Increase in numbers of β-lactam-resistant invasive *Streptococcus pneumoniae* in Brazil and the impact of conjugate vaccine coverage

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A comprehensive investigation of invasive *Streptococcus pneumoniae* was carried out in Brazil as part of the programme of the national epidemiological surveillance system. The investigation provided data on the trends of resistance to antimicrobial agents. A total of 6470 isolates of *S. pneumoniae* collected in the country from 1993 to 2004 were tested. During this period of time, the number of penicillin-resistant strains rose from 10·2 to 27·9 %. The proportions of intermediate and high-level resistant strains in 1993, which were 9·1 and 1·1 %, respectively, rose to 22·0 and 5·9 % in 2004. Geometric mean MICs for penicillin increased after the year 2000, to 0·19 μg ml^{-1} in 2004; most of these isolates were from patients with pneumonia and from children under 5 years old, and belonged to serotype 14. There was a significant increase in the number of isolates belonging to serotypes included in the 7-valent conjugate vaccine from children under 5 years old: from 48·6 % in 1993 to 69·6 % in 2004, mainly related to an increase in the frequency of serotype 14 isolates. From 2000 to 2004, meningitis isolates showed higher resistance rates to cefotaxime (2·6 %) compared to non-meningitis isolates (0·7 %); percentages of isolates resistant to trimethoprim-sulfamethoxazole, tetracycline, erythromycin, chloramphenicol and rifampicin were 65, 14·6, 6·2, 1·3 and 0·7 %, respectively. No levoflaxin resistance was observed. Multidrug resistance was identified in 4·6 % of isolates, of which 3·8 % were resistant to three classes, 0·7 % to four classes and 0·1 % to five classes of antimicrobial agent. The study provides valuable information that may support empirical antimicrobial therapy for severe *S. pneumoniae* infections in Brazil, and emphasizes the need for conjugate pneumococcal vaccination.

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INTRODUCTION

Infections caused by *Streptococcus pneumoniae* have become an issue of global concern because of their high morbidity, and the increase of antimicrobial resistance in pneumococcal isolates (Klugman, 1990; Simonsen *et al.*, 2004). The frequent use of antibiotics in young children has been identified as the major risk factor for resistance selection in pneumococci (Kellner *et al.*, 1998). Serotypes which colonize and cause infection in children, such as 6A, 6B, 9V, 14, 19A, 19F and 23F, are associated with antibiotic resistance (Dagan *et al.*, 2003; Klugman & Koornhof, 1988).

Abbreviations: CI, confidence interval; GMC, geometric mean of MIC; IAL, Adolfo Lutz Institute; I, intermediate resistance; MDR, multidrug resistance; NSP, non-susceptible to penicillin; R, resistant; S, susceptible; SIREVA, Sistema Regional de Vacunas; 7-val, 7-valent; 9-val, 9-valent.

The inclusion of the current 7-valent (7-val) pneumococcal conjugate vaccine in immunization programmes for young children has been widely recommended, and this vaccine has the advantage of including most pneumococcal serotypes associated with antimicrobial resistance (Dagan *et al.*, 2003; CDC, 2006). Although this vaccine has been licensed in Brazil, it has not been introduced into the national immunization program. β-Lactams are still the reference drugs for treatment of pneumococcal infections. The decreased susceptibility to these antibiotics is associated with alterations in penicillin-binding proteins in bacterial cell walls (Dowson *et al.*, 1994). The gradual process of increase in resistance to β-lactams is shown by MIC values. Since the 1990s, decreased susceptibility rates to penicillin have been described in many countries, with prevalence rates sometimes higher than 40 % and, occasionally, MICs higher than

8 μg ml^{-1} (Appelbaum, 2002; Klugman, 1990; Schrag *et al.*, 2004). Nevertheless, in the past few years, some studies have detected a significant decrease in the rates of resistant pneumococci in Europe and the USA with the introduction of the 7-val conjugate vaccine together with a reduction in antibiotic consumption levels (Doern *et al.*, 2005; Kaplan *et al.*, 2004; Oteo *et al.*, 2004).

Since the 1980s, surveillance of *S. pneumoniae* disease has been globally improved in order to generate data that assist clinical practices and enhance public health interventions. During the period 1993–1999 in Brazil, for invasive isolates from children under five years old, resistance to penicillin was about 20 %, with no significant increase for a number of years (Brandileone *et al.*, 1997; Di Fabio *et al.*, 2001). More recently, however, we have observed significant increases in high (300 %) and intermediate (61 %) resistance to penicillin among strains isolated between 1998 and 2003 in Brazil (Brandileone *et al.*, 2005). The goal of this study was to evaluate the presence of different levels of resistance to *β*-lactams among 6470 *S. pneumoniae* isolates recovered from invasive disease in Brazil from 1993 to 2004. We also attempted to assess whether variations in the proportions of resistant serotypes reflected the serotypes present in the 7-val conjugate vaccine and the extent of vaccine coverage over this period.

METHODS

Surveillance of *S. pneumoniae* and clinical samples. Since 1993, with the entry of Brazil into the Sistema Regional de Vacunas (SIREVA) project for Latin America (Di Fabio *et al.*, 1997), pneumococcal isolates from invasive disease recovered by the National Public Health Surveillance Network coordinated by the Ministry of Health of Brazil, have systematically been sent with demographic data to the Adolfo Lutz Institute (IAL), São Paulo, Brazil, which is the National Reference Centre for meningitis and *S. pneumoniae*. The IAL is a public health laboratory of the State of São Paulo.

Bacterial isolates. Between January 1993 and December 2004, 6470 pneumococci were voluntarily sent by 72 hospitals and 23 public health laboratories throughout Brazil, for serotyping and antimicrobial susceptibility testing at IAL. Most of the strains were isolated in the south-east (57 %) and in the north-east (22·5 %) of Brazil, followed by the south (13·8 %), the central-west (5·7 %) and the north (1 %). Participating centres from the northern region of the country have only recently joined the pneumococcal surveillance network, which explains the low number of isolates from this region.

Identification, serotyping and susceptibility testing. The identification of *S. pneumoniae* was confirmed by standard methodology (Facklam & Washington, 1991), and the isolates were stored lyophilized. Pneumococci were serotyped by the Neufeld–Quellung reaction with antisera obtained from the Staten Serum Institut. Multiple isolates of the same serotype from different clinical sites of the same patient were treated as a single isolate. The percentages of isolates from children under 5 years old that belonged to serotypes included in the 7-val (4, 9V, 6B, 14, 18C, 19F and 23F) and 9-valent (9-val, 7-val plus serotypes 1 and 5) conjugate vaccines were calculated for each year studied to estimate the potential coverage of the vaccines.

Susceptibility testing to oxacillin, trimethoprim-sulfamethoxazole, tetracycline, erythromycin, chloramphenicol, rifampicin, vancomycin and levofloxacin (Oxoid) was performed by the disc-diffusion method (Clinical and Laboratory Standards Institute/National Committee for Clinical Laboratory Standards, 2005). Isolates collected from 1993 to 2004 were initially screened for susceptibility to penicillin by using 1 μg oxacillin discs (Oxoid). Isolates presenting inhibition zones <19 mm to oxacillin were examined by MIC testing of penicillin (Sigma) and cefotaxime (Sigma) by the broth microdilution method. The interpretive criteria of the Clinical and Laboratory Standards Institute/National Committee for Clinical Laboratory Standards (2005) were used as a means of assigning susceptibility categories (i.e. S for susceptible, I for intermediate or R for resistant) to both disc-diffusion and MIC results. For the MIC analysis, we assumed 0·06 μg ml^{-1} to be the MIC value of penicillin for all isolates S to oxacillin (inhibition zones $\geqslant 20$ mm); the same rationale was applied to MIC values of cefotaxime (Clinical and Laboratory Standards Institute/National Committee for Clinical Laboratory Standards, 2005). Isolates categorized as I or R to penicillin were considered to be non-susceptible to penicillin (NSP). For the other drugs, both I and R were considered as resistant. Multidrug resistance (MDR) was defined as resistance to three or more classes of antimicrobial drugs.

Data analysis. All information relating to each isolate was recorded in a data file by using EpiInfo software version 6.04 (Centers for Disease Control and Prevention). The geometric mean of MIC (GMC) values for penicillin, with respective 95 % confidence intervals (95 % CIs), were calculated for every year and stratified by clinical diagnosis (pneumonia and meningitis), age group (<5 and $\geqslant 5$ years old), and by those serotypes mostly related to NSP (6B, 14 and 23F). Chi square or Fisher exact tests were used for comparison of proportions when appropriate. Statistical analysis was performed by using SPSS software version 10.0 (SPSS Inc.). P values $<0{\cdot}05$ were considered statistically significant.

RESULTS AND DISCUSSION

Among the 6470 invasive *S. pneumoniae* recovered during 12 years (1993–2004) of surveillance of pneumococcal disease, 69·3 % were isolated from meningitis (blood and cerebrospinal fluid), 19·1 % from pneumonia (blood and pleural fluid), and 11·6 % from bacteraemia, arthritis or abdominal abscess (blood or other normally sterile fluids). Data on patient age were available for 5520 isolates, 42·8 % of which were children under 5 years old. Overall, no significant changes were found over the years in the frequency of isolates relative to clinical diagnosis or age group (Table 1).

From 1993 to 2004, the frequency of NSP increased from 10·2 to 27·8 %. A significant increase was observed after 1999, for both I (from 12·4 to 22·0 %) and R isolates (from 2·4 to 5·9 %) (Table 1). The GMC of penicillin steadily increased after 1999, reaching values above 0·12 μg ml^{-1} in 2002, and 0·19 μg ml^{-1} in 2004 ($P<0{\cdot}05$) (Fig. 1). These GMCs contrast with those from the period 1993–1999, when most GMC values were around 0·06 μg ml^{-1}. The year 2000 was a transitional period towards the progressive emergence of isolates with elevated MICs, reaching the highest values after 2001. The frequency of isolates stratified by MICs of penicillin through the period 1999–2004 is summarized in Table 2. No isolate with an MIC of penicillin $>4{\cdot}0$ μg ml^{-1} was identified. During the period 2000–2004, a higher prevalence of NSP was detected in the south-east (NSP = 28·1 %; I = 20·4 %, R = 7·7 %) and south of Brazil (NSP = 27·7 %; I = 22·5 %, R = 5·2 %) compared with the

Table 1. *S. pneumoniae* isolates by age of patients, clinical diagnosis, susceptibility to penicillin, and estimated coverage of the 7- and 9-valent conjugate vaccines, Brazil, 1993–2004

Values shown are percentages of the total number of isolates.

Year	No. of isolates	Proportion <5 years old	Clinical diagnosis			NSP*		Vaccine coverage†	
			Meningitis	Pneumonia	Other	I	R	7-val	9-val
1993	175	40·0	81·1	17·1	1·7	9·1	1·1	48·6	70·8
1994	331	52·9	60·1	29·0	10·9	13·2	0·3	52·2	73·1
1995	378	56·6	61·4	25·9	12·7	13·5	0·8	52·1	71·8
1996	362	47·2	76·5	14·6	8·9	21·9	0·3	59·1	75·5
1997	430	47·2	75·1	14·7	10·2	11·9	0·9	64·6	76·5
1998	468	51·7	68·2	26·5	5·4	13·2	2·0	61·2	74·0
1999	509	43·4	72·3	18·5	9·3	12·4	2·4	58·9	75·0
2000	597	47·6	68·2	20·3	11·6	17·7	3·1	59·8	72·8
2001	730	43·3	74·1	16·4	9·5	18·1	5·2	69·4	79·4
2002	751	40·7	68·4	16·2	15·4	20·0	5·5	68·5	75·7
2003	871	35·1	66·9	18·3	14·8	19·5	7·2	60·8	70·7
2004	868	31·2	67·2	17·4	15·4	22·0	5·9	69·6	78·9
Total	6470	42·8	69·3	19·1	11·6	17·2	3·7	62·0	74·9

*I, MIC 0·12–1·0 μg ml^{-1}; R, MIC ⩾2·0 μg ml^{-1}, as defined by the Clinical and Laboratory Standards Institute/National Committee for Clinical Laboratory Standards (2005).

†Proportion of isolates from children up to 5 years belonging to serotypes in the conjugate vaccines: 7-val serotypes are 4, 6B, 9V, 14, 18C, 19F, 23F; 9-val serotypes are the serotypes in 7-val plus 1 and 5.

central-west (NSP = 20·5 %; I = 18·8 %, R = 1·7 %), the north-east (NSP = 16·1 %; I = 15·4 %, R = 0·7 %) and the north (NSP = 7·9 %; I = 7·9 %); R isolates were not detected in the north of Brazil. These findings probably reflect the fact that the most populated and developed urban centres are located in the southern and south-eastern regions of Brazil, where better health services can be found and the consumption of antibiotics by the community is higher.

An analysis of GMC of penicillin stratified by clinical diagnosis, age group and serotype is displayed in Fig. 2. After 2000, GMCs significantly increased to values higher than 0·24 μg ml^{-1} (0·27 μg ml^{-1} in 2004) for pneumonia isolates, whereas a minor rise was observed for meningitis isolates (0·16 μg ml^{-1} in 2004) ($P>0·05$) (Fig. 2a). The GMCs for isolates from children aged <5 years also increased significantly after 2000 (0·31 μg ml^{-1} in 2004), contrasting with those from children aged ⩾5 years, which remained below 0·06 μg ml^{-1} until 2001 (0·14 μg ml^{-1} in 2004) ($P>0·05$) (Fig. 2b). Therefore, the analysis of the GMCs of penicillin by infection site and by age group demonstrated that the increase of resistance since the year 2000 in Brazil has been strongly associated with isolates recovered from pneumonia cases and from patients <5 years old. The association of penicillin resistance with younger age is not surprising, since there is a higher empirical use of antimicrobial agents for respiratory tract infections in children (Dagan *et al.*, 1992, 2003; Kellner *et al.*, 1998; Mandell *et al.*, 2002).

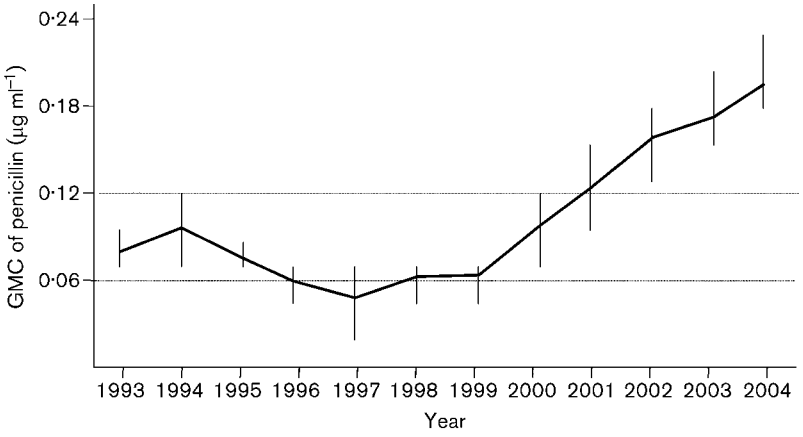

Fig. 1. GMC values of penicillin with 95 % CIs for *S. pneumoniae* isolates from 1993 to 2004 in Brazil.

Table 2. *S. pneumoniae* isolates and MIC values of penicillin from 1999 to 2004

Values shown are percentages of the total number of isolates.

MIC of penicillin (μg ml^{-1})	Year (no. of isolates)					
	1999 (509)	2000 (597)	2001 (730)	2002 (751)	2003 (871)	2004 (868)
0·12	4·9	8·1	7·6	10·8	7·8	7·9
0·25	5·7	5·5	5·3	5·6	7·1	6·3
0·5	1·0	2·3	2·2	1·6	1·2	4·0
1·0	0·8	1·7	2·7	1·5	3·6	3·7
2·0	2·0	2·3	3·7	4·3	6·0	4·4
4·0	0·4	0·8	1·4	1·1	1·2	1·5

The serotypes associated with NSP were 14 (49·6 %), 6B (16·6 %), 23F (15·2 %), 19A (6·6 %), 19F (4·3 %), 23B (2·0 %), 6A (1·6 %) and 9V (1·5 %). The serotypes expressing resistance to penicillin in this study are consistent with the worldwide pattern and in accordance with previous reports from Brazil (Brandileone *et al.*, 1997; Di Fabio *et al.*, 2001; Kertesz *et al.*, 1998). On the one hand, serotype 14 isolates presented a striking tendency to increasing GMCs of penicillin, from 0·12 μg ml^{-1} in 1996 to 0·74 μg ml^{-1} in 2004 ($P<0·05$) (Fig. 2c). On the other hand, serotype 23F isolates presented higher GMCs in the period 1994–1995, reaching the highest level in 1995

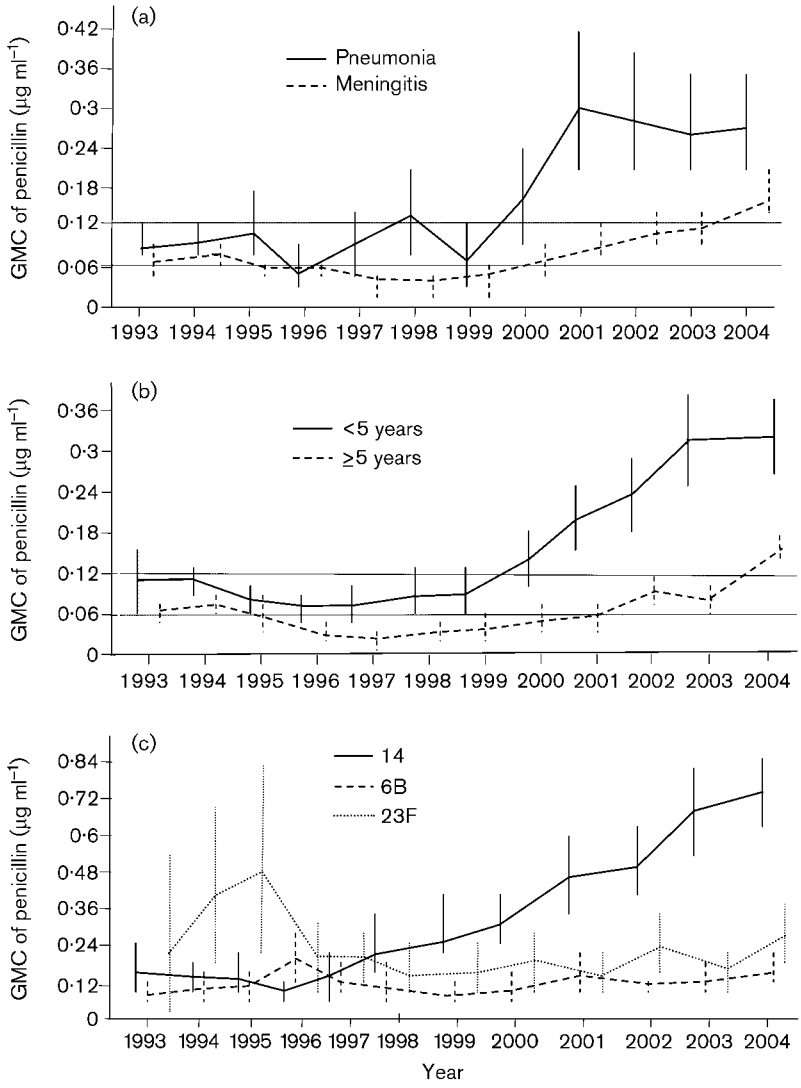

Fig. 2. GMC values of penicillin with 95 % CIs for *S. pneumoniae* isolates by clinical diagnosis (a), age group of patient (b), and the main serotypes associated with penicillin resistance (c) from 1993–2004, Brazil.

(0·48 μg ml^{-1}), then falling sharply, and remaining relatively stable (0·27 μg ml^{-1} in 2004). This peak of GMC values associated with serotype 23F isolates may have occurred because of the emergence of a specific clone, since most of these isolates were recovered from the State of São Paulo and were resistant to trimethoprim-sulfamethoxazole. Serotype 6B isolates had relatively stable GMCs: around 0·12 μg ml^{-1} during the whole period (0·15 μg ml^{-1} in 2004) (Fig. 2c).

Serotype 14 was responsible for 50 % of all NSP isolates in the country. Among the NSP isolates, the prevalence of isolates belonging to serotype 14 increased from 41·3 % (139/336) in 1993–1999 to 54·1 % [204/377, odds ratio (OR) = 1·7 (95 % CI, 1·23 to 2·27)] in 2000–2004. Studies of the molecular surveillance of *S. pneumoniae* have demonstrated that resistant isolates circulating within a geographical area belong to a small number of multi-resistant clones (Klugman, 2002; McGee *et al.*, 2001). In fact, a nationwide study of penicillin-resistant pneumococci by molecular typing in Brazil has identified the emergence and rapid dissemination of two international clones, Spain^{9V}-3 and Tennessee^{14}-18 (McGee *et al.*, 2001), both expressing the capsule belonging to serotype 14. These clones became predominant after 1998, mainly in the southern and south-eastern regions, accounting for the highest penicillin-resistance rates in Brazil (Brandileone *et al.*, 1998). Thus, the predominance of these clones differs according to geographical region, probably as a result of socio-economic differences among Brazilian regions, and maybe reflecting regional differences in antimicrobial usage (Klugman, 2002; McCormick *et al.*, 2003; Okeke *et al.*, 2005a, b).

The influence of antibiotic use on increasing resistance in the most common serotypes over time, especially in the paediatric ones, has been well described by other authors (Feikin & Klugman, 2002). In Brazil, this effect has led to the increase of resistance to penicillin associated with serotype 14 isolates, particularly in isolates from children up to 5 years old. Thus, as serotype 14 is included in the formulation of conjugate vaccines, we investigated the impact of these changes in resistance on the potential coverage of the conjugate vaccines over this time period. Data on distribution of serotypes obtained during the previously reported survey show that serotype 14 is the most frequent serotype among children up to 5 years old (Brandileone *et al.*, 2003). The individual analysis of the NSP serotypes also indicates a significant rise in the prevalence of serotype 14 in children <5 years old, from 25·1 % (336/1339) in 1993–1999 to 32·1 % [326/1015, OR = 1·4 (95 % CI, 1·17 to 1·7)] in 2000–2004. For the other NSP serotypes, no difference in prevalence rates was observed. The percentage of isolates belonging to serotypes included in the 7-val and 9-val conjugate vaccines by year is displayed in Table 1. This observation shows an increase in the coverage of the 7-val vaccine of 21 % over time, from 48·6 % in 1993 to 69·6 % in 2004, whereas the increase of the 9-val vaccine was approximately 8 % (Table 1). Analysis of our data reveals that the increasing penicillin resistance associated with serotype 14 isolates from invasive disease of children appeared to be associated with the increase of 7-val vaccine coverage. The 7-val conjugate vaccine has presented a potential coverage of 90 % for NSP isolates.

It is worth noting that the number of pneumococcus isolates sent to IAL has increased over the years, mainly as a result of the increase in the number of institutions (from 28 centres in 1993 to 95 in 2004) taking part in the national surveillance of invasive pneumococcal disease. However, it is unlikely that the higher number of isolates resistant to penicillin after 1999 may be explained by selection bias in referral isolates, since institutions more recently included in the surveillance are located in the central-west and northern regions of Brazil, where lower resistance was observed.

Table 3 presents the prevalence of pneumococcus by MIC of penicillin, and by age group and clinical diagnosis. Similar MIC values were found for the 5–64 and >64 years age groups, whereas for the <5 years age group these values

Table 3. Percentages of *S. pneumoniae* isolates by MIC of penicillin and clinical diagnosis, 2000–2004, Brazil

The number of isolates is shown in parentheses. See Results and Discussion for meaning of bold type.

MIC of penicillin (μg ml^{-1})	Age group					
	<5 years		5–64 years		>64 years	
	Meningitis (915)	Non-meningitis (568)	Meningitis (915)	Non-meningitis (412)	Meningitis (88)	Non-meningitis (81)
0·12–4·0*	33·1	43·1	13·0	20·0	13·6	18·5
2·0†	5·3	**11·4**	1·0	**2·2**	1·1	**3·7**
4·0†	1·1	**4·6**	0	**0·7**	0	**1·2**

*Non-susceptible MIC breakpoints to penicillin defined by the Clinical and Laboratory Standards Institute/National Committee for Clinical Laboratory Standards (2005): I, 0·12–1·0 μg ml^{-1}; R ⩾2·0 μg ml^{-1}.

†Non-susceptible MIC breakpoints to penicillin for all isolates other than those from meningitis recommended by the Drug-Resistant *Streptococcus pneumoniae* Therapeutic Working Group (Heffelfinger *et al.*, 2000): I, 2·0 μg ml^{-1}; R, ⩾4·0 μg ml^{-1}.

were higher. Higher MICs were found among isolates from non-meningitis cases than among isolates from meningitis. Despite this finding, therapeutic success has been achieved with the use of penicillin to treat *S. pneumoniae* pneumonia (Biscay, 2002; Song *et al.*, 2004). The correlation between clinical outcomes of pneumonia cases and the MIC of penicillin breakpoints from the Clinical and Laboratory Standards Institute (CLSI) has become a global issue. Taking into account the recommendation made by the Drug-Resistant *Streptococcus pneumoniae* Therapeutic Working Group (DRSPTWG) (Heffelfinger *et al.*, 2000), which suggests alternative MIC breakpoints to penicillin for pneumonia isolates (NSP: I, 2·0 μg ml^{-1}; R, >4·0 μg ml^{-1}), the prevalence of NSP among these isolates drops three- to sevenfold compared to CLSI breakpoints (shown in bold type in Table 3). Therefore, it seems reasonable to recommend penicillin as an effective drug for pneumococcal pneumonia in regions of Brazil with a low prevalence of resistance to penicillin (MIC <4·0 μg ml^{-1}), as recommended by the DRSPTWG and other investigators (Biscay, 2002; Heffelfinger *et al.*, 2000; Klugman *et al.*, 2004; Song *et al.*, 2004). There is a distinct scenario, however, for meningitis isolates expressing MICs higher than 0·06 μg ml^{-1}, which is the critical breakpoint for treatment. In fact, the most serious effect of penicillin resistance in pneumococci is encountered in meningitis due to the poor penetration of the antibiotic into the cerebrospinal fluid, so that it is difficult to achieve the required bactericidal concentration (Bashir *et al.*, 2003; Tunkel *et al.*, 2004).

Table 4 shows percentages of pneumococci stratified by MICs of cefotaxime from 1999 to 2004. Lower percentages of isolates expressing an MIC of cefotaxime of 1·0 μg ml^{-1} were found among meningitis isolates than among isolates from other clinical diagnoses. However, when the I and R levels of resistance are taken into account, higher percentages of isolates non-susceptible to cefotaxime were obtained from meningitis isolates [mean rate 2·4 % (95 % CI, 1·9 % to 3·1 %)] than from isolates from other clinical diagnoses [mean rate 0·7 % (95 % CI, 0·3 % to 1·4 %)] [OR = 3·42 (95 % CI, 1·8 to 8·8)]. No isolates expressing an MIC of cefotaxime >2·0 μg ml^{-1} were identified. Resistance to cefotaxime for meningitis isolates was 4·8 % in the south, 3·1 % in the south-east, and <1·0 % in the other regions of Brazil. Therefore, on the whole, we found high susceptibility to cefotaxime among isolates from Brazil, supporting the unique use of third-generation cephalosporins to treat meningitis caused by NSP (Mandell *et al.*, 2002). At the present time, third-generation cephalosporins are the drugs of choice for treatment of meningitis in Brazil in areas with a high prevalence of NSP (MIC >0·1 μg ml^{-1}) (Biscay, 2002; Tunkel *et al.*, 2004). All isolates resistant to cefotaxime were also resistant to penicillin.

Among the 3817 isolates recovered during the period 2000–2004, 65 % showed resistance to trimethoprim-sulfamethoxazole, 14·6 % to tetracycline, 6·2 % to erythromycin, 1·3 % to chloramphenicol, and 0·7 % to rifampicin. No significant differences in resistance to these drugs were observed over time. None of the isolates was resistant to levofloxacin and vancomycin.

MDR was identified in 180 (4·6 %) of 3817 isolates, showing resistance to three (3·8 %), four (0·7 %) or five (0·1 %) classes of antimicrobial agents. The predominant MDR pattern was resistance to trimethoprim-sulfamethoxazole/tetracycline/erythromycin (n=81), followed by resistance to trimethoprim-sulfamethoxazole/tetracycline/penicillin (n=26). A total of 992 (26·3 %) isolates were resistant to two drugs, with trimethoprim-sulfamethoxazole/penicillin (n=762, 76·8 %) and trimethoprim-sulfamethoxazole/tetracycline (n=163, 16·4 %) as predominant patterns. The high resistance to trimethoprim-sulfamethoxazole found in this surveillance study is due primarily to the wide use of this drug for empirical treatment of respiratory tract infections, as recommended by the World Health Organization. Although macrolide resistance is increasing worldwide, reaching more than 25 % in the USA and Europe (Reinert *et al.*, 2005; Stephens *et al.*, 2005), in Brazil the rate of resistance to this class of antibiotic is still low.

In view of the antimicrobial resistance trends among invasive *S. pneumoniae* isolates in Brazil after 1999, there

Table 4. Percentages of *S. pneumoniae* isolates by MIC of cefotaxime and clinical diagnosis, and year, Brazil

Bold type shows percentage of I and R levels for meningitis and for all isolates other than meningitis.

MIC of cefotaxime* (μg ml^{-1})	Meningitis (no. isolates)						Non-meningitis (no. isolates)					
	1999 (368)	2000 (407)	2001 (541)	2002 (514)	2003 (583)	2004 (583)	1999 (141)	2000 (190)	2001 (189)	2002 (237)	2003 (288)	2004 (285)
⩽0·5	98·7	97·6	98·1	97·3	97·4	97·1	95·1	94·3	90·6	90·0	91·7	91·0
1·0	**1·3**	**2·4**	**1·5**	**2·9**	**2·4**	**2·9**	3·5	4·7	8·4	8·4	8·0	9·0
2·0	**0**	**0**	**0·4**	**0·2**	**0·2**	**0**	**1·4**	**1·0**	**1·0**	**1·6**	**0·3**	**0**

*MIC breakpoints of cefotaxime for isolates from meningitis (S, ⩽0·5 μg ml^{-1}; I, 1·0 μg ml^{-1}; R, ⩾2·0 μg ml^{-1}), and for all isolates other than meningitis (S, ⩽1·0 μg ml^{-1}; I, 2·0 μg ml^{-1}), as defined by the Clinical and Laboratory Standards Institute/National Committee for Clinical Laboratory Standards (2005).

is a need for systematic national surveillance as well as dissemination of the data to provide regional information for empirical treatment of pneumococcal infections, and to promote public health strategies for appropriate antimicrobial use. Furthermore, the findings of this surveillance reinforce the use of the conjugate vaccine in young children and infants as an important tool to reduce the spread of vaccine serotypes associated with resistance.

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