 3% of isolates from pediatric patients in Salvador. These differences may be due, in part, to true geographical differences in serotype prevalences, but they most likely reflect differences in surveillance methodologies and the patient populations sampled (11, 12).

On the basis of the results of the present surveillance study, 74% of the patients <5 years of age with pneumococcal meningitis were infected with isolates with serotypes represented in the seven-valent conjugate vaccine; this proportion is similar to that found for pediatric patient groups in the United States and other countries (23). Furthermore, conjugate vaccines may have an important additional benefit in reducing the transmission of antibiotic-resistant strains. In our study, 94% of the penicillin-nonsusceptible isolates from pediatric patients were restricted to four serotypes (serotypes 14, 6B, 23F, and 19F). These serotypes are represented in conjugate vaccines that have been shown to be effective in reducing nasopharyngeal carriage of antibiotic-resistant strains with serotypes covered by the vaccines (6, 20). In developing countries like Brazil, the significant burden associated with the treatment of penicillin-resistant pneumococcal disease in the future may justify serious consideration of the use of efficacious, albeit costly, conjugate vaccines.

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3. *Nasopharyngeal Carriage of* Streptococcus pneumoniae *in a community: Results of a Household-based Survey.*

Objetivo: Com o objetivo de determinar a prevalência e os fatores de risco para a colonização nasofaringeana pelo *S. pneumoniae* em indivíduos saudáveis na comunidade, 39 domicílios no bairro de Nordeste de Amaralina foram selecionados e seus moradores incluídos na investigação que se procedeu com uma única coleta de swab nasofaringeano.

a. Determinar a prevalência de colonização nasofaringeana em indivíduos saudáveis.
b. Identificar fatores de risco para a colonização nasofaringeana pelo *S. pneumoniae*.
c. Determinar a resistência à penicilina e a outros agentes antimicrobianos nos pneumococos isolados de nasofaringe.
d. Determinar a prevalência dos sorotipos colonizadores e avaliar a provável cobertura vacinal.
e. Avaliar o comportamento epidemiológico dos isolados de pneumococos através de técnicas de biologia molecular para identificar fatores facilitadores da colonização e disseminação do *S. pneumoniae* na família.

Nasopharyngeal Carriage of *Streptococcus pneumoniae* in a community: Results of a Household-based Survey

Running Title: Household Survey of *S. pneumoniae*

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Abstract

Conjugate vaccines may be an important intervention in addressing the emergence of antimicrobial resistant *Streptococcus pneumoniae* in developing countries. To investigate nasopharyngeal carriage of antimicrobial resistant pneumococci in healthy individuals from Salvador, Brazil, we conducted a cross-sectional study among residents of 39 randomly selected households in an urban slum community. Among 262 subjects, 95 (36%) had pneumococcal carriage. Of these, 9 (10%) and 28 (30%) had isolates with intermediate-resistance to penicillin and resistance to TMP/SMX, respectively. The most prevalent serotypes were 19F (13%) and 6A (11%), followed by 14 (7%), 23F (7%), 18C (6%) and 19A (5%). Seven percent were non-typeable. The theoretical vaccine coverage of the 7-valent conjugate vaccine is 49% (46 of the 94) for all individuals and 57% (19 of the 33) for children less than 5 years of age. Penicillin nonsusceptible isolates belonged to serotypes 19F, 14, 6B, 6A, 23F, 46 and non-typeable. Seven isolates (78% of penicillin nonsuceptible isolates) were represented in the 7-valent conjugate vaccine. BOX-PCR and PFGE typing showed that penicillin-nonsusceptible strains had unrelated fingerprint patterns. In contrast to studies of isolates from patients with invasive disease in Brazil, penicillin-nonsusceptible pneumococci associated with carriage do not appear to be clonally related. Furthermore, a limited proportion of these strains have serotypes represented in the conjugate vaccine.

Introduction

Streptococcus pneumoniae is one of the major causes worldwide of severe infections such as meningitis, septicemia, and respiratory tract infections. Risk groups for pneumococcal infections are young children under the age of 2 years, elderly people, and immunocompromised patients [1]. Pneumococcus is often part of the nasopharyngeal flora and all humans are likely to be colonized with this organism at least once during infancy [2]. The colonization of infants provides an important reservoir for the spread of this organism to other susceptible individuals who may develop disease [3, 4].

In Salvador, a city in northeastern Brazil, active surveillance for pneumococcal meningitis during a two-year period found an annual incidence of 31.7 and 8 cases per 100,000 person-years in children under one and five years of age, respectively [5]. Children under the age of two years had a significantly increased risk of acquiring penicillin nonsusceptible isolates than children two years or older. Furthermore, a penicillin non-susceptible serotype 14 clone was identified and may have disseminated to widely geographic areas in Brazil.

It was hypothesized that the exposure to *S. pneumoniae* nonsusceptible to penicillin may occur in their residence, whereas factors identified in developed countries as attendance at a day care center, chronic care facility, nursing homes and previous hospitalizations [6], were not associated with meningitis due to penicillin nonsusceptible isolates. A cross-sectional study was conducted in a community to provide epidemiological data in conjunction with phenotypic and genotyping methods to evaluate clonality and factors facilitating the colonization and spread of *S. pneumoniae* within families.

Patients and Methods

Study Population. The survey was carried out from July 2000 to May 2001 in Salvador, state of Bahia, Brazil in a densely populated, low-income community. The census district was composed of 18 census tracts and has a population of 23,980 [7]. One census tract with 1338 inhabitants residing in 296 domiciles was chosen because it contained only residential buildings. The perimeter of the target community was defined as a single census tract based on 1996 census information and the area was then stratified into four areas based on the location of the four main stairways. Within each of these areas, beginning from the main street, every fifth house was selected for inclusion in the study. If the fifth house declined to participate then the sixth or seventh house was selected until at least ten households were enrolled. A total of 39 (13% of 296) households were sampled. All persons at home at the time of the survey who were living continuously in the household for more than one month were eligible. Written informed consent was obtained from the head of household.

At the time of sample collection, a questionnaire was administered to all study subjects; information for children was obtained from the parent or legal guardian. Information collected included demographic data, underlying medical conditions, hospitalizations, occurrence of symptoms for an upper respiratory tract infection (URTI) in the previous month, recent antibiotic therapy, number of family members, childcare arrangements, school attendance, siblings, and habits such as smoking.

Isolation of Pneumococci. Nasopharyngeal specimens were collected with calcium alginate swabs (Calgiswab type 1. Spectrum USA) and immediately inoculated onto tryptic soy agar plates with 5% sheep blood and 5.0 μg/ml gentamicin. Plates were incubated at 35°C in 5%

CO_2-enriched atmosphere for up to 48 hours. Three to five colonies exhibiting morphologic characteristics of *S. pneumoniae* were isolated. Pneumococci were identified according to Gram stain morphology, optochin sensitivity (Difco Laboratories, Detroit, USA) and bile solubility (BBL Microbiology Systems, Cockeysville, USA).

Antibiotic Susceptibility testing. The disk-diffusion method was performed according to National Committee for Clinical Laboratory Standards (NCCLS) recommendations [8] to determine susceptibility of isolates to oxacillin, cefotaxime, tetracycline, erythromycin, clindamycin, trimethoprim-sulfamethoxazole, vancomycin, and chloramphenicol (BBL Microbiology Systems, Cockeysville, USA). Isolates exhibiting an inhibition zone diameter less than a 20 mm surrounding a 1μg oxacillin disk were further tested for penicillin and ceftriaxone MIC by the E-test (AB Biodisk, Solna, Sweden) [9]. Isolates with a penicillin MIC value $\geq$ 0.1 μg/ml were considered penicillin-nonsusceptible. Isolates with a ceftriaxone MIC value $\geq$ 0.5μg/ml were considered ceftriaxone-nonsuscpetible. *S. pneumoniae* ATCC 49619 was used as the control strain in the study.

Serotyping. Serogrouping and serotyping was done on overnight cultures of the individual colonies by the capsular swelling method with type-specific antisera (Centers for Disease Control and Prevention, Atlanta, Ga.). Isolates negative by all pooled sera and to omniserum were defined as nontypeable.

BOX A PCR strain typing. The Box A typing method was performed as described by Ko et al. [5]. Briefly, pneumococcal DNA was amplified by PCR (7 min at 94°C [predenaturation]; 35 cycles of 1 min at 94°C, 1 min at 53°C, 8 min at 65°C and a final step for 16 min at 65°C, using BOX-A primer [10]. The amplified products were separated on a 1.5% agarose gel.

Gels were stained with ethidium bromide, and photographed under ultraviolet transillumination, and the banding patterns were evaluated visually. The digitized gel images were also analysed by the Windows version of the Gel compar software version 4 (Applied Maths, Kortrijk, Belgium). Comparison of the banding patterns was performed using the Dice similarity coefficient and dendogram were constructed using the unweighed-pair-group method using average linkages (UPGMA) and with the. The bands were assigned manually, according to densitometric curves and the accompanying copy of photograph. A tolerance of 1.5% in band positions was applied during comparison of the DNA patterns.

Pulse-field gel electrophoresis (PFGE) method. Chromosomal DNA fragments generated by *SmaI* digestion were prepared and analyzed as described elsewhere [11]. A CHEF-DRII apparatus (Bio-Rad, Hercules, CA) was used for running the gels. Running conditions were 23 hours at 14 °C at a voltage of 6 V/cm ramped with initial forward time of 2s and final forward time of 30s. Interpretation of relatedness of isolates was performed according to the Tenover criteria [12].

The genotypes of the pneumococcal isolates were also compared with a local collection of pneumococcal strains from a prospective surveillance for all cases of meningitis identified in Salvador, since 1996 to determine whether the major resistant or susceptible clones were present in this community (unpublished observation).

Statistical analysis. The epidemiological database was created and analysed with the use of Epi-Info Version 6.04 (Center for Disease Control and Prevention, Atlanta, GA). Fisher's exact test or χ^2 test was used to measure associations between variables for categorical data. Odds ratios were adjusted (using the GENMOD procedure in SAS) and generalized

estimating equations for the dependence among individuals in the same household. The two-sample Wilcoxon rank-sum test was used to compare differences in means for continuous variables. $P<0.05$ was accepted as statistically significant.

Results

In a densely populated urban community with an estimated population of 23,980 39 (out of 296) households were randomly selected House visits were conducted between July, 2000 and May, 2001 to identify eligible residents, collect household information and perform nasopharyngeal swabs. Of 282 eligible residents, 262 (93%) consented to be enrolled in the study. The median monthly household income was 171 U.S. dollars and the mean of inhabitants was 7 (range, 3 to 14). Among households visited, 79% (31 of 39) had at least one child aged less than 5 years of age and 36% had at least one child less than 2 years.

The adjusted prevalence of *S. pneumoniae* carriage was 36% (95% Confidence Interval [CI]: 28%, 44%), adjusting for sample design. Analyzed by household, 25 of 39 (64%) had two or more inhabitants colonized. The prevalence of carriage decreased significantly with increasing age (figure 1). The prevalence was 65% (35 of 50) among children less than 5 years, 44% (43 of 95) among 5 – 17, and 18% (19 of 117) among adults older than 17 years.

Of the 95 pneumococcal isolates obtained, 51 (54%) were fully susceptible to all antimicrobial drugs tested. The remaining 44 (46%) showed decreased susceptibility to at least one of the nine antibiotics tested: 18 (19%) were resistant to two or more antibiotics and 5 of these (5% of 95) were resistant to 3 or more antibiotics (Table 1). Among 22 isolates identified to be oxacillin resistant, only 9 (41% of 22) were nonsusceptible to penicillin when tested by E-test. All 9 isolates that were penicillin nonsusceptible were also found to be nonsusceptible to cotrimoxazole. In addition to penicillin and cotrimoxazole resistance, 4 of these strains showed resistance to other antibiotics: tetracycline and chloramphenicol (n=1), tetracycline (n=1), chloramphenicol (n=1), erythromycin (n=1).

Among the 95 isolates tested, the frequency resistance to antibiotics other than penicillin was 29% for cotrimoxazole, 15% for tetracycline, 4% for chloramphenicol and 2% for erythromycin. Resistance to ceftriaxone, vancomycin, rifampin or clindamycin was not detected.

Among the 9 penicillin-nonsusceptible isolates, 5 (54%) were recovered from children less than 5 years old. Risk factors for carriage of penicillin nonsusceptible pneumococci were not examined because of the small number of individuals who were found to carry such strains. No significant differences in cotrimoxazole resistance between pneumococci isolated from children and adults were found (figure 1).

Serotyping was performed for 94 (99%) of 95 pneumococcal isolates. In all, 32 different serotypes were identified. The most prevalent were 19F (13% of 94) and 6A (11%). Serotypes 14, 23F and non-typeable isolates each accounted for 7%, while serotypes 19A and 18C accounted for 6% and 5%, respectively. In households in which more than one inhabitant was colonized, 21 of 33 inhabitants shared the same serotype. We also checked for multiple colonization by picking 5 well-separated colonies from primary cultures of nasopharyngeal specimens. For multiple colonies typed from the same individual, all colonies were phenotypically and genetically identical.

Based on the serotype distribution of all isolates identified from nasopharyngeal carriage, the theoretical percentage of coverage with the 7-valent conjugate vaccine is 49% and for isolates from children less than 5 years of age, the percentage of coverage is 57%. Type 6A, which cross-reacts with type 6B was included in the vaccine type group. No children less than 5

years of age was colonized with two of the 7 vaccine serotypes, 4 and 18C, while only 3% of the children were colonized with serotypes 9V and 6B.

Penicillin nonsusceptible strains belonged to serotypes 19F (n=2), 14 (n=2), 6B (n=1), 6A (n=1), 23F (n=1), 46 (n=1) and non-typeable (n=1). As 7 of 9 penicillin nonsusceptible isolates were vaccine serotypes, the theoretical coverage of the 7-valent vaccine for penicillin-resistant disease would be 78%, based on this small sample.

In univariate analysis accounting for the clustered study design, age less than 17 years, school attendance and an URTI in the last month were associated with nasopharyngeal carriage of *S. pneumoniae* (Table 2). For children less than or equal 2 years, breastfeeding was negatively associated with pneumococcal carriage. None of the other variables was significantly associated with pneumococcal nasopharyngeal carriage. Independent risk factors for carriage in multivariate logistic regression were age group less than 5 years old, URTI in the last month and school attendance, while breastfeeding was protective (Table 2).

Ninety-four of the *S. pneumoniae* isolates were evaluated using Box-A PCR, which generated 53 different DNA patterns. PFGE was used to confirm the clonal relationship between the isolates. Fifty-two isolates (55%) had unique patterns, while 42 isolates belonged to 15 clonal groups ranging in size from two to four isolates. Eight of these 15 clonal groups contained multiple serotypes (Table 3). Exceptions for 2 isolates serotype 14, which were identical by Box-A and PFGE, the other 7 penicillin nonsusceptible isolates had distinct DNA patterns.

In 23 (68% of 34) domiciles were found individuals colonized with clustered isolates (Figure 2). Within 10 domiciles were found 2 or more individuals colonized with isolates belonged to

same clonal group. The isolates at 4 of these domiciles had different serotypes despite their clonally related DNA pattern (Figure 3).

Comparison of the BoxA PCR patterns with those generated from 362 invasive pneumococcal isolates demonstrated that 17 (18% of 94) isolates were identical or closely related with previously studied meningitis isolates, being 5 cluster patterns (15 isolates) and 2 non-clusters patterns (Table 3). Two penicillin nonsusceptible serotype 14 isolates from colonized individuals were closely related to a previously studied serotype 14 penicillin nonsusceptible clone from meningitis cases. In addition, 10 strains found in the carriage study were clonally related to 3 penicillin susceptible clones responsible for meningitis cases in Salvador (Figure 4).

Discussion

S. pneumoniae was carried by 36% of the 262 healthy individuals enrolled in the study. The high prevalence (65%) of pneumococcal carriage among children less than 5 years old was similar to those reported in developing countries, [13, 14] including one study in urban northeastern Brazil that reported carriage of 50% among both healthy children and children with pneumonia [15]. However, the prevalence found in our study is higher than the 35% reported among children with acute rhinopharyingitis in São Paulo, Brazil [16].

The prevalence of penicillin nonsusceptible isolates in our study (9%) agrees with the 16% prevalence among isolates reported by Ferreira et al [16] for São Paulo, but was much lower than the 49% reported by Rey et al [15] for Fortaleza. Results obtained from different studies in different populations may not be comparable because these studies have used different sampling methodologies, with not all samples being representative of a population.

Previously, surveillance for antimicrobial resistance and serotype distribution among pneumococci in a community survey of nasopharyngeal carriage isolates of *S. pneumoniae* was found to correlate well with invasive isolates [17]. Our results support this. In our community, 9% of nasopharyngeal isolates from healthy individuals were penicillin-nonsusceptible, consistent with the 15% prevalence of penicillin-nonsusceptible isolates from meningitis cases in this community [18]. Among both invasive and non-invasive isolates in this area, a high rate of resistance to cotrimoxazole was observed. Among children less than 5 years old, 36% of the isolates were resistant and 12% had intermediate resistance. This suggests a major problem with respect to treatment of community-acquired pneumonia in developing countries, where WHO recommends the use of cotrimoxazole as a first-line agent.

A majority (57%) of noninvasive isolates from children < 5 were serotypes included in the current 7-valent pneumococcal conjugate vaccine. This percentage is lower than the 74% found among isolates from meningitis cases in this area [18]. We could not compare these data with other Brazilian regions, because this study is the first that we know of to estimate the serotype distribution in the community using nasopharyngeal isolates. Surveys of nasopharyngeal carriage of *S. pneumoniae* in healthy children could rapidly provide a large number of isolates than invasive disease surveillance and has been recommended to predict features of invasive diseases.

Currently, routine vaccination of all children with the pneumococal-CRM197 conjugate vaccine is our best strategy for reducing the burden of early-childhood pneumococcal disease. Vaccination interrupts carriage of homologous serotypes, which may be indirect evidence of potential efficacy against invasive disease. Continued surveillance of the distribution of

pneumococcal serotypes and patterns of drug resistance is necessary and will dictate the development of future approaches.

Univariate and multivariate analyses indicate that age, previous infections of the upper respiratory tract in the last month and school attendance may favor *S. pneumoniae* carriage, while breastfeeding has a protective effect. Unlike other authors' findings [3, 19, 20], nasopharyngeal carriage of *S. pneumoniae* were not related with day care attendance, antibiotic use in the last month and crowded environment, such as the number of people per house. These differences may be because of sample size or epidemiologic characteristics of our population.

Box-A PCR and PFGE investigated the epidemiological behavior of the pneumococcal strains isolated from the healthy individuals in a community. Previous studies have used these techniques to identify clusters of closely related isolates within a population [21, 22]. We used the criterion of a ≤ 3 band difference within a PFGE and Box-A type, which corresponded to a level of similarity on the dendogram ($\geq 85\%$), above which isolates were assigned to the same PFGE type. We believe that a more stringent criterion – grouping isolates with banding pattern differences compatible with a single genetic event [12] is preferable. The prevalence of predominant PFGE types would have been higher as well as the serotype diversity within a clonal group.

Among 94 isolates, 68 different genotypes were observed of which 15 genotypes represented 42 isolates closely related. In contrast to studies of isolates from patients with invasive disease in Brazil [5, 23], penicillin-nonsusceptible pneumococci associated with nasopharyngeal carriage do not appear to be clonally related, being only 22% (2 of the 9

isolates) identical by Box-A and PFGE typing. The most surprising finding in this data was that 8 (53%) clonal groups were composed by multiples serotypes, indicating that serotype shift may occur more frequently when colonizing than causing disease. When we looked at the clonality by domicile, we found two or more individuals colonized by clonal groups in 10 (29%) households and we also found genetic similarities between different serotypes, indicating that a serotype shift might have occurred in at least four of these domiciles. Evidence that capsular transformation takes place in day care centers has earlier been shown [24].

A strength of this study was the availability of a large molecular database for comparison of the PFGE and Box-A patterns from population-based surveillance of meningitis in Salvador. Seventeen (18%) isolates from nasopharyngeal carriage were homologous to previously studied meningitis isolates. Among noninvasive isolates, it was possible to identify genetic similarity with 4 predominant clonal groups responsible for meningitis cases in Salvador during more than 5 years of surveillance. These 4 groups included one penicillin nonsusceptible and 3 penicillin susceptible. These findings may explain the persistence of these clones through several years in our community.

With the advent of pneumococcal conjugate vaccines, a reduction in pneumococcal disease and carriage is expected. In a study reported by Mbelle et al.[25] the conjugate vaccine reduced morbidity and carriage of penicillin resistant pneumococci of vaccine serotypes; however, carriage of nonvaccine serotypes increased. Serotype shift might be a method by which the pneumococci would escape the immune response generated to specific serotypes. This could have implications for the long term efficacy of conjugate vaccines that protect

against only a limited number of serotypes. Detailed molecular epidemiological monitoring of pneumococcal colonization and infection is required to survey the vaccine efficacy.

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Table 1. Drug resistance in nasopharyngeal *Streptococcus pneumoniae* isolates from healthy individuals in Salvador, Brazil

Drug resistance[1]	Isolates, no. (%)			
	Children < 5 yrs (n = 33)	Children 5 – 17 yrs (n = 43)	Adults > 17 yrs (n = 19)	Total (n = 95)
Oxacillin	13 (39)	8 (19)	1 (5)	22 (23)
Penicillin	5 (15)	3 (7)	1 (5)	9 (9)
Erythromycin	1 (3)	1(2)	0 (0)	2 (2)
Tetracycline	8 (24)	4 (9)	2 (10)	14 (15)
Chloramphenicol	2 (6)	0 (0)	1 (5)	3 (3)
Cotrimoxazole	16	15	7	38
Intermediate	4 (12)	5 (12)	2 (10)	11 (11)
Resistant	12 (36)	10 (23)	5 (26)	27 (28)
≥ 2 drugs[2]	11 (33)	4 (9)	3 (16)	18 (19)
≥ 3 drugs[3]	3 (9)	0 (0)	2 (10)	5 (5)

NOTE. Breakpoints (μg/ml) used to define susceptibility categories were those recommended by the National Committee for Clinical Laboratory Standards (ref).

1) Disk diffusion method was used to determine susceptibility to all drugs tested except to penicillin. The E-test method was used to determine penicillin MICs for all oxacillin resistant isolates. The proportion of penicillin nonsusceptible isolates was defined as having a MIC ≥ 0.1 μg/ml to penicillin. All drugs were categorized as resistant except for penicillin and cotrimoxazole that includes breakpoints intermediate and resistant as nonsusceptible.

2) Resistance to 2 or more antibiotics

3) Resistance to 3 or more antibiotics

Table 2: Univariate and multivariate analysis of characteristics potentially associated with pneumococcus carriage.

Characteristics		Pneumococcal carriage		Univariate analysis	Multivariate analysis [a]
		Yes N=95	No N=167	OR (95% C.I.)	OR (95% C.I.)
Age (years)		No (%) of isolates			
	< 2	10 (11)	5 (3.0)	7.9 (2.2-28.7)	14.1 (5.2 – 38.2)
	2 – 4	23 (24)	12 (7.2)	8.9 (4.1-19.4)	8.0 (3.5 – 18.6)
	5 – 17	43 (45)	52 (31.1)	4.7 (2.6-8.7)	1.5 (0.6 – 3.8)
	> 17	19 (20)	98 (58.7)	1.0	1.0
Male Sex		48 (50.5)	65 (39.0)	1.4 (1.0 – 2.0)	1.1 (0.7 – 1.8)
Chronic underlying illness		13 (13.7)	31 (18.6)	0.7 (0.3 – 1.4)	
URTI in the last month		40 (42.0)	38 (22.7)	2.1 (1.3 - 3.6)	1.6 (0.9 – 2.8)
Antibiotic use in the last month		11 (11.6)	15 (9.0)	1.1 (0.5 – 2.6)	
Breastfeeding [b]		4 (4.0)	4 (2.4)	0.1 (0.02 – 0.7)	0.2 (0.05-1.0)
Smokes		4 (4.0)	17 (10.2)	0.6 (0.2 – 1.5)	
Day care attendance (< 5 years)		8 (8.0)	2 (1.2)	1.3 (0.3 – 5.3)	
School attendance (<18 years)		39 (41.0)	41 (24.5)	2.1 (1.4 – 3.0)	2.7 (1.2 – 6.0)
Smokes exposure		43 (45.0)	69 (41.3)	1.1 (0.5 – 2.3)	
Number of people/house					
	< 5	7 (7.4)	14 (8.4)	1.0	
	5-10	58 (61.0)	99 (59.3)	1.1 (0.5 – 2.6)	
	>10	30 (31.6)	54 (32.3)	1.0 (0.4 –2.7)	
Household contact with age:					

	< 5 years	69 (72.6)	129 (77.2)	0.7 (0.3 – 1.4)
	< 10 years	89 (93.7)	149 (89.2)	1.7 (0.5 –4.6)
Household contact w/ pneumococcal carriage		86 (90.5)	148 (88.6)	1.2 (0.5 – 3.1)

NOTE: Odds ratio and 95% CI adjusted for design effect and weighted for number of person per household.

[a] Full model includes Age, sex, at least one episode of URTI in the last month, breastfeeding and school attendance.

b Information obtained for children ≤ 2 years;

Table 3. Distribution of *S. pneumoniae* isolates (N=95) from healthy individuals in Salvador, Brazil, according to their BoxA and PFGE typing patterns.

BoxA/PFGE pattern	No (%) of Isolates (N= 94)	Serotypes (n)	Related typing patterns identified during meningitis surveillance (serotype)
Cluster[1]	42 (45)		
D	4	19F (3), 6B	
P	4	18C(2), 21, 10A	Yes (18C)
L	4	19F, 14, 18C(2)	Yes (18C, 18F, 18B)
B	3	NT	
C	3	23F	Yes (23F)
H	3	19A, 9N, 34	
I	3	6A (2), 15B	
J	3	14, 5, 23B	
N	3	6A	
A	2	14	Yes (14)
E	2	16F	Yes (16F)
F	2	19F, 9N	
G	2	5, 18B	
K	2	23F	
O	2	19A	
Non-cluster	52 (55)		Yes[2]

1) Cluster was defined as groups of isolates closely related – that is, PFGE patterns differing by ≤ 3 bands.

2) 2 isolates serotype 3 were cluster with clonal groups of serotype 3 identified during meningitis surveillance in Salvador.

3) Box represents cluster groups identified with different serotypes.

Table 4. Molecular typing patterns, serotypes and antimicrobial resistance of pneumococci isolated from subjects in representative household.

Household ID	Subject	Molecular strain type	Serotype	Resistance
2	**A**	**H**	**9N**	
	B	**H**	**34**	
	C	PF	5	
	D	I	15B	
	E	O	19A	
	F	PH	6A	TMP, TET
	G	AI	14	TET
	H	AQ	9V	
4	**A**	**G**	**5**	**TMP**
	B	**G**	**18B**	**TMP**
	C	U	18C	
	D	BC	22F	
6	**A**	**L**	**19F**	
	B	**L**	**14**	**TMP**
	C	**L**	**18C**	**TMP**
	D	PJ	6A	
7	**A**	**J**	**14**	
	B	**J**	**5**	
19	**A**	**C**	**23F**	**TMP, TET**
	B	**C**	**23F**	**TMP, TET**
	C	BD	11A	TMP
	D	P	18C	TMP
	E	AC	46	P, TMP, ERI
22	**A**	**B**	**NT**	
	B	**B**	**NT**	
29	**A**	**E**	**16F**	
	B	**E**	**16F**	
	C	AB	19F	P, TMP
	D	AH	6B	TMP
	E	AD	17F	
30	**A**	**N**	**6A**	**TMP, TET, CHL**
	B	**N**	**6A**	**TET, CHL**
	C	**N**	**6A**	**P, TMP, TET, CHL**
	D	PI	34	TMP
38	**A**	**D**	**19F**	**TMP**
	B	**D**	**19F**	**TMP**

	C	P	21	TMP
	D	V	23F	P, TMP
	E	AT	15C	TMP
	F	BA	NT	
39	**A**	**K**	**23F**	**TMP, TET**
	B	**K**	**23F**	**TMP, TET**

NOTE: Antibiotic abbreviations: P, penicillin; TMP, cotrimoxazole; TET, tetracycline; CHL, chloramphenicol;

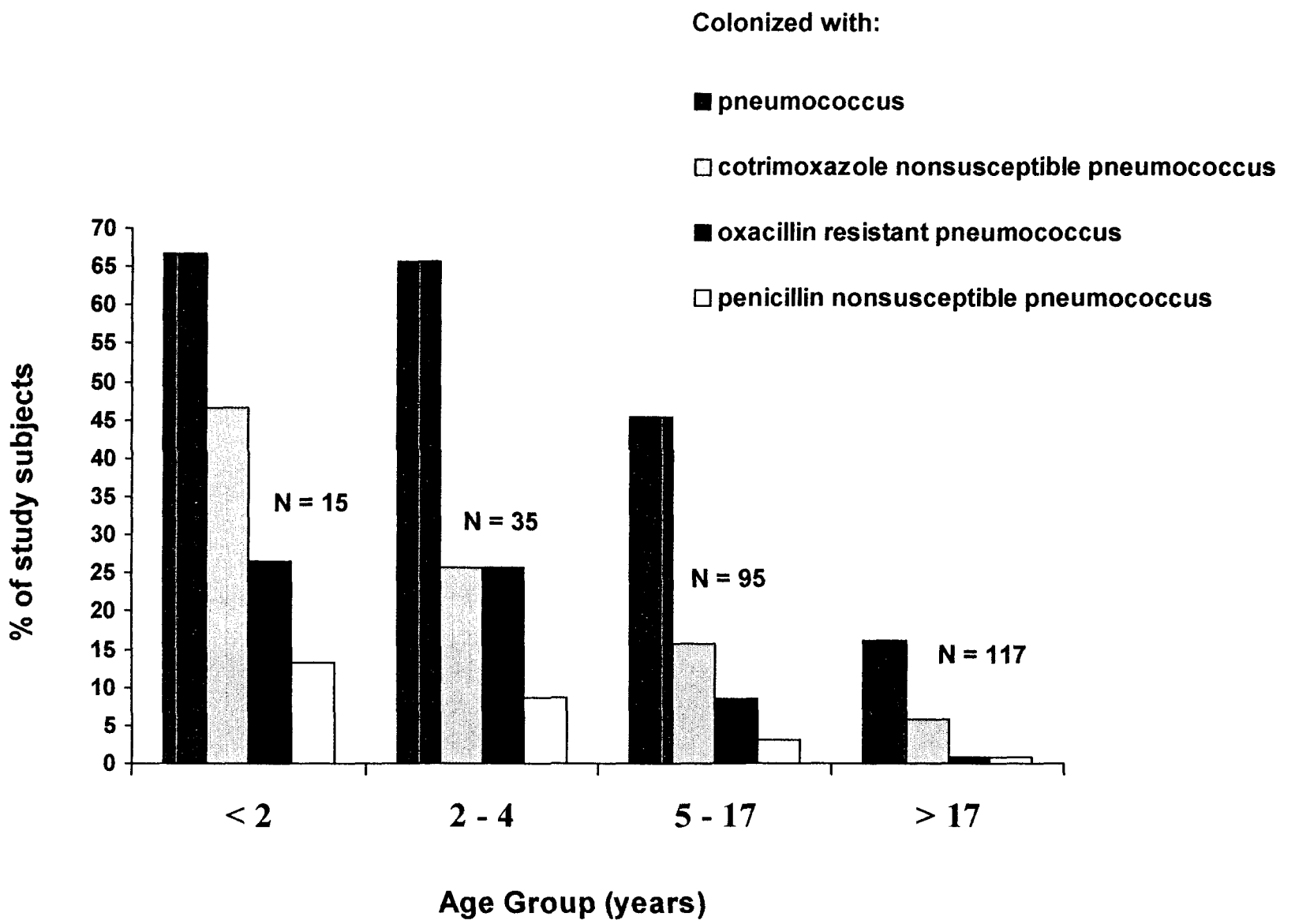
FIGURE LEGENDS

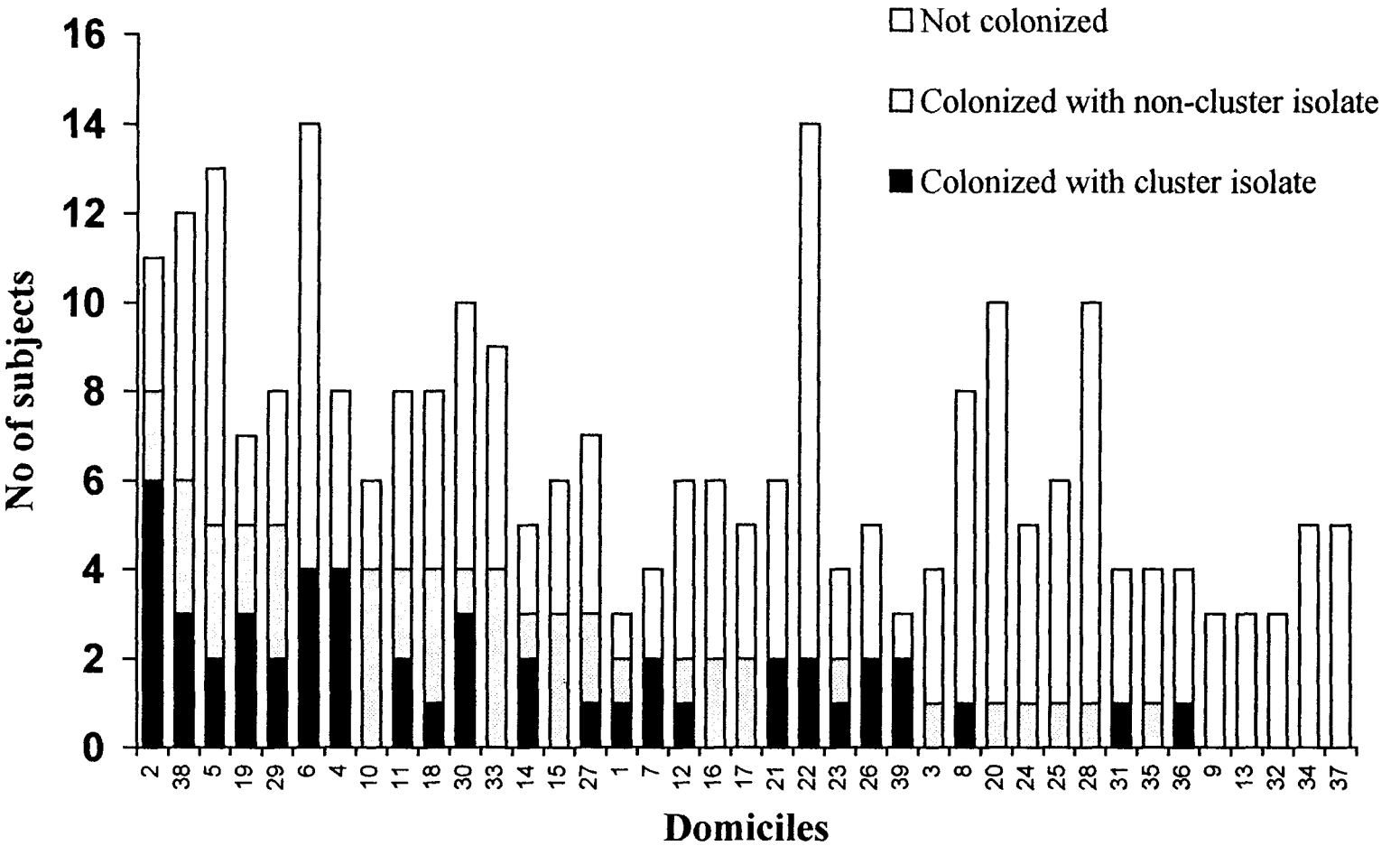
Figure 1: Proportion of individual colonized with *S. pneumoniae* by age group and antimicrobial susceptibility (N=95/262)

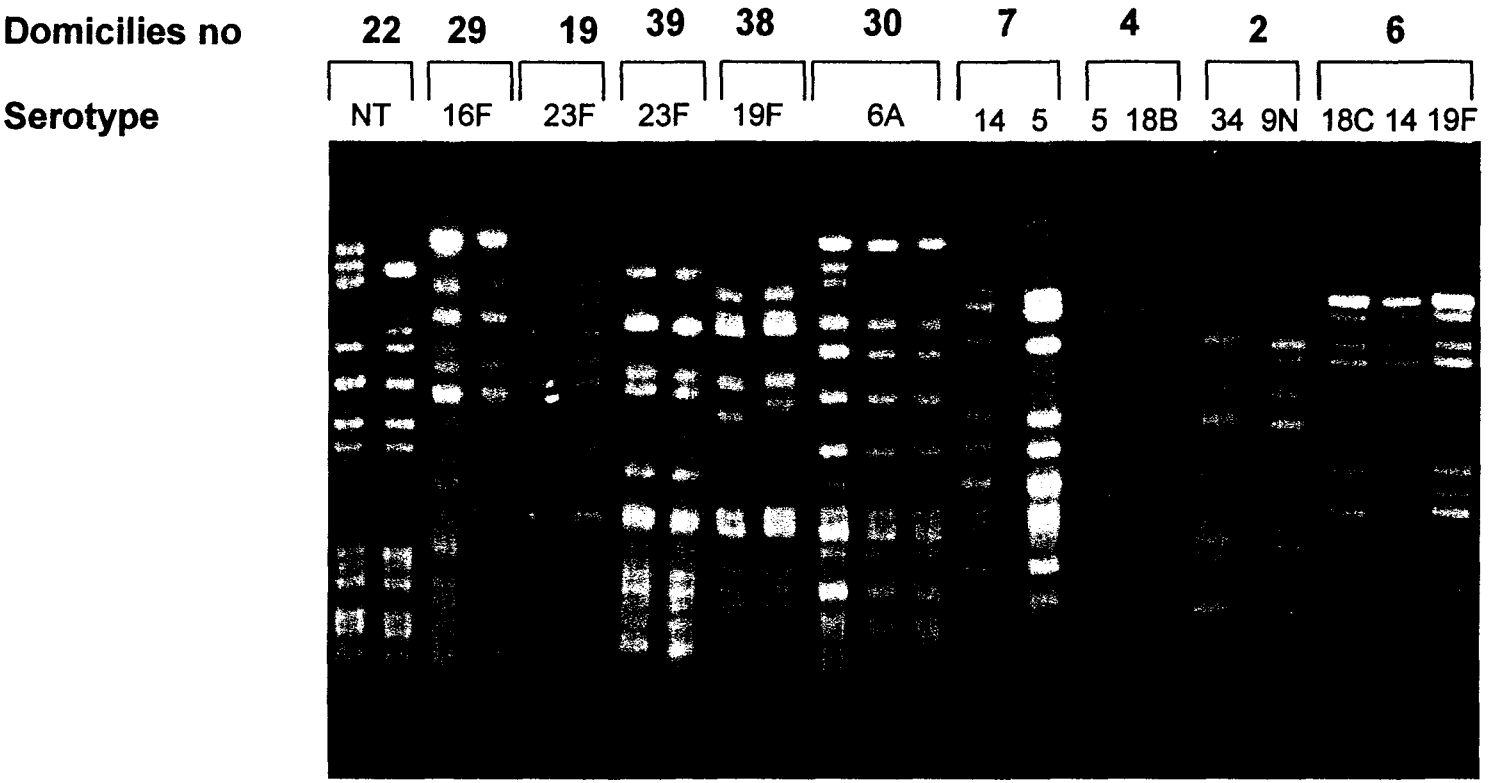
Figure 2: Distribution of clustered isolates per domiciles

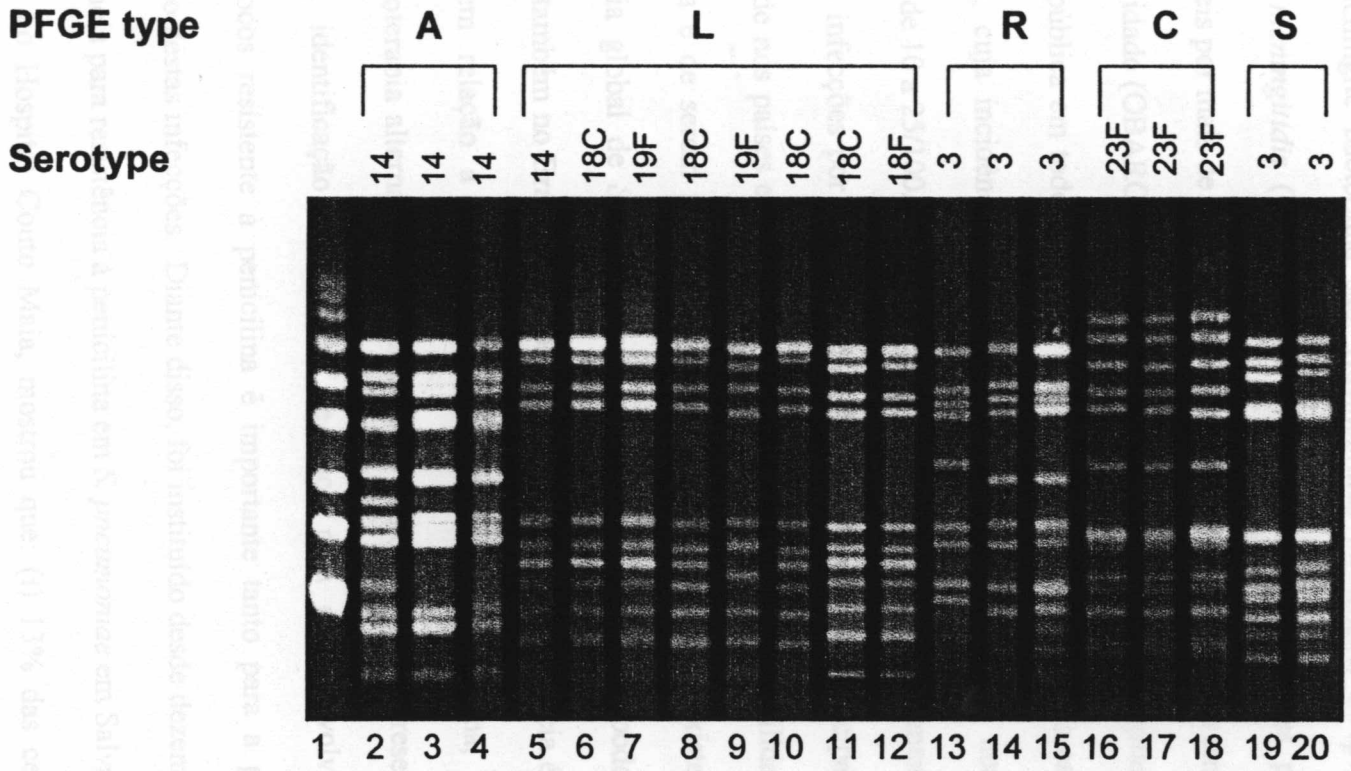
Figure 3: Pulse-field gel electrophoresis of *Sma*I-restricted chromosomal DNA from nasopharyngeal isolates of *Streptococcus pneumoniae* from healthy individuals. Domiciles 22, 29, 19, 39, 38 and 30 are those where isolates with same capsular type and clonally related DNA pattern were found. Domiciles 7, 4, 2 and 6 are those where isolates belonging to different capsular type but an clonally related pattern were found.

Figure 4: Pulse-field gel electrophoresis of *Sma*I-restricted chromosomal DNA. Lane 1, contained a molecular size marker; lanes 2, 9 – 12, 13, 15, 18 and 19 fingerprinting patterns for strains isolated during active surveillance for meningitis cases; lanes 3 –8, 14, 16, 17 and 19 contain nasopharyngeal isolates of S. pneumoniae from healthy individuals.


Colonized with:
pneumococcus
cotrimoxazole nonsusceptible pneumococcus
oxacillin resistant pneumococcus
penicillin nonsusceptible pneumococcus
% of study subjects
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
N = 15
N = 35
N = 95
N = 117
< 2
2 - 4
5 - 17
> 17
Age Group (years)


Not colonized
Colonized with non-cluster isolate
Colonized with cluster isolate
No of subjects
0
2
4
6
8
10
12
14
16
2 38 5 19 29 6 4 10 11 18 30 33 14 15 27 1 7 12 16 17 21 22 23 26 39 3 8 20 24 25 28 31 35 36 9 13 32 34 37
Domiciles


Domicilies no
22 29 19 39 38 30 7 4 2 6
Serotype
NT 16F 23F 23F 19F 6A 14 5 5 18B 34 9N 18C 14 19F


PFGE type
A
L
R
C
S
Serotype
14 14 14 14 18C 19F 18C 19F 18C 18C 18F 3 3 3 23F 23F 23F 3 3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

4 DISCUSSÃO

A meningite bacteriana apesar dos avanços e do desenvolvimento da antibioticoterapia nos últimos 50 anos continua sendo responsável por taxas inaceitáveis de mortalidade e seqüelas (QUAGLIARELO & SCHELD, 1993). As três bactérias que mais freqüentemente causam meningite bacteriana são: *Haemophilus influenzae, Streptococcus pneumoniae* e *Neisseria meningitidis* (WENGER et al., 1990). As infecções por *S. pneumoniae* são responsáveis por mais de 1 milhão de mortes a cada ano no mundo em crianças com menos de 5 anos de idade (OBARO et al., 1996). A infecção por *N. meningitidis* é um grave problema de saúde pública em todo o mundo, com mais de 350.000 casos a cada ano (RAYMOND et al., 1997), cuja incidência varia de 1 a 3/100.000 habitantes nos países desenvolvidos, chegando de 10 a 25/100.000 habitantes nos países em desenvolvimento.

As infecções por *S. pneumoniae* permanecem como principal causa de morbidade e mortalidade nos países em desenvolvimento, apesar da disponibilidade de antibioticoterapia apropriada e de serem preveníveis com o uso de vacinas. Existem evidências de que a emergência global de *S. pneumoniae* resistente à penicilina pode ser um problema em ascensão também no Brasil. O impacto potencial desta resistência é grande em nosso meio, não só em relação à falência do tratamento com penicilina, mas a necessidade de antibioticoterapia alternativa e o aumento de custo que isto representaria para o sistema de saúde. A identificação dos fatores de risco para o desenvolvimento de doença por pneumococos resistente à penicilina é importante tanto para a prevenção como para o tratamento destas infecções. Diante disso, foi instituído desde dezembro de 1995, um sistema de vigilância para resistência à penicilina em *S. pneumoniae* em Salvador, Bahia. Este estudo, realizado no Hospital Couto Maia, mostrou que: (i) 13% das cepas isoladas foram não sensíveis à penicilina; (ii) o uso anterior de antibióticos e idade menor que 2 anos foram fatores de risco para aquisição de meningite pneumocócica não sensível à penicilina; (iii) os

sorotipos dos isolados não sensíveis à penicilina estão presentes na vacina heptavalente conjugada que poderia ser utilizada numa intervenção potencial.

Há relato de cepas de *Haemophilus influenzae* resistentes a ampicilina e a outros antibióticos como o cloranfenicol. Nos países em desenvolvimento os regimes para tratamento para meningite bacteriana em crianças são baseados em antibióticos de baixo custo (CENTER..., 1996). Entretanto, resistência antimicrobiana tem emergido nas bactérias comumente causadoras de meningite na população pediátrica. De forma análoga ao que ocorria com o pneumococo, foi extremamente importante avaliar a prevalência de *H. influenzae* resistente em nosso meio, e os resultados sugerem que o regime inicial recomendado para tratamento de meningite bacteriana no Brasil pode não ser apropriado para o tratamento de meningite por *H. influenzae* [(REIS *et al.*, 2002), em anexo][1].

Com a introdução da vacina contra *H. influenzae* tipo B foi observado uma modificação na epidemiologia das meningites bacterianas. A freqüência de meningite por *Haemophilus influenzae* tipo b diminuiu drasticamente, essa redução chegou a 82% entre 1985 e 1991 em crianças com menos de 5 anos de idade (ADAMS et al., 1993). No Brasil, a vacina contra *H. influenzae* tipo b (Hib) tornou-se disponível na rede pública em 1999, e através do sistema de vigilância ativa para meningite bacteriana nós tivemos a oportunidade para avaliar a incidência de meningite por *H. influenzae* não-b antes e após a introdução da vacina Hib na rotina de imunização [(RIBEIRO et al., 2003), em anexo][2].

O uso na rotina de medidas preventivas para doença meningocócica é limitado. Em alguns países a vacina polissacaridica tetravalente A, C, Y e W-135 é administrada em situações especiais e em casos de epidemias. Esta vacina não é indicada para uso de rotina na população em geral e não protege contra o sorogrupo B. Avanço substancial na prevenção da

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[1, 2]Estes trabalhos foram desenvolvidos com participação da autora Joice Neves Reis Pedreira, no período de realização das atividades concernentes ao Trabalho de Doutorado que constam da presente Tese, sendo por isso anexado a este volume.

meningite bacteriana dificilmente ocorrerá antes da implantação na rotina de vacinas eficazes contra *N. meningitidis* e *S. pneumoniae*. Para isso é importante a identificação de fatores de risco para a aquisição de meningite bacteriana, identificando desta forma, a população a ser vacinada inicialmente.

A compreensão não só de fatores de risco para infecção, mas do mecanismo de resistência e das rotas de transmissão destas cepas na comunidade, parece essencial para proposta de medidas visando à diminuição da transmissão na comunidade e o manejo das infecções que surgirem. Com esta finalidade, foram realizados estudos moleculares associados com dados epidemiológicos para melhor compreender a emergência de resistência desenvolvida por estes patógenos (MCGEE et al., 2001, DAVIES et al., 1999).

Vários estudos têm demonstrado que a emergência global de pneumococos multi-resistentes e altamente resistentes à penicilina se deve em grande parte à disseminação de clones individuais altamente resistentes. Este fenômeno tem sido particularmente evidente pela disseminação mundial do clone de *S. pneumoniae* sorotipo 23F ($Spain^{23F}$-1), primeiramente descrito na Espanha no inicio dos anos 80 (MUNOZ et al., 1991). Este clone tem sido identificado nos Estados Unidos (LAIBLE et al., 1991; CASTANEDA et al., 1998), África do Sul (CASTANEDA et al., 1998), América do Sul (ROSSI et al., 1998) e em vários países europeus (SIBOLD et al., 1992). No estudo relatado no manuscrito 1 foi identificado um único grupo clonal de isolados de sorotipo 14 não sensível à penicilina e ao trimetoprim-sulfametoxazole, que foi responsável por mais de 50% dos casos de meningite por *S. pneumoniae* não sensível à penicilina durante aquele período. Também foi verificado que isolados clínicos de outras 4 cidades brasileiras: São Paulo, Recife, Rio de Janeiro e Brasília tinham padrões idênticos ou relacionados aos isolados de Salvador através dos métodos de Box PCR e PFGE, sugerindo a circulação de um clone específico em nosso país. Após constatar a persistência deste clone na comunidade durante os anos subseqüentes de vigilância

epidemiológica ativa, isolados representativos do clone foram submetidos à rede de Epidemiologia Molecular de Pneumococos (K. Klugman, minuta do 6th encontro de PMEN, p.7, 2002 – Anexo 3).

Embora muitos estudos tenham utilizado métodos de tipagem molecular para estabelecer clonalidade entre os isolados de pneumococos resistentes (DAVIES et al., 1999, ROSSI, 1998, CASTANEDA, 1998), poucos têm atentado para examinar a heterogeneidade genética entre isolados de pneumococos sensíveis aos antimicrobianos. A distribuição clonal das cepas de pneumococos sensíveis e resistentes isolados de pacientes com meningite durante sete anos de vigilância epidemiológica no Hospital Couto Maia foi investigada através dos métodos de Box-A PCR e PFGE. De um total de 477 pacientes, 453 (95%) foram analisados quanto à susceptibilidade antimicrobiana e distribuição de sorotipos, e em 362 isolados foram aplicados os métodos de tipagem molecular. Os resultados prévios deste trabalho serão apresentados no 103^{Q} congresso da sociedade americana de microbiologia que se realizará em Washington, DC no período de 18 a 22 de maio de 2003 (Anexo 4) e posteriormente serão incluídos em um manuscrito.

Estes resultados reforçam a importância da informação obtida de estudos populacionais com respeito à orientação de medidas preventivas como vacinação e controle do uso indiscriminado de antibióticos. Entretanto investigações hospitalares encerram limitações metodológicas devido às elevadas taxas de mortalidade e cultura negativa. Por um outro lado, os estudos sobre colonização tem sido recomendado como instrumento útil na monitorização da prevalência de *S. pneumoniae* resistente na comunidade (CENTER..., 1996). Vários estudos tem encontrado uma boa correlação entre os isolados de nasofaringe e invasivos dentro de uma mesma população (KELLNER et al., 1998). Os nossos resultados confirmam esta correlação quanto ao perfil de susceptibilidade antimicrobiana, porém diferenças na distribuição de sorotipos e na diversidade genética precisam ser investigados em

uma população maior e por um período de vigilância mais amplo.

5 CONCLUSÕES

1. A vigilância hospitalar ativa identificou que 13% dos casos de meningite pneumocócica são devidos a isolados não sensíveis à penicilina.
2. Com base na distribuição de sorotipos das cepas causadoras de meningite em pacientes menores de 5 anos estima-se que a taxa de cobertura pela vacina heptavalente conjugada seria de 74% para esta população.
3. O mesmo trabalho de vigilância identificou a circulação de um clone especifico (Brasil[14]) em nossa comunidade, o qual pode ser responsável pela emergência de *S. pneumoniae* não sensível à penicilina no Brasil.
4. Quatro grupos clonais, sendo um não sensível e 3 sensíveis à penicilina, foram identificados em todos anos de vigilância ativa, indicando a persistência de um limitado repertório de grupos clonais como responsáveis pelos casos de meningite em Salvador, Bahia.
5. O estudo de colonização em indivíduos saudáveis na comunidade identificou que 9% dos pneumococos colonizadores foram não sensíveis à penicilina, enquanto que em isolados de meningite esta taxa foi de 13%, reafirmando a boa correlação bacteriológica nos diferentes espécimes clínicos.
6. Em contraste com o estudo de meningite, pneumococos não sensíveis à penicilina associados com colonização não demonstraram clonalidade.
7. Uma limitada proporção (57%) de sorotipos colonizadores isolados de crianças menores de 5 anos estão representados na vacina heptavalente conjugada.

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7 ANEXOS

Antimicrobial Resistance in *Haemophilus influenzae* Isolted during Population-Based Surveillance for meningitis in Salvador, Brazil.

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Antimicrobial Resistance in *Haemophilus influenzae* Isolated during Population-Based Surveillance for Meningitis in Salvador, Brazil

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Antimicrobial susceptibility was determined for 150 *Haemophilus influenzae* isolates obtained during population-based surveillance for meningitis in Salvador, Brazil. Ten (6.7%) isolates were resistant to ampicillin and chloramphenicol. Of these, two isolates, a β-lactamase and non-β-lactamase producer, were resistant to amoxacillin-clavulinic acid. These findings indicate that present antibiotic regimens in Brazil may not be appropriate for the treatment of *H. influenzae* meningitis.

Haemophilus influenzae remains a major public health problem in the post-*H. influenzae* type b (Hib) conjugate vaccine era, responsible each year for more than 3 million cases of invasive disease and 400,000 deaths worldwide (11). In developing countries, where the *H. influenzae* disease burden is greatest, treatment regimens for bacterial meningitis are based on low-cost antibiotics because of the economic constraints associated with purchasing third-generation cephalosporins; in Brazil, the combination of ampicillin and chloramphenicol is standard initial therapy for suspected bacterial meningitis in the pediatric population (9). However, antimicrobial resistance has emerged in the developing world in bacterial pathogens commonly associated with meningitis (5). In South America, more than 20% of *Streptococcus pneumoniae* clinical isolates are nonsusceptible to penicillin (4). Furthermore, findings from reference laboratory collection surveys (2) and laboratory-based surveillance (14) indicate that *H. influenzae* is resistant to commonly used antibiotics, albeit at levels lower than those observed in developed countries (6). Yet little, if any, information on antimicrobial resistance in *H. influenzae* disease is available from population-based surveillance in South America. Data from surveillance become ever more critical in guiding therapeutic decisions since few hospitals in South America have the laboratory infrastructure to monitor antimicrobial resistance in local catchment populations.

Active population-based surveillance for bacterial meningitis was established in Salvador, a city of more than 2 million inhabitants in northeastern Brazil. According to the state health secretary's regulations, suspected cases of meningitis from the metropolitan region of Salvador are referred to the state infectious disease reference hospital. More than 95% of the case notifications for meningitis from the metropolitan region are identified at this site (case notification records, Secretary of Health for the State of Bahia). Between March 1996 and October 2000, the study team prospectively identified 524 consecutives cases of meningitis with *H. influenzae* isolated from the cerebrospinal fluid. *H. influenzae* was identified based on Gram stain morphology and growth requirement for hemin and β-NAD. Patients were enrolled into the study according to protocols approved by the institutional review board committees of the New York-Presbyterian Hospital and Oswaldo Cruz Foundation/Brazilian Ministry of Health. A sample of 150 (29%) isolates was randomly selected from the 524 identified during surveillance in order to achieve 4% precision for an observed prevalence value of 10% in the sample. The slide agglutination method was performed with type-specific antisera (Difco Laboratories, Detroit, Mich.) and identified 145 (95% of 150), 2 (1.3%), 2 (1.3%), and 1 (0.6%) isolates as serotypes b, a, nonencapsulated, and f, respectively.

The broth microdilution method was used to determine MICs (10). We evaluated susceptibility to antimicrobial agents used in the treatment of bacterial meningitis and acute respiratory infections for which *H. influenzae* is an etiologic agent: ampicillin, amoxicillin-clavulanic acid, cefotaxime, cefaclor, cefuroxime, chloramphenicol, ofloxacin, rifampin, tetracycline, co-trimoxazole (Sigma Chemical Co., St. Louis, Mo.), and azithromycin (Pfizer Ltd., Sao Paulo, Brazil). Antibiotics were added to the *Haemophilus* test medium (Difco Laboratories) in doubling dilution to give final concentrations ranging from 0.008 to 64 μg/ml. Microdilution trays were inoculated with a saline (0.9%) suspension of isolate strains to produce a final inoculum density of approximately 5×10^5 CFU/ml (100-μl final volume) and were examined for growth after 20 to 24 h of incubation at 35°C in ambient air. The MIC was defined as the lowest concentration of the antibiotic that inhibited growth. *H. influenzae* ATCC 49247 and 49766 were included as quality control standards in each assay (10). MIC determination showed that 10 (6.7% of 150) of the isolates were resistant to ampicillin, 16 (10.7%) to tetracycline, 12 (8.0%) to chloramphenicol, and 8 (5.3%) to co-trimoxazole (Table 1). All of the isolates tested were susceptible to cefuroxime, cefotaxime, ofloxacin, and azithromycin.

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TABLE 1. Distribution of MICs for 150 *H. influenzae* isolates from meningitis cases in Salvador, Brazil[a]

MIC (μg/ml)	No. of strains										
	AMP	CEC	CXM	AZM[b]	CEF	CHL	TET	RIF	SXT	OFL	AMC
0.008					78		1	1		3	
0.016	4				63					10	
0.031	2				4	1			2	60	2
0.062		1		1				1	9	77	
0.125	11		1	1					30		1
0.25	71	5	1	1	2		1	13	63		4
0.5	46	16	101	50	1	10	30	131	28		103
1	5	44	42	48	2	106	91	3	6†	35	
2	1†	45	5	2		18	9		4†		3
4		34				3†	2†		4*		
8	2*	4					1*		2*		2*
16	4*	1†				9*	10*	1*	2*		
32	3*					3*	5*				
64	1*										

[a] Data are presented as the number of strains. Breakpoints are those defined by the National Committee for Clinical Laboratory Standards. †, intermediate susceptible; *, resistant. AMP, ampicillin; CEC, cefaclor; CXM, cefuroxime; AZM, azithromycin; CEF, cefotaxime, CHL, chloramphenicol; TET, tetracycline; RIF, rifampin; SXT, co-trimoxazole; OFL, ofloxacin; and AMC, amoxicillin-clavulanic acid.

[b] Only 103 isolates were tested.

Multidrug resistance, defined as being intermediate or resistant to two or more classes of antibiotics, was found in 9.3% (14 of 150) of the isolates tested. The most frequently identified pattern was resistance to ampicillin, chloramphenicol, and tetracycline (10 isolates, 6.7%) and was followed by resistance to chloramphenicol and tetracycline (3, 2.0%). All 14 multidrug-resistant isolates were nonsusceptible to chloramphenicol, and 10 (6.7% of 150) isolates were resistant to ampicillin and chloramphenicol.

Nine of 10 (90%) ampicillin-resistant isolates were identified as β-lactamase producers, while one was β-lactamase negative and ampicillin resistant (BLNAR), according to the nitrocefin disk method (Cefinase; BBL Microbiology Systems, Cockeysville, Md.). All 139 ampicillin-susceptible isolates were β-lactamase nonproducers, including the five isolates for which cefotaxime MICs were relatively elevated (0.25 to 1.0 μg/ml). Two isolates were resistant to amoxicillin-clavulanic acid; one was BLNAR while the other was β-lactamase positive and amoxicillin-clavulanic acid resistant (BLPACR). The amoxicillin-clavulanic acid MICs for the BLNAR and BLPACR isolates were 8.0/4.0 μg/ml, whereas the cefotaxime MIC for only the BLNAR isolate was relatively elevated (1.0 μg/ml).

Our study found that a significant proportion (6.7%) of patients in Salvador, Brazil, developed meningitis due to strains resistant to ampicillin and chloramphenicol, antibiotics used routinely for the empirical treatment of bacterial meningitis in Brazil. These population-based findings corroborate those obtained from reference laboratory surveys in Brazil (2, 14) and South America (3), albeit in certain countries, such as Argentina, Chile, and Mexico (7), ampicillin resistance was identified in more than 20% of the clinical isolates. Together, these findings indicate that the choice of antibiotic regimen for the empirical treatment of bacterial meningitis may need to be reevaluated in Brazil and parts of South America where ampicillin and chloramphenicol are used. During surveillance in Salvador, all isolates were susceptible in vitro to third-generation cephalosporins, indicating that they would be an appropriate alternative. However, the cost of treating a patient with these alternative regimens can be up to 10 times greater than that for ampicillin and chloramphenicol (12) and represents a significant burden in regions such as Salvador, where the per capita public sector health expenditure is less than U.S. $20 each year.

Identification of *H. influenzae* BLNAR and BLPACR strains during surveillance in Salvador appears to be the first reported from Brazil and South America. The mechanism for resistance in BLNAR strains is believed to be associated with modifications in the penicillin binding proteins (13) and/or decrease in cell wall permeability (8). BLNAR strains may be resistant to β-lactams in combination with a β-lactamase inhibitor, as observed for the amoxicillin-clavulanic acid-resistant BLNAR isolate identified in this study and to first- and second-generation cephalosporins (1). Recent large surveys have not identified BLNAR and BLPACR among *H. influenzae* clinical isolates in South America (3, 6, 7). The isolation of these resistant strains in Salvador indicates that a new mechanism for drug resistance in *H. influenzae* may have recently emerged in this region.

Immunization with Hib conjugate vaccines is cost effective and is the priority for reducing the burden of *H. influenzae* disease and addressing antimicrobial-resistant Hib in South America. All ampicillin-resistant strains identified during surveillance for meningitis in Salvador were Hib. Hib conjugate vaccine campaigns in South America, such as that which was recently initiated in Brazil, will therefore provide the benefit of reducing future costs associated with treating antimicrobial-resistant Hib disease.

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Prevention of *Haemophilus influenzae* Type b (Hib) Meningitis and Emergence of Serotype Replacement with Type a Strains after Introduction of Hib Immunization in Brazil.

Prevention of *Haemophilus influenzae* Type b (Hib) Meningitis and Emergence of Serotype Replacement with Type a Strains after Introduction of Hib Immunization in Brazil

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Surveillance for *Haemophilus influenzae* meningitis cases was performed in Salvador, Brazil, before and after introduction of *H. influenzae* type b (Hib) immunization. The incidence of Hib meningitis decreased 69% during the 1-year period after initiation of Hib immunization (from 2.62 to 0.81 cases/100,000 person-years; $P < .001$). In contrast, the incidence for *H. influenzae* type a meningitis increased 8-fold (from 0.02 to 0.16 cases/100,000 person-years; $P = .008$). Pulsed-field gel electrophoretic analysis demonstrated that *H. influenzae* type a isolates belonged to 2 clonally related groups, both of which were found before Hib immunization commenced. Therefore, Hib immunization contributed to an increased risk for *H. influenzae* type a meningitis through selection of circulating *H. influenzae* type a clones. The risk attributable to serotype replacement is small in comparison to the large reduction in Hib meningitis due to immunization. However, these findings highlight the need to maintain surveillance as the use of conjugate vaccines expands worldwide.

A major public health advance has been the development and widespread use of *Haemophilus influenzae* type b (Hib) polysaccharide conjugate vaccines. Hib is an important cause of meningitis, pneumonia, and epiglottitis in the pediatric population and is responsible each year for >2 million cases of invasive disease and 300,000 deaths worldwide among children aged <5 years [1]. Hib infection rates have been reduced dramatically in countries that have implemented Hib conjugate vaccine programs as part of routine infant immunization [1, 2]: in the United States, annual cases of Hib meningitis among children aged <5 years decreased from >10,000 cases to <200 cases within a 10-year period after licensure of Hib conjugate vaccines [3].

In addition to preventing Hib invasive disease, several studies have shown that conjugate vaccines are effective in reducing nasopharyngeal colonization [4–7] and therefore may confer protection to populations not targeted for immunization through herd immunity [8]. On the other hand, reduction of Hib carriage may open ecological niches for *H. influenzae* non–type b strains

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Informed consent was obtained from patients or guardians according to procedures approved by the institutional review boards of the Oswaldo Cruz Foundation, Brazilian Ministry of Health, and Weill Medical College of Cornell University, and human experimentation guidelines of the Brazilian Ministry of Health, New York–Presbyterian Hospital, and US Department of Health and Human Services were followed in the conduct of the clinical research.

The authors do not have any agreements or relationships that would represent a conflict of interest with respect to the study.

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and therefore potentially increase the risk of colonization and invasive disease by these strains [9, 10]. *H. influenzae* non–type b strains are generally believed to be less pathogenic than Hib [11] and are an infrequent cause of severe invasive disease [12–14]. However, reports have shown that these strains can cause outbreaks of meningitis and bacteremia [11, 15]. Furthermore, in the conjugate vaccine era, the incidence of *H. influenzae* non–type b invasive disease has increased in certain geographical locations [16–18]. In Salvador, Brazil, through active surveillance for meningitis, we had the opportunity to examine the incidence of *H. influenzae* non–type b disease before and after introduction of routine Hib immunization. In the present study, we provide evidence for serotype replacement with *H. influenzae* type a associated with the use of the Hib conjugate vaccine.

METHODS

Study site. The metropolitan region of Salvador is comprised of 30 municipalities in Northeast Brazil, with a total population of 3,208,893 inhabitants [19]. State health secretary protocol requires that suspected cases of meningitis from metropolitan Salvador be referred to the state infectious disease hospital for diagnosis and assessment of the need for isolation procedures. Notification of a case of meningitis to state health officials is mandatory, and this hospital reports 98% of the cases among residents of metropolitan Salvador [20].

In August 1999, the Hib conjugate vaccine was introduced as part of the routine infant immunization program in Brazil. Children aged <1 year were scheduled to receive 3 vaccine doses given at 2-month intervals. Children aged 12–23 months were scheduled to receive a single vaccine dose. Between August and December 1999 in metropolitan Salvador, 71,213 vaccine doses (*Haemophilus* b CRM-197 protein conjugate vaccine [HbOC]; Wyeth-Lederle) were administered to a target population of 58,412 children aged <1 year, and 22,488 vaccine doses were administered to a target population of 60,051 children aged 12–23 months [21]. In 2000, 162,303 doses (*Haemophilus* b tetanus toxoid protein conjugate vaccine [PRP-T]; Pasteur-Merieux) were administered to a target population of 59,261 children aged <1 year, and 14,461 doses were administered to a target population of 60,486 children aged 12–23 months. On the basis of information obtained from immunization cards, 72% of the children aged <1 year completed the 3-dose schedule in 2000. Of the children aged 12–23 months, 24% received the 1-dose schedule in 2000.

Surveillance. Active surveillance for *H. influenzae* meningitis was performed at the state infectious disease hospital between 9 March 1996 and 8 September 2000. A case was defined by the isolation of *H. influenzae* from the blood or cerebrospinal fluid of a patient with clinical signs and symptoms of meningitis. Clinical laboratory records were reviewed during the 5 workdays of the week to identify culture-positive case patients. A standardized data entry form was used to obtain information about demographic characteristics, clinical presentation, and outcome after discharge from the medical rec-

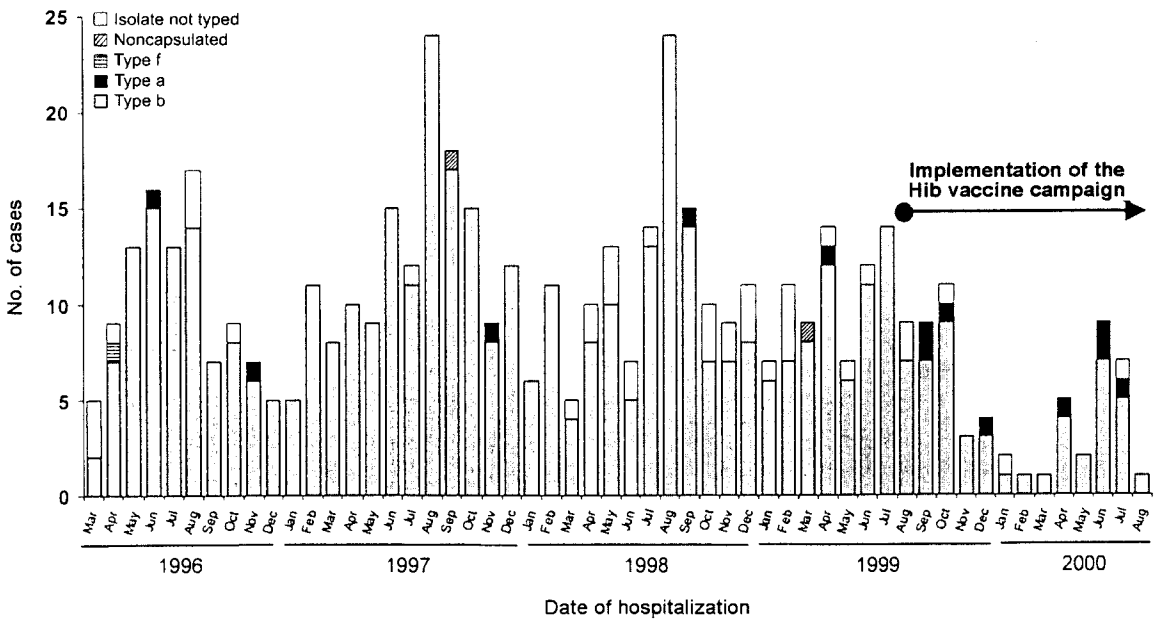

Figure 1. Monthly distribution of 522 *Haemophilus influenzae* meningitis cases identified during surveillance from March 1996 to August 2000 in Salvador, Brazil. Case patients were stratified according to the serotype status of the clinical isolate: *H. influenzae* type b *(gray bars)*, type a *(black bars)*, type f *(horizontal hatched bars)*, noncapsulated *(cross-hatched bars)*, and not typed, because the isolate was unavailable for serotyping *(white bars)*.

ord. Immunization cards were reviewed to obtain information on the timing and number of Hib conjugate vaccine doses administered before hospitalization.

Laboratory investigation. *H. influenzae* was identified according to Gram-stain morphology and growth requirement for factors V and X. Biotyping was performed by use of the indole spot (Difco Laboratories) and ornithine decarboxylase and urease (BBL Microbiology Systems) tests. Commercial antiserum (Difco Laboratories) was used to determine capsular serotype. Each isolate was tested for slide agglutination with the complete panel of type a– to type f–specific antisera and a saline control. Isolates were serotyped at 2 laboratories in Brazil, and those identified as a *H. influenzae* non–type b serotype and those with discordant results were reanalyzed at the Centers for Disease Control and Prevention (CDC). A seminested polymerase chain reaction (PCR) method was used to amplify serotype-specific and nonspecific DNA sequences from the *H. influenzae* capsular loci [22]. Isolates were defined as noncapsulated if agglutination was not observed with the 6 type–specific antisera and if PCR capsular loci sequences conserved among serotypes were not detectable by PCR [22].

Pulsed-field gel electrophoresis (PFGE) was performed with *Sma*I-digested DNA [23, 24] of *H. influenzae* type a strains from Salvador and with those obtained during reference laboratory–based surveillance in Brazil and the United States. PFGE typing patterns were defined according to the criteria of Tenover et al. [25]. Closely related (1–3-band difference) and identical patterns were assigned a unique letter and number code, respectively.

Statistical analysis. Data entry and statistical analyses were performed with Epi Info version 6.04 software (CDC). Fisher's exact test or the χ^2 test was used to compare proportions, and the Kruskal-Wallis test was used to compare continuous data. Cumulative incidence was calculated on the basis of the number of case patients from metropolitan Salvador and population counts from the 1996 national census. The pre- and postvaccine periods were defined as the 3.5-year interval before and 1-year interval after 9 September 1999, respectively. Rates from the prevaccine period were used as the expected value to calculate the probability, according to the Poisson distribution, of observing postvaccine period rates.

RESULTS

Active surveillance identified 522 case patients with *H. influenzae* meningitis during the 4.5-year period between 9 March 1996 and 8 September 2000 (figures 1 and 2). In 483 (93%) of these case patients, isolates were serotyped by slide agglutination; 467 (96.7%) of the 483 isolates were Hib, 13 (2.7%) were *H. influenzae* type a, 2 (0.4%) were noncapsulated, and 1 (0.2%) was *H. influenzae* type f. PCR-based detection of

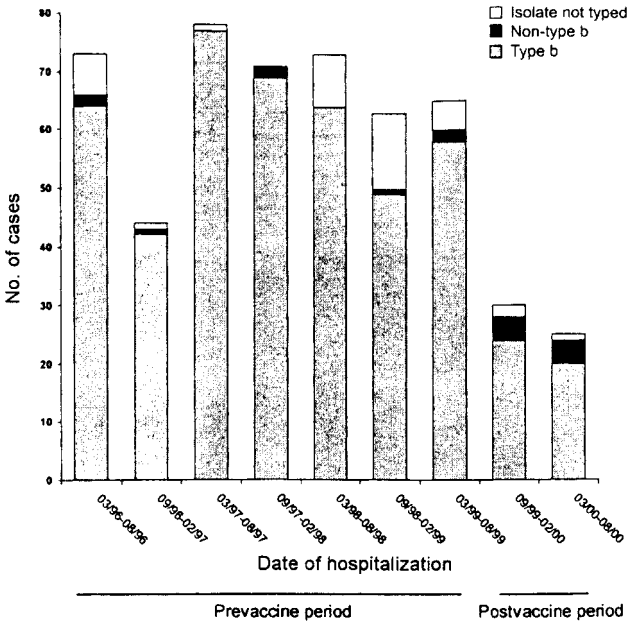

Figure 2. Distribution of 552 *Haemophilus influenzae* meningitis cases according to identification during 6-month surveillance periods, March 1996 to August 2000. Case patients were stratified according to the serotype status of the clinical isolate: *H. influenzae* type b *(gray bars)*, non–*H. influenzae* type b (non-Hib; *black bars*), and not typed, because the isolate was unavailable for serotyping *(white bars)*. Periods before (prevaccine) and after (postvaccine) initiation of the Hib immunization campaign are noted as lines below the figure. Dates are month/year.

capsular loci sequences confirmed the serotype of all *H. influenzae* non–type b isolates (results not shown). Isolates were serotyped from 431 (92%) of 467 cases identified during the prevaccine period and 52 (95%) of 55 of the cases identified during the postvaccine period. The proportion of *H. influenzae* type a cases increased from 5 (1.2%) of 431 to 8 (15.4%) of 52 ($P < .001$) after introduction of routine Hib immunization, but no significant increase was observed in the proportion of cases due to other *H. influenzae* non–type b isolates.

The incidence of *H. influenzae* meningitis among the 357 (68%) of the 522 patients who resided within metropolitan Salvador, decreased 64% during the 1-year period after the introduction of Hib immunization (pre- and postvaccine rates: 2.88 and 1.03 cases/100,000 person-years, respectively; $P <$ .001; table 1). This decrease was a result of the significant reduction in the incidence of Hib meningitis among children aged <2 years (77%) and those aged 2–4 years (49%). However, the incidence of *H. influenzae* type a meningitis increased 8-fold after introduction of routine Hib immunization (pre- and postvaccine rates; 0.02 and 0.16 cases/100,000 person-years, respectively; $P = .008$). There was a significant difference between the rates of *H. influenzae* type a meningitis between pre- and postvaccine periods in the target population for Hib immunization (children aged <2 years; 0 and 1.77 cases/100,000

Table 1. Annual incidence of *Haemophilus influenzae* meningitis in Salvador, Brazil, before and after introduction of routine *H. influenzae* type b (Hib) immunization

Type of infection, age group	Prevaccine period	Postvaccine period	RR (95% CI)	*P*
All *H. influenzae* meningitis cases (n = 357)[a]				
0–4 years	30.08 (308)	9.91 (29)	0.33 (0.23–0.48)	<.001
<2 years	57.59 (228)	15.91 (18)	0.28 (0.17–0.45)	<.001
2–4 years	12.74 (80)	6.13 (11)	0.48 (0.26–0.90)	.022
5–9 years	1.02 (11)	0.98 (3)	0.95 (0.27–3.42)	1.00
Overall	2.88 (324)	1.03 (33)	0.36 (0.25–0.51)	<.001
With Hib isolates (n = 320)				
0–4 years	27.54 (272)	8.20 (24)	0.30 (0.20–0.45)	<.001
<2 years	53.80 (213)	12.38 (14)	0.23 (0.13–0.40)	<.001
2–4 years	10.98 (69)	5.57 (10)	0.51 (0.26–0.98)	.042
5–9 years	0.93 (10)	0.65 (2)	0.70 (0.15–3.19)	1.00
Overall	2.62 (294)	0.81 (26)	0.31 (0.21–0.46)	<.001
With *H. influenzae* type a isolates (n = 7)				
0–4 years	0.20 (2)	1.03 (3)	5.25 (0.88–31.42)	.08
<2 years	0.00 (0)	1.77 (2)	—	.049
2–4 years	0.32 (2)	0.56 (1)	1.75 (0.16–19.30)	.53
5–9 years	0.00 (0)	0.33 (1)	—	.22
Overall	0.02 (2)	0.16 (5)	8.75 (1.70–45.10)	.008

NOTE. Data are cases per 100,000 person-years (no. of cases), unless otherwise noted. CI, confidence interval; RR, relative risk; —, values could not be determined.

[a] Includes 2 case patients with noncapsulated isolates and 28 with isolates that were not serotyped.

person-years, respectively; P = .049), but not in the other age groups (table 1).

H. influenzae type a meningitis cases did not cluster spatially with respect to the neighborhood of residence during pre- and postvaccine periods. There were no significant differences with respect to age, sex, prior hospitalization, attendance at day care centers, and underlying chronic diseases between *H. influenzae* type a and *H. influenzae* non–type a meningitis cases (table 2).

Isolates from the 13 *H. influenzae* type a meningitis cases belonged to 2 distinct groups of closely related PFGE typing patterns (A [4 isolates] and B [9 isolates]; figure 3; table 3). Pattern A isolates were biotype I, and those with pattern B were biotypes II or III (table 3). *H. influenzae* type a patterns were unrelated to the 4 molecular typing patterns found in PFGE analyses of 15 of 44 Hib isolates obtained during the postvaccine period (results not shown). Two of 4 pattern A and 3 of 9 pattern B *H. influenzae* type a strains were isolated from case patients identified in Salvador before the introduction of Hib immunization. Furthermore, 7 of the 8 *H. influenzae* type a clinical isolates identified during national laboratory-based surveillance from other Brazilian cities during 1992–1998 had PFGE patterns (A [3 isolates] and B [4 isolates]) identical to those of *H. influenzae* type a isolates from Salvador (figure 3). PFGE patterns A and B found in *H. influenzae* type a strains from Brazil were unrelated to those of *H. influenzae* type a clinical isolates or reference strains from the United States.

Information on Hib immunization status was obtained from 14 (52%) of 27 *H. influenzae* meningitis case patients who were identified in the postvaccine period and were in the vaccine target population. Of the 4 case patients with *H. influenzae* type a meningitis interviewed, 2 had completed a 3-dose Hib immunization schedule, and 2 had received 2 doses of the vaccine (table 3). In contrast, of the 10 interviewed case patients with Hib meningitis, 4 had not received any doses of the conjugate vaccine, and 6 had received 1 dose before acquiring their illness.

The clinical manifestations seen in the 13 *H. influenzae* type a meningitis cases were similar in severity to those seen in the 549 *H. influenzae* non–type a cases (547 with Hib strains and 2 with noncapsulated strains) for whom clinical information and isolate serotype were obtained (table 2). Significant differences were not observed in mortality from meningitis due to *H. influenzae* type a and *H. influenzae* non–type a strains (case-fatality ratios, 23% vs. 16%, respectively; $P > .05$) or disease caused by these strains during the periods before or after the initiation of the Hib immunization campaign (results not shown). Neurological sequelae, such as hydrocephalus and auditory deficits, were observed at similar frequencies among survivors with *H. influenzae* type a (20%) and *H. influenzae* non–

Table 2. Characteristics of case patients with *Haemophilus influenzae* type a and *H. influenzae* non–type a meningitis identified between 1996 and 2000.

Characteristic	*H. influenzae* type a	*H. influenzae* non–type a
Median age in years (range)	1 (0–15)	1 (0–53)
Male sex	7 (54)	260 (57)
Underlying disease[a]	1 (10)	26 (6)
Seizures	4 (31)	127 (28)
Focal neurological signs	4 (31)	110 (24)
CSF examination[b]		
Leukocyte count, median cells $\times 10^3/\mu L$ (range)	7.8 (0.8–10.0)	5.8 (0.03–31.0)
Glucose level, median mg/dL (range)	20 (20–39)	20 (20–60)
Protein level, median mg/dL (range)	260 (150–500)	300 (30–500)
ICU admission	3 (23)	96 (21)
Days in ICU,[c] median (range)	7 (3–8)	2 (1–33)
Case-fatality rate	3 (23)	75 (16)
Days of hospitalization, median (range)		
Among those who died	2 (1–3)	2 (1–16)
Among survivors	16 (12–40)	16 (10–75)
Neurological sequelae on discharge[d]	2 (20)	96 (25)

NOTE. Data are no. (%) of case patients, unless otherwise noted. Information on isolate serotype and clinical characteristics was obtained from 472 (90%) of 522 patients with *H. influenzae* meningitis identified during surveillance. Of these 472 patients, 13, 457, and 2 patients had *H. influenzae* type a, *H. influenzae* type b, and noncapsulated isolates. CSF, cerebrospinal fluid; ICU, intensive care unit.

[a] Percentages were calculated on the basis of 10 patients with *H. influenzae* type a isolates and 442 patients with *H. influenzae* non–type a isolates for which information on underlying disease was available.

[b] Results of CSF examination are shown for 13 patients with *H. influenzae* type a isolates and for 454 patients with *H. influenzae* non–type a isolates.

[c] Median days were calculated for patients admitted to the ICU. There was a significant difference ($P < .05$) between the 2 groups for this but not other characteristics.

[d] Sequelae among 394 survivors included ataxia (48), motor deficit (20), auditory deficit (15), and hydrocephalus (15).

type a (25%) meningitis (tables 2 and 3). *H. influenzae* type a case patients did not have outcomes different from those of *H. influenzae* non–type a case patients with respect to admission to the intensive care unit (ICU) or duration of hospitalization, although those who were admitted to the ICU did have a longer duration of stay than did *H. influenzae* non–type a cases (7 vs. 2 days; $P = .02$).

DISCUSSION

The present study's findings demonstrate the major public health impact of Hib immunization 1 year after its introduction in Brazil. The benefits are similar to those observed previously in countries, mostly in the developed world, that have adopted Hib conjugate vaccines [1]. Within the first year of the campaign in Salvador, overall Hib meningitis rates decreased 77% among children aged <2 years. Serious neurological sequelae were identified during hospitalization in >20% of children with *H. influenzae* meningitis. In addition to decreased meningitis and mortality rates, a major impact of Hib immunization in Salvador was the prevention of long-term morbidity and social burdens due to neurological sequelae.

In parallel, surveillance in Salvador identified a small but significant increase in *H. influenzae* type a meningitis rates. *H. influenzae* non–type b has been described to be the cause of invasive disease [11, 14–18, 26, 27]. However, strong evidence of serotype replacement has not been detected anywhere since Hib conjugate vaccines were introduced in the late 1980s [10]. In the present study, the evidence that serotype replacement occurred after introduction of routine Hib immunization in Salvador, Brazil is as follows: (1) a significant increase in the incidence of *H. influenzae* type a meningitis cases was observed after the initiation of the Hib conjugate vaccine campaign; (2) all *H. influenzae* type a strains belonged to 2 clonal groups present in Salvador before the introduction of the Hib conjugate vaccine; and (3) *H. influenzae* type a meningitis was documented in subjects who had previously been immunized with the conjugate vaccine. Because surveillance was limited to those with meningitis, the study's findings may not apply to the other forms of invasive *H. influenzae* disease.

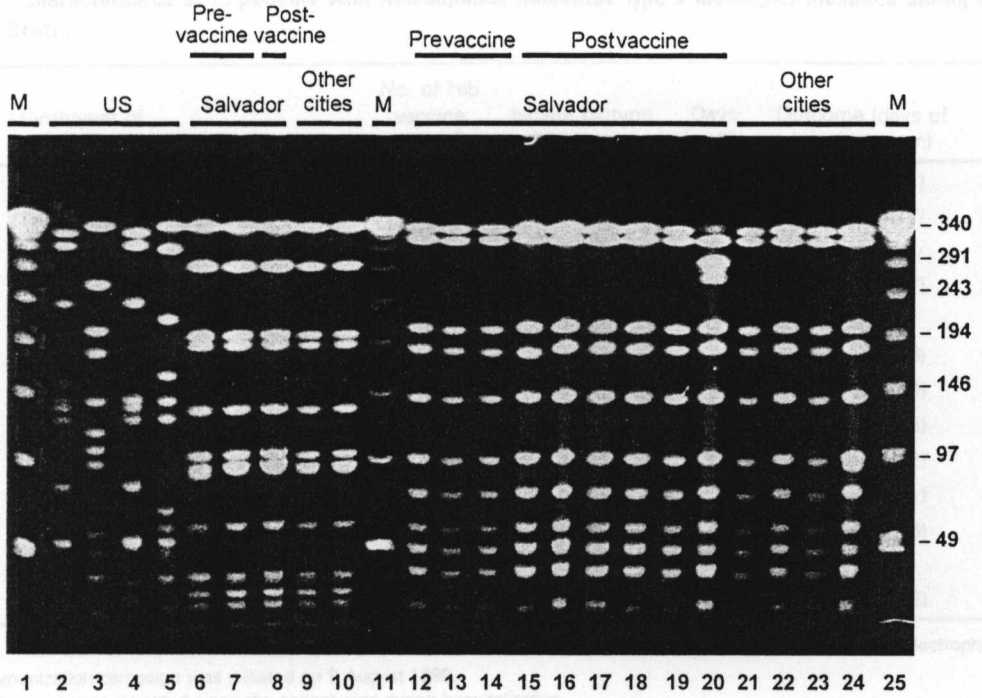

Figure 3. Pulsed-field gel electrophoresis (PFGE) analysis of *Sma*I-digested DNA from *Haemophilus influenzae* type a isolates. *H. influenzae* type a strains were isolated from meningitis case patients that were identified before *(lanes 6–7* and *12–14)* and after *(lanes 8* and *15–20)* introduction of routine *H. influenzae* type b (Hib) immunization. These isolates belonged to 2 closely related PFGE patterns (A1, *lanes 6–8;* B1 and B2, *lanes 12–19* and *20,* respectively). *H. influenzae* type a clinical isolates from other Brazilian cities had PFGE patterns identical to the A1 and B1 pattern (A1, Curitiba, *lane 9,* and São Paulo, *lane 10;* B1, São Paulo, *lanes 21, 23,* and *24;* and Recife, *lane 22*). The 2 closely related patterns observed for *H. influenzae* type a isolates from Salvador were unrelated to those for Hib isolates obtained during surveillance in Salvador (not shown), the *H. influenzae* type a reference strain (ATCC 9006), and isolates from the United States (US) (*lanes 2* and *3–5,* respectively). The position and size (kb) of fragments in the molecular mass standards (M; *lanes 1, 11* and *25*) are shown on the right.

Improved surveillance is an alternative explanation for the serotype shift identified after the introduction of the vaccine. However, increases in the rate of disease due to non-Hib other than *H. influenzae* type a were not observed. Moreover, differential case ascertainment for *H. influenzae* type a and non–type a meningitis cases is unlikely, given the similarity between patient groups and clinical presentations (table 2). Outbreaks of *H. influenzae* type a disease have been reported [11, 15], but *H. influenzae* type a cases identified in this study were not clustered in space or time and did not occur in specific risk groups, other than the pediatric population targeted by the immunization campaign. In addition, the similarity of host characteristics between *H. influenzae* type a and *H. influenzae* non–type a cases indicates that the increase in *H. influenzae* type a disease was not associated with a change in the population at risk for *H. influenzae* meningitis. Introduction of a new hypervirulent strain was not observed, because *H. influenzae* type a isolates from the postvaccine period had PFGE patterns identical to those of isolates from the prevaccine period.

Serotype replacement has not been previously detected for *H. influenzae* nasopharyngeal carriage [4, 6, 7] or invasive disease [12, 28] in the postvaccine era. Few reports have described increased rates of *H. influenzae* non–type b invasive disease in regions where Hib conjugate vaccines have been used [16–18], but molecular typing studies were not performed to determine whether increased rates were due to serotype replacement. Furthermore, long-term surveillance in countries such as the United States, which have used conjugate vaccines for >10 years, has not found sustained increases in the rates of *H. influenzae* non–type b invasive disease [29]. We demonstrated that 2 clonally related groups of strains were responsible for transmission of *H. influenzae* type a meningitis in Salvador. Identification of serotype replacement in meningitis cases, therefore, appears to be due in part to the presence of circulating virulent *H. influenzae* type a clones not found in other locations. The presence or lack of circulating virulent *H. influenzae* non–type b clones may be an explanation why increased rates of *H. influenzae* non–type b disease after Hib immunization have been observed in few epidemiological settings. However, we found that *H. influenzae* type a strains from geographically disparate regions of Brazil had PFGE patterns identical to those in Salvador, which indicates that dissemination of these clonal groups is not a local phenomenon that is restricted to our surveillance region. With increasing reports

Table 3. Characteristics of 13 patients with *Haemophilus influenzae* type a meningitis identified during surveillance in Salvador, Brazil.

Patient	Month/year of hospitalization	Age	Sex	No. of Hib vaccine doses[a]	Isolate biotype (PFGE type)	Days in ICU	Outcome (days of hospitalization)	Neurological sequelae on discharge
1	07/1996	4 years	M	0	II (B1)	0	Discharged (21)	Auditory deficit
2	11/1996	3 years	M	0	II (B1)	0	Discharged (12)	None
3	12/1997	9 months	F	0	I (A1)	0	Death (2)	—[b]
4	09/1998	2 years	M	0	II (B1)	0	Discharged (17)	None
5	04/1999	4 months	F	0	I (A1)	3	Death (3)	—[b]
6	09/1999	3 years	F	0	II (B1)	0	Discharged (16)	None
7	09/1999	5 months	F	ND	II (B1)	8	Discharged (40)	Hydrocephalus
8	10/1999	15 years	M	0	I (A1)	0	Discharged (13)	None
9	12/1999	18 months	F	2	I (A1)	0	Discharged (12)	None
10	04/2000	5 months	M	2	II (B1)	7	Discharged (31)	None
11	06/2000	12 months	M	3	III (B1)	0	Discharged (16)	None
12	07/2000	9 years	M	0	II (B1)	0	Death (1)	—[b]
13	07/2000	8 months	F	3	II (B2)	0	Discharged (12)	None

NOTE. Hib, *Haemophilus influenzae* type b; ICU, intensive care unit; ND, not determined; PFGE, pulsed-field gel electrophoresis.

[a] Hib immunization campaign was initiated on 9 August 1999.

[b] Sequelae were not recorded since the patient died during hospitalization.

of virulent *H. influenzae* type a [11, 15, 30] and *H. influenzae* type f [26, 27] strains isolated from different regions of the world and with the expanding global use of Hib conjugate vaccines, serotype replacement may become an emerging and more widespread possibility.

Clinically, the virulence of *H. influenzae* type a strains was indistinguishable from that of Hib: the case-fatality ratios among *H. influenzae* type a and *H. influenzae* non–type a meningitis patients were 23% and 16%, respectively (table 2). Increased virulence among *H. influenzae* type a, which was generally considered to be a rare cause of invasive disease in the prevaccine era [11, 15, 31], appears to be associated with partial deletion of 1 of 2 *bexA* copies within duplicated *cap* loci [15] and/or amplification of *cap* loci [30]. Virulent *H. influenzae* type a strains identified in this study were responsible for sporadic meningitis cases in the prevaccine period. Introduction of the Hib immunization contributed to an increase in the rates of meningitis due to these strains. Our study found that cases of *H. influenzae* type a meningitis occurred among infants who previously had received ≥2 vaccine doses. We propose that, in Salvador, the use of Hib conjugate vaccines provided circulating virulent type a strains an increased opportunity to replace Hib during nasopharyngeal colonization.

Although surveillance in Salvador identified a significant increase in the rate of *H. influenzae* type a meningitis that resulted from serotype replacement, the impact of this finding is small in comparison to substantial and large reduction in the burden of Hib meningitis attributable to the use of the conjugate vaccine (table 1). Without question, the public health priority for *H. influenzae* disease is the widespread introduction of the Hib conjugate vaccine in developing countries, where the cost of conjugate vaccines has thus far precluded their use. More than 10 years after the introduction of Hib conjugate vaccines, immunization schedules contributed to <2% reduction in the global burden of Hib disease [1]. This situation is expected to improve as efforts progress to reduce vaccine costs, as was done recently in Brazil [32], and as more developing countries, such as those in Latin America, adopt Hib immunization programs. However, the finding of this study suggests that, as global immunization coverage expands, continued surveillance for *H. influenzae* will be needed to monitor potential increases in disease due to serotype replacement.

Acknowledgments

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Riley for critical advice during study implementation and manuscript preparation; and, most of all, the study patients and their families.

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Minuta do 6th encontro da rede de Epidemiologia Molecular de *S. pneumoniae* (PMEN), p.7, 2002.

SIXTH MEETING
PNEUMOCOCCAL MOLECULAR EPIDEMIOLOGY NETWORK (PMEN)
SAN DIEGO,CA - 28 SEPTEMBER 2002

MINUTES

1. Welcome and apologies

Prof. Keith Klugman (chairman) opened the meeting and thanked everyone for attending the sixth meeting of the Pneumococcal Molecular Epidemiology Network.

Present

Keith Klugman
Waleria Hryniewicz
Lesley McGee
Karl Kristinsson
Angela Brueggemann
Barbara Wiley
Karen Rudolph
Ian Morrissey
Nurith Porat
Cynthia Whitney
Linda McDougal
Sandra Richter
David Livermore
Donald Low
Lucia Teixeira
George Syrogiannopoulos
David Greenberg
Ron Dagan
Jim Kellner
Joice Pedreira

Apologies

Bernard Beall
Edouard Bingen
Richard Facklam
Marilyn Roberts
Regina Hakenbeck
Birgitta Henriques
Peter Hermans
Gunilla Kallenius
Alexander Tomasz
Amanda Leach
Jean Claude Lefevre
Josefina Linares
Marguerite Lovgren
Vicki Luna
Robert George
Minjun Chen
Burcin Sener
Fred Tenover
Brian Spratt
Dominique Caugant
Irving Nachamkin
Greg Tyrell
Nicolas Legakis

2. New members

Dr Klugman welcomed the new members of the network who attended the meeting – George Syrogiannopoulos (Greece), Ian Morrissey (UK), Ron Dagan (Israel), Nurith Porat (Israel), David Greenberg (Israel), Karen Rudolph (Alaska), Jim Kellner (Canada), Cynthia Whitney (USA), as well as the new members who were unable to attend:
Irving Nachamkin (USA)
Dominique Caugant (Norway)

3. Matters arising

A number of issues were raised prior to the meeting to allow some input from those members unable to attend the meeting. These were discussed and accepted as follows:

3.1 There has been some interest shown in accepting antibiotic susceptible clones that meet all other epidemiological criteria for inclusion into the PMEN? The original aim of the PMEN was to provide nomenclature and

NEW CLONES PROPOSED AT 6TH MEETING OF PMEN

Clone	Reference No./ ATCC No.	Contributor	Serotype	Antibiotic susceptibilities				MLST	Ref
				PEN/CTX	ERY/CLIN	TET/CHL	SXT		
Portugal[6B]	DCC1508	De Lencastre	6B	0.12/0.06	>8/>8	>8/8	1	ST386 32-28-1-1-15-52-14 5/7 loci to Poland[6B]-20	JID 2000 182 1153-1160
Alaska[6B]	000UZLUB	Rudolph	6B	0.25/0.25	>8/0.12	0.25/4	8		JCM 1998 36:2703-7
USA[6B]	V71	Hollingshead	6B	0.5/0.25	>8/>8	>8/4	2		
Greece[6B]	594/T2PB/217	Syrogiannopoulos	6B	0.015/0.015	>8/>8	>8/16	4		CID 1997 25:188-194 Int J Antimicrob Agents 2000 16:219-24
Brazil[14]	11A/JJA/238A	Pedreira/Ko	14	0.25/0.25	0.12/0.12	0.25/4	8		CID 2000 30:78-86
Israel[1]	1578/34569	Porat/Dagan	1	0.03/0.03	0.06/0.12	0.25/4	8		
Greece[21]	P33	Syrogiannopoulos	21	R			R		CID 1997 25:188-194 JCM 2000 38:4361-4366
Australia[6B]		Leach	6B	R	R	R/R	R		Epidemiol Infect 2001 126:25-9

Molecular Epidemiology of Pneumococcal Meningitis during Seven-Year Surveillance in Salvador, Brazil.

American Society for Microbiology • 1752 N Street, NW • Washington, DC 20036-2904

21 February 2003

Joice Reis
Gonçalo Moniz Research Center, Oswaldo Cruz Foundation, MS
Centro de Pesquisas Gonçalo Moniz
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Brazil

Re: Abstract number: 5200

Dear Dr. Reis

I am pleased to inform you that your abstract has been accepted for a **poster** presentation at the 103rd General Meeting, which will be held at the Washington Convention Center, from May 18 through May 22, 2003 in Washington, DC.

Following is information pertaining to the above abstract:

Abstract Title: Molecular Epidemiology of Pneumococcal Meningitis During Seven-Year Surveillance in Salvador, Brazil
Session No.: 128
Room/Day/Time: Poster Hall / 5/20/03 9:00 AM
Poster Number (to be included in your poster title): C-123

Poster Placement: **The size of the posterboard is 4 feet tall by 8 feet wide (1.2 m x 2.4 m).**

Two poster sessions are scheduled each day (except Thursday). The morning session is from 9:00 a.m. to 12:00 p.m. and the presenter must stand at the poster from 10:30 a.m. until 12:00 p.m. The afternoon session is from 1:00 p.m. to 4:00 p.m. and the presenter must stand at the poster from 1:00 p.m. until 2:30 p.m. The period between 12:00 and 1:00 p.m. is reserved for removing the morning posters and placing the afternoon posters. The poster area of the Exhibit Hall is only open to the public from 9:00 a.m. to 4:00 p.m. **Therefore, you must bring this letter with you and show it to Security in order to place your poster between 7:30 and 9:00 a.m. for the morning session or to remove it between 4:00 and 5:30 p.m. for the afternoon session. On Thursday, admittance will be until 12:30 p.m. for poster removal/retrieval.**

Please recall that you agreed to present your poster as scheduled. If you fail to do so, you will be prohibited from submitting abstracts to ASM-sponsored meetings for 3 years. If unable to present your poster, notify ASM before March 7, 2003, so that your abstract will not be published.

Please check our website at http://www.asmusa.org/mtgsrc/mtgs.htm for information regarding presentation hints. Select "Oral and Poster Presentation Guidelines" from the sidebar. In addition, confirmation notices for student travel grant awardees will be sent under separate cover. While you are at the website, do not forget to register for the General Meeting. The link to the registration company can be found under "The 2003 General Meeting Program."

We look forward to your participation and to seeing you in Washington, DC.

Sincerely,

Daniel Sahm

Dan Sahm, Chair
General Meeting Program Committee

103rd General Meeting, May 18 - 22, 2003, Washington, D.C.

Control/Tracking Number : 03-GM-A-5200-ASM
Activity : Abstract
Current Date/Time : 12/18/2002 9:16:15 PM

Molecular Epidemiology of Pneumococcal Meningitis During Seven-Year Surveillance in Salvador, Brazil

J. N. Reis[1,2], S. M. Cordeiro[1], E. L. Gouveia[1], R. M. Pinheiro[1], K. Salgado[3], T. A. Thompson[4], S. P. Smith[5], L. W. Riley[5], M. G. Reis[1], A. I. Ko[1,6];
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S. pneumoniae remains a major cause of morbidity and mortality in developing countries. Much of this disease could be eliminated with widespread use of currently available vaccines. From December 1995 through August 2002 active population-based surveillance for meningitis in Salvador Brazil identified patients that had positve CSFcultures positive for *S. pneumoniae*. Interviews and medical chart review were performed to collect demographic and clinical information. Serotyping, Box A PCR and PFGE were used to investigate the clonal distribution of isolates. Of 477 patients, 453 (95%) had isolates with complete susceptibility and serotype information. Of these, 59 (13%) had intermediate resistance to penicillin. The most prevalent serotypes were 14 (14%), 3 (10%), 19F (8%), 6B (8%), 6A (7%), 23F (6%), 4 (5%), 18C (5%) and 8 (4%). Reduced susceptibility to penicillin was associated with serogroups 14 (38 isolates), 6 (9), 23 (7), and 19 (5). The estimated rate of coverage of the 7-valent conjugate vaccine, was 70% among patients < 5 years of age and 91% among those with penicillin nonsusceptible isolates. Of the 362 isolates that were genotyped, 284 (78%) were distributed among 69 cluster fingerprint patterns with two or more isolates whereas 78 (12%) had non cluster patterns. Of the 69 cluster patterns, 9 had more than 6 isolates and accounted for 129 (36%) isolates. The 4 predominant clonal groups included 1 penicillin nonsusceptible of strains with serotype 14 (33 isolates) and 3 penicillin susceptible of strains with serotypes 18C (22 isolates) and 3 (2 groups, 18 and 15 isolates). Each of these groups were identified in all surveillance years. These findings indicate that persistent transmission of a restricted repertoire of clonally related groups of *S. pneumoniae* is a major cause of penicillin susceptible and non susceptible pneumococcal meningitis in Salvador, Brazil. The majority of these clonally related groups have serotypes represented in the 7-valent conjugate vaccine, therefore indicating that introduction of this vaccine in routine childhood immunization in Brazil would reduce the impact of pneumococcal meningitis.

Topic (Complete): C13 Molecular Typing, Epidemiology and Surveillance: S. pneumoniae and Streptococcus spp.
Keyword (Complete): S. pneumoniae ; Molecular Epidemiology