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Increasing prevalence of antimicrobial resistance among isolates of *Streptococcus pneumoniae* from the PROTEKT surveillance study, and comparative *in vitro* activity of the ketolide, telithromycin

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The prevalence of resistance to a range of antimicrobials was determined for isolates of *Streptococcus pneumoniae* examined in the PROTEKT (Prospective Resistant Organism Tracking and Epidemiology for the Ketolide Telithromycin) surveillance study (1999–2000) using NCCLS testing methods and interpretative criteria. Of 3362 pneumococcal isolates collected from 69 centres in 25 countries, 22.1% overall were resistant to penicillin G, with the highest rates of resistance found among isolates from Asia (53.4%), France (46.2%) and Spain (42.1%). Erythromycin A resistance occurred in 31.1% of isolates overall with the highest rates found in Asia (79.6%), France (57.6%), Hungary (55.6%) and Italy (42.9%). Marked geographical differences in the prevalence of both penicillin G (the Netherlands 0%; South Korea 71.5%) and erythromycin A (Sweden 4.7%; South Korea 87.6%) resistance were observed. Asia was characterized by the highest prevalence of resistance, overall, with only eight of 19 antimicrobials (co-amoxiclav, linezolid, vancomycin, teicoplanin, quinupristin/dalfopristin, levofloxacin, moxifloxacin and telithromycin) retaining high activity against isolates of *S. pneumoniae* from this region. Notable rates of resistance to clarithromycin, azithromycin, co-trimoxazole and tetracycline were observed in the majority of countries submitting isolates of *S. pneumoniae* to the PROTEKT surveillance study. Fluoroquinolone resistance was low (1%), overall, although 14.3% of 70 isolates from Hong Kong were resistant to levofloxacin and moxifloxacin, all but one of these isolates belonging to a single clone of the 23F serotype. Although, at present, apparently limited to pockets of clonal spread, continued vigilance with regard to the evolution of fluoroquinolone resistance is indicated. Telithromycin (MIC_{90} 0.12 mg/L; 99.9% of isolates susceptible) and linezolid (MIC_{90} 2 mg/L; 100% of isolates susceptible) were the two most active oral agents tested, both compounds retaining activity against isolates of fluoroquinolone-resistant *S. pneumoniae*. The results of the PROTEKT surveillance study 1999–2000 emphasize the widespread evolution of resistance to a variety of antimicrobials amongst isolates of *S. pneumoniae* and demonstrate the potential of telithromycin as a therapeutic option for the treatment of community-acquired respiratory tract infections caused by this organism.

Introduction

Although a common component of the normal human respiratory flora, *Streptococcus pneumoniae* is also one of the most important bacterial pathogens. Infection with this organism is among the most frequent causes of community-acquired respiratory tract infections (CARTIs), including community-acquired pneumonia (45% of cases), otitis media (30–40%) and sinusitis (20–35%), as well as being one of the leading causes of meningitis and bacteraemia.[1–3] Indeed, it has been estimated that each year in the USA, *S. pneumoniae* infections account for 500 000 cases of pneumonia, 55 000 cases of bacteraemia and 6000 cases of meningitis.[4,5]

While β-lactams, most notably penicillin G, have been the mainstay of antimicrobial treatment for CARTIs, resistance to these agents has reached alarming levels in many regions. Isolates of *S. pneumoniae* with reduced susceptibility to penicillin G were first reported during the 1960s in Australia and Papua New Guinea.[3,6] By the early 1970s penicillin G-intermediate and -resistant strains had been detected in

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Poland and Germany, with Japan, South Africa, Switzerland and the UK also reporting the presence of such strains during the mid to late 1970s.[6] Subsequently, penicillin G-resistant strains of *S. pneumoniae* have rapidly spread worldwide and their prevalence continues to increase.[7–12] Of even more concern is the degree to which these isolates are cross-resistant, not only to other β-lactam drugs, such as the cephalosporins, but also to non-β-lactam classes, e.g. the macrolides.[10] Resistance to fluoroquinolones is also emerging among *S. pneumoniae*, albeit currently at relatively low levels.[13]

In this era of increasing antimicrobial resistance, treatment of CARTIs should ideally be pathogen directed, based on identification and susceptibility testing of the causative organism. In reality, however, an aetiologically specific diagnosis, let alone susceptibility results, is rarely available to assist in the selection of therapy, which is consequently largely empirical. In view of the importance of *S. pneumoniae* in CARTIs and the traditionally empirical approach to the treatment of these infections, knowledge of local antimicrobial resistance patterns is essential to ensure rational prescribing.[14] These issues highlight the need for continuing surveillance of antimicrobial resistance at local, national and international levels, as well as the need for research into new antibacterial agents with the ability to overcome *S. pneumoniae* resistance mechanisms and which do not induce or select further resistance.

The ketolides are a new family of antibacterial agents belonging to the macrolide–lincosamide–streptogramin class.[15,16] Telithromycin, the first ketolide antibacterial, has demonstrated clinical efficacy across a range of CARTIs, and potent *in vitro* activity against the pathogens commonly implicated in these infections, including drug-resistant *S. pneumoniae*.[17–19] Data from both *in vitro* and human studies suggest that this agent also has a low potential for the selection or induction of resistance, and has minimal effect on normal human gut flora.[20,21] In 1999, the PROTEKT (Prospective Resistant Organism Tracking and Epidemiology for the Ketolide Telithromycin) surveillance study was established to track longitudinally the susceptibility of common respiratory pathogens from patients with CARTIs to current and new antimicrobials, including the new ketolide, telithromycin. This paper describes the widespread prevalence of resistance and the comparative activity of common and newly developed antimicrobial agents against isolates of *S. pneumoniae* collected during the first year (1999–2000) of the PROTEKT study.

Materials and methods

Collecting centres

During the 1999–2000 respiratory season, 69 centres in 25 countries participated in PROTEKT: Canada (7), USA (8), Mexico (4), Brazil (7), Argentina (2), Germany (7), the Netherlands (1), Sweden (1), UK (2), Eire (1), Belgium (1), France (4), Portugal (2), Spain (2), Switzerland (2), Italy (2), Austria (1), Turkey (1), Hungary (1), Poland (1), Hong Kong (1), Japan (6), South Korea (2), Australia (2) and Indonesia (1).

Bacterial isolates

Each centre recruited into the PROTEKT study was requested to collect a minimum of 60 isolates of *S. pneumoniae* from patients with any of six types of community-acquired infection, namely sinusitis, otitis media, pharyngitis, pneumonia, acute bacterial exacerbations of chronic bronchitis and acute exacerbation of chronic obstructive airways disease. Sources for isolation of RTI pathogens were cultures from blood, sputum, bronchoalveolar lavage, middle ear fluid, nasopharyngeal swab or aspirate, and sinus aspirate. Patients with nosocomial RTIs and those with cystic fibrosis were excluded, and duplicate strains or strains originating from existing collections were not included in the study. Demographic data collected included the age and sex of the patient, infection, culture source, in/outpatient status, specimen accession number and date of sample collection. Criteria for isolate storage, transportation and identification, and confirmation of isolate identification have been described in detail previously.[22]

Antimicrobial susceptibility testing

MICs were determined at a central laboratory (GR Micro Ltd, London, UK) for a panel of existing and new antimicrobials, using previously described methods.[22] In brief, we used the NCCLS broth microdilution method with lyophilized microtitre plates (Sensititre system; Trek Diagnostics) and an inoculum of $3–7 \times 10^4$ cfu in 100 μL medium. MIC endpoints were read as the lowest concentration of antimicrobial that totally inhibited macroscopically visible growth of the inoculum.

Isolates were tested against the following antimicrobials: penicillin G, co-amoxiclav (2:1 ratio), cefaclor, cefixime, cefpodoxime, cefuroxime, azithromycin, clarithromycin, erythromycin A, telithromycin, clindamycin, levofloxacin, ciprofloxacin, moxifloxacin, co-trimoxazole (1:19 ratio), linezolid, quinupristin–dalfopristin (30:70 ratio), teicoplanin, tetracycline and vancomycin. Percentage susceptibilities were calculated based on NCCLS breakpoints, although it should be noted that NCCLS breakpoints applied to telithromycin are tentative and not yet approved (Table 1).

Results

A total of 3362 isolates of *S. pneumoniae* were collected from 25 countries within Western Europe (n = 1322), North America (n = 687), Latin America (n = 518), Asia (n = 515), Eastern Europe (n = 199) and Australasia (n = 121). Informa-

Table 1. Interpretative categories for *S. pneumoniae*[a]

Antimicrobial agent	Interpretative categories (mg/L)		
	susceptible	intermediate	resistant
Co-amoxiclav	≤2	4	≥8
Azithromycin	≤0.5	1	≥2
Cefaclor	≤1	2	≥4
Cefixime	susceptibility reported as for penicillin G		
Cefpodoxime	≤0.5	1	≥2
Cefuroxime	≤1	2	≥4
Clarithromycin	≤0.25	0.5	≥1
Clindamycin	≤0.25	0.5	≥1
Co-trimoxazole	≤0.5	1–2	≥4
Erythromycin A	≤0.25	0.5	≥1
Levofloxacin	≤2	4	≥8
Linezolid	≤2		≥8
Moxifloxacin	≤1	2	≥4
Penicillin G	≤0.06	0.12–1	≥2
Quinupristin/dalfopristin	≤1	2	≥4
Teicoplanin	≤8	16	≥32
Telithromycin	≤1	2	≥4
Tetracycline	≤2	4	≥8
Vancomycin	≤1	–	–

[a]All breakpoints given are NCCLS, with the exception of telithromycin for which NCCLS-approved breakpoints are not yet available (tentative breakpoints given).

tion on culture source, patient age and gender, and treatment setting were available for approximately 45–87% of the isolates collected, and this is summarized in Table 2.

Prevalence of antimicrobial resistance

Susceptibility of *S. pneumoniae* to penicillin G and to erythromycin A is shown in Table 3 by region and by country.

Strains of *S. pneumoniae* with reduced susceptibility to penicillin G were evident in all of the participating countries, at an overall prevalence of 36.2%. There was, however, marked variation between countries in the prevalence of such isolates, ranging from 3.9% in the Netherlands to 81% in South Korea. Of those isolates with reduced susceptibility to penicillin G, the resistant phenotype was found to dominate over the intermediate phenotype in 12 of the 25 countries, with particularly high rates of penicillin G resistance being reported in all three countries within Asia (44.5–71.5%), as well as in France (46.2%) and Spain (42.1%). The Netherlands and Indonesia were the only countries not to report any isolates with full resistance to penicillin G, although the number of isolates submitted by the latter is too small to draw any meaningful conclusions. Overall, 22.1% of isolates were resistant to penicillin G, with 7.9% of isolates having a penicillin G MIC ≥ 4 mg/L.

Erythromyin A resistance rates were also very high (31.0% overall), and in most countries (19/25) they exceeded rates of penicillin G resistance. Again, Asian countries had a very high prevalence of erythromycin A resistance, averaging 79.6%, while for isolates collected from North America, Western Europe, Latin America, Eastern Europe and Australasia, the prevalence of erythromycin A resistance ranged from 11.6 to 29.1%. Across Europe, rates of resistance of *S. pneumoniae* isolates to erythromycin A reached 55.6% in Hungary and 57.6% in France. Other European countries with high levels of resistance included Belgium (32.1%), Eire (26.4%), Italy (42.9%) and Spain (28.6%).

Fluoroquinolone resistance (defined as levofloxacin MIC ≥ 8 mg/L) was detected in nine of the countries participating in PROTEKT in 1999–2000, reaching rates of 14.3% in Hong Kong (Figure 1). However, the overall prevalence of fluoroquinolone resistance was low, averaging 1.0%. Similar resistance rates were noted for moxifloxacin.

Comparative in vitro activity of the test agents

MIC and percentage susceptibility data for the 19 antimicrobials tested against *S. pneumoniae* are reported by region in Table 4. These data highlight striking differences between regions in terms of susceptibility of *S. pneumoniae* not only to

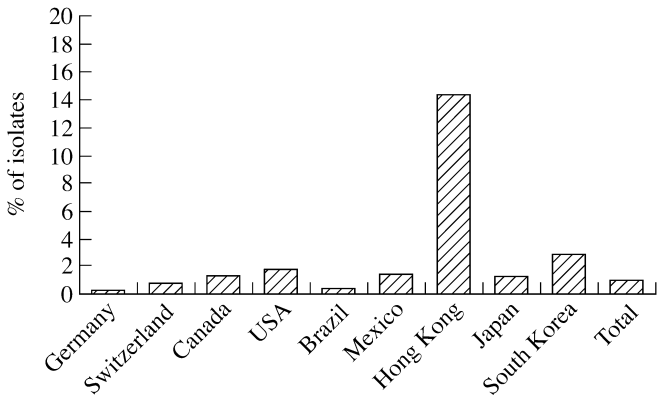

Figure 1. Prevalence of fluoroquinolone resistance among *S. pneumoniae* ($n = 3362$) collected in the PROTEKT study (1999–2000). (Only those countries where fluoroquinolone-resistant isolates were detected are shown.)

penicillin G and erythromycin A, but also to other commonly used agents. In particular, Asian countries had a very high prevalence of resistance to many of the antimicrobials tested, with only eight of the 19 agents being active against >50% of all 515 *S. pneumoniae* isolates: co-amoxiclav (87.6%), linezolid (100%), teicoplanin (100%), vancomycin (100%), quinupristin/dalfopristin (96.3%), moxifloxacin (96.7%), levofloxacin (95.3%) and telithromycin (100%).

The pattern of resistance to the cephalosporins tended to parallel that to penicillin G, the highest and lowest susceptibilities being recorded for isolates collected from Australasia and Asia, respectively. Based on MIC and susceptibility data, co-amoxiclav proved the most active of the β-lactams tested, although susceptibility to this agent was reduced somewhat in both Asia and North America (87.6% and 90.5%, respectively) compared with the other regions (97.5–100%).

Table 2. Patient demographics and culture source of 3362 isolates of *S. pneumoniae* collected in the PROTEKT study (1999–2000)

Parameter	Group	Number	Percentage of total with data	Percentage of total collected
Age	0–2	440	15.30	13.09
	3–14	362	12.60	10.77
	>14–65	1261	43.90	37.51
	>65	809	28.20	24.06
	NR	490	–	14.57
Gender	male	1831	63.10	54.46
	female	1073	36.90	31.92
	NR	458	–	13.62
Source	blood	502	17.30	14.93
	BAL	258	8.90	7.67
	sputum	1482	51.10	44.08
	sinus	137	4.70	4.07
	ear	203	7.00	6.04
	MEF	30	1.00	0.89
	nasopharyngeal	234	8.10	6.96
	throat	54	1.90	1.61
	NR	462	–	13.74
Infection site	AECB	448	24.40	13.33
	COAD	141	7.70	4.19
	pneumonia	742	40.40	22.07
	sinusitis	158	8.60	4.70
	otitis media	265	14.40	7.88
	tonsillitis/pharyngitis	81	4.40	2.41
	NR	1527	–	45.42
In/outpatient	in	1514	51.50	45.03
	out	1425	48.50	42.39
	NR	423	–	12.58

NR, not recorded; AECB, acute exacerbation of chronic bronchitis; COAD, chronic obstructive airways disease; BAL, bronchoalveolar lavage; MEF, middle ear fluid.

Table 3. Number (%) of *S. pneumoniae* strains by penicillin G and erythromycin A susceptibility interpretative category in each continent and country

Region/country	Total no. of isolates tested	Penicillin G susceptibility[a] [no. (%) of isolates]			Erythromycin A susceptibility[b] [no. (%) of isolates]		
		susceptible	intermediate	resistant	susceptible	intermediate	resistant
Western Europe	1322	996 (75.3%)	115 (8.7)	211 (16.0)	998 (75.5)	2 (0.2)	322 (24.4)
Austria	57	53 (93.0%)	1 (1.8)	3 (5.3)	49 (86.0)	1 (1.8%)	7 (12.3)
Belgium	28	23 (82.1%)	1 (3.6)	4 (14.3)	19 (67.9)	0 (0)	9 (32.1)
Eire	53	31 (58.5)	4 (7.5)	18 (34.0)	39 (73.6)	0 (0)	14 (26.4)
France	184	70 (38.0)	29 (15.8)	85 (46.2)	77 (41.8)	1 (0.5)	106 (57.6)
Germany	325	298 (91.7)	20 (6.2)	7 (2.2)	274 (84.3)	0 (0)	51 (15.7)
Italy	119	101 (84.9)	6 (5.0)	12 (10.1)	68 (57.1)	0 (0)	51 (42.9)
The Netherlands	51	49 (96.1)	2 (3.9)	0 (0)	47 (92.2)	0 (0)	4 (7.8)
Portugal	106	76 (71.7)	19 (17.9)	11 (10.4)	89 (84.0)	0 (0)	17 (16.0)
Spain	133	62 (46.6)	15 (11.3)	56 (42.1)	95 (71.4)	0 (0)	38 (28.6)
Sweden	64	58 (90.6)	1 (1.6)	5 (7.8)	61 (95.3)	0 (0)	3 (4.7)
Switzerland	111	97 (87.4)	9 (8.1)	5 (4.5)	101 (91.0)	0 (0)	10 (9.0)
UK	91	78 (85.7)	8 (8.8)	5 (5.5)	79 (86.8)	0 (0)	12 (13.2)
Eastern Europe	199	118 (59.3)	54 (27.1)	27 (13.6)	141 (70.9)	0 (0)	58 (29.1)
Hungary	54	19 (35.2)	28 (51.9)	7 (13.0)	24 (44.4)	0 (0)	30 (55.6)
Poland	68	50 (73.5)	9 (13.2)	9 (13.2)	52 (76.5)	0 (0)	16 (23.5)
Turkey	77	49 (63.6)	17 (22.1)	11 (14.3)	65 (84.4)	0 (0)	12 (15.6)
North America	687	468 (68.1)	72 (10.5)	147 (21.4)	525 (76.4)	2 (0.3)	160 (23.3)
Canada	350	276 (78.9)	37 (10.6)	37 (10.6)	292 (83.4)	2 (0.6)	56 (16.0)
USA	337	192 (57.0)	35 (10.4)	110 (32.6)	233 (69.1)	0 (0)	104 (30.9)
Latin America	518	300 (57.9)	139 (26.8)	79 (15.3)	438 (84.6)	1 (0.2)	79 (15.3)
Argentina	55	40 (72.7)	6 (10.9)	9 (16.4)	49 (89.1)	0 (0)	6 (10.9)
Brazil	260	172 (66.2)	67 (25.8)	21 (8.1)	242 (93.1)	1 (0.4)	17 (6.5)
Mexico	203	88 (43.3)	66 (32.5)	49 (24.1)	147 (72.4)	0 (0)	56 (27.6)
Australasia	121	95 (78.5)	21 (17.4)	5 (4.1)	106 (87.6)	1 (0.8)	14 (11.6)
Australia	114	91 (79.8)	18 (15.8)	5 (4.4)	99 (86.8)	1 (0.9)	14 (12.3)
Indonesia	7	4 (57.1)	3 (42.9)	0 (0)	7 (100)	0 (0)	0 (0)
Asia	515	165 (32.0)	75 (14.6)	275 (53.4)	104 (20.2)	1 (0.2)	410 (79.6)
Hong Kong	70	29 (41.4)	1 (1.4)	40 (57.1)	20 (28.6)	0 (0)	50 (71.4)
Japan	308	110 (35.7)	61 (19.8)	137 (44.5)	67 (21.8)	1 (0.3)	240 (77.9)
South Korea	137	26 (19.0)	13 (9.5)	98 (71.5)	17 (12.4)	0 (0)	120 (87.6)
Total	3362	2142 (63.7)	476 (14.2)	744 (22.1)	2312 (68.8)	7 (0.2)	1043 (31.0)

[a]Penicillin G susceptibility interpretative criteria: susceptible, ≤0.06 mg/L; intermediate, 0.12–1 mg/L; resistant, ≥2 mg/L.
[b]Erythromycin A susceptibility interpretative criteria: susceptible, ≤0.25 mg/L; intermediate, 0.5 mg/L; resistant, ≥1 mg/L.

Of the macrolide–lincosamide–streptogramin antimicrobials, telithromycin proved the most active overall (99.9% susceptibility). In contrast, high rates of resistance were reported for both clarithromycin (30.6%) and azithromycin (30.7%); the activity of these agents and patterns of resistance being essentially equivalent to those of erythromycin A across the regions studied. Resistance to clindamycin averaged 19.7%. Clindamycin resistance was reported most frequently for isolates collected from Asia and from Europe, where resistance rates were three-fold and seven-fold higher, respectively, than for the other regions. All regions reported a high prevalence of resistance to co-trimoxazole, ranging from 15.7% in Australasia to 45.6% in Latin America (28.6% overall). Overall, resistance to tetracycline averaged 29.7%, the highest prevalence being reported in Asia (81.2%). All isolates collected were susceptible to linezolid, teicoplanin and vancomycin.

Of the oral agents tested, telithromycin and linezolid proved to have the most potent anti-pneumococcal activity, based on MIC and percentage susceptibilities. *S. pneumoniae* MIC distributions for telithromycin compared with β-lactams, macrolides and fluoroquinolones are shown in Figures 2–4.

Table 4. *In vitro* activity of 19 antimicrobial agents against 3362 isolates of *S. pneumoniae* collected from across five continents in the PROTEKT study (1999–2000)[a]

	Asia ($n = 515$)			Australasia ($n = 121$)			Europe ($n = 1521$)			Latin America ($n = 518$)			North America ($n = 687$)		
Antimicrobial	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S
Telithromycin[b]	0.06	0.5	100	0.008	0.015	100	0.015	0.06	99.9	0.008	0.06	100	0.015	0.25	100
Penicillin G	2	4	32.0	0.015	0.25	78.5	0.015	2	73.2	0.06	2	57.9	0.015	2	68.1
Co-amoxiclav	1	4	87.6	0.03	0.25	100	0.03	2	97.5	0.03	2	98.7	0.03	2	90.5
Cefaclor	64	>64	21.6	1	8	81.0	1	>64	70.2	1	64	63.7	1	>64	63.5
Cefixime	32	64	32.0	0.25	4	78.5	0.25	32	73.2	0.5	32	57.9	0.25	32	68.1
Cefpodoxime	2	4	36.3	0.12	0.25	93.4	0.12	2	80.6	0.12	v2	78.4	0.12	4	75.3
Cefuroxime	4	8	36.7	0.03	0.5	92.6	0.06	8	80.6	0.12	4	77.0	0.06	8	75.3
Azithromycin	64	>64	20.2	0.12	4	87.6	0.12	>64	75.1	0.12	8	84.8	0.12	16	76.4
Clarithromycin	32	>32	20.4	0.03	1	88.4	0.03	>32	75.0	0.03	4	84.8	0.06	8	76.4
Clindamycin	2	>4	49.5	0.06	0.12	94.2	0.06	>4	80.1	0.06	0.12	93.1	0.06	0.12	91.1
Erythromycin A	64	>64	20.2	0.06	2	87.6	0.06	>64	74.9	0.06	4	84.6	0.06	16	76.4
Levofloxacin	1	1	95.3	1	1	100	1	1	99.8	1	1	99.2	1	1	98.0
Moxifloxacin	0.12	0.25	96.7	0.12	0.25	100	0.12	0.25	99.9	0.12	0.25	99.2	0.12	0.25	98.7
Co-trimoxazole	1	16	35.0	0.25	4	70.2	48.8	11.2	66.3	2	16	36.5	0.5	16	61.6
Tetracycline	>16	>16	18.4	0.25	>16	86.6	60.6	32	75.9	0.25	>16	75.1	0.25	>16	86.2
Quinupristin/dalfopristin	0.5	1	96.3	0.5	1	99.2	0.5	1	98.9	0.5	0.5	99.8	0.5	1	99.6
Linezolid	1	1	100	1	1	100	1	1	100	1	2	100	1	2	100
Teicoplanin	0.06	0.12	100	0.06	0.12	100	0.06	0.12	100	0.06	0.12	100	0.06	0.12	100
Vancomycin	0.5	0.5	100	0.5	0.5	100	0.5	0.5	100	0.5	v0.5	100	0.5	0.5	100

[a]For susceptibility criteria see Table 1.
[b]Inhibited by ≤1 mg/L (tentative NCCLS susceptibility breakpoint).

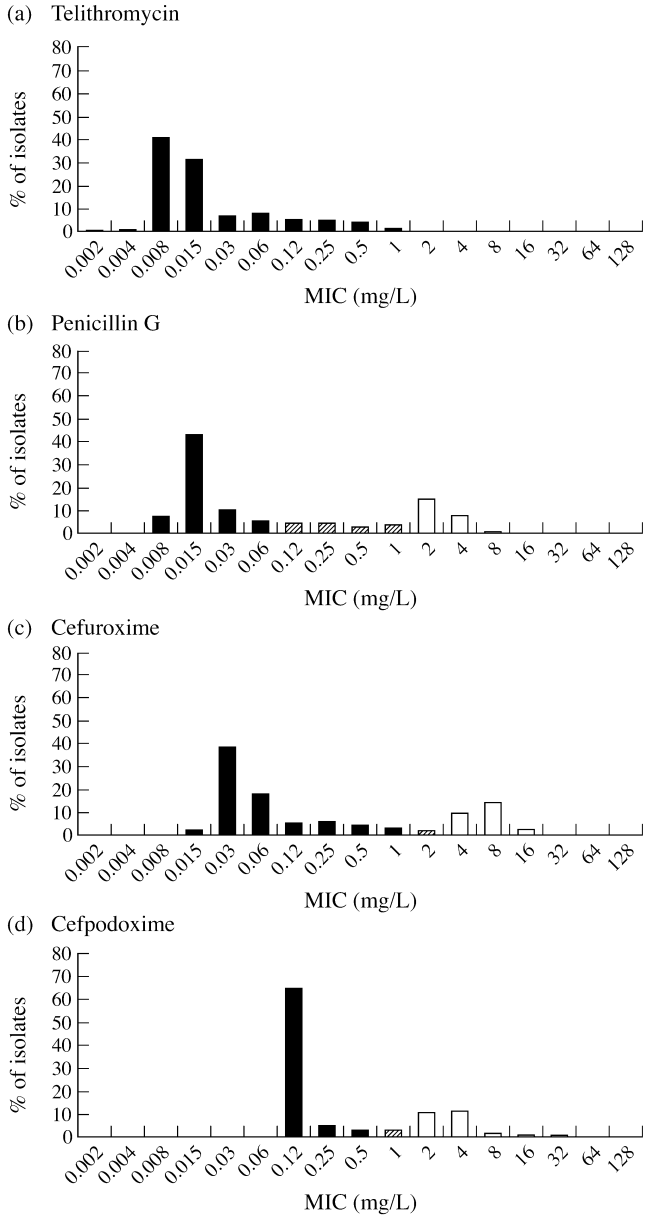

Figure 2. MIC distributions for telithromycin and β-lactams against 3362 isolates of *S. pneumoniae* collected in total in the PROTEKT study (1999–2000). Key: black bars, susceptible; shaded bars, intermediate; white bars, resistant

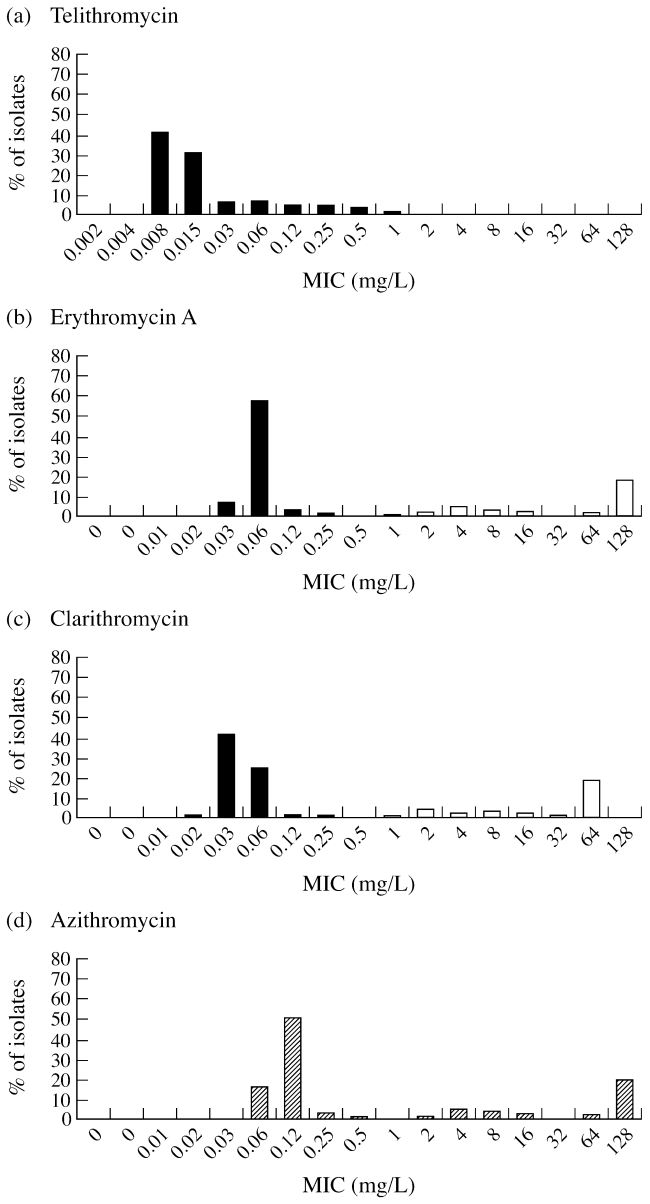

Figure 3. MIC distributions for telithromycin and macrolides against 3362 isolates of *S. pneumoniae* collected in the PROTEKT study (1999–2000). For key see Figure 2.

Cross- and parallel-resistance

In total, 12.6% of penicillin G-susceptible *S. pneumoniae* isolates were found to be resistant to erythromycin A, with rates rising to 49.2% and 72.4% for penicillin G-intermediate and -resistant isolates, respectively. Table 5 summarizes the activity of representatives of the major groups of antimicrobials against *S. pneumoniae* isolates collected in PROTEKT according to the penicillin G susceptibility phenotype, and by region. With the exception of levofloxacin and telithromycin, resistance to the drugs listed in Table 5 was notably higher among penicillin G-non-susceptible isolates than among penicillin G-susceptible isolates. A similar cross-resistance profile was observed between penicillin G and other β-lactams, and between penicillin G and macrolides (data not shown). Again there was marked geographical variation in the prevalence of antimicrobial resistance among the penicillin G resistance phenotypes.

Table 6 shows the extent to which *S. pneumoniae* isolates were multi-resistant to commonly used oral antimicrobials. Approximately 50% of all erythromycin A-resistant strains were also resistant to penicillin G and co-trimoxazole, while 63.2% and 76.5% of erythromycin A-resistant strains were

Table 5. *In vitro* activity of selected antimicrobial agents against 3362 isolates of *S. pneumoniae* collected in the PROTEKT study (1999–2000), according to penicillin G susceptibility and continent[a]

	Asia ($n = 515$)			Australasia ($n = 121$)			Europe ($n = 1521$)			Latin America ($n = 518$)			North America ($n = 687$)		
Antimicrobial	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S
Telithromycin[b]															
Pen-S	0.03	0.25	100	0.008	0.015	100	0.008	0.015	100	0.008	0.015	100	0.008	0.015	100
Pen-I	0.06	0.5	100	0.008	0.015	100	0.015	0.06	100	0.015	0.25	100	0.015	0.12	100
Pen-R	0.06	0.5	100	0.015	0.03	100	0.015	0.5	99.2	0.008	0.12	100	0.06	0.5	100
Co-amoxiclav															
Pen-S	0.03	0.06	100	0.03	0.03	100	0.03	0.03	100	0.03	0.03	100	0.03	0.03	100
Pen-I	0.25	1	98.7	0.25	0.5	100	0.12	1	100	0.12	1	100	0.25	2	100
Pen-R	2	4	77.0	2	2	100	2	8	84.0	2	2	91.4	2	>4	55.8
Cefpodoxime															
Pen-S	0.12	0.5	95.2	0.12	0.12	100	0.12	0.12	100	0.12	0.12	100	0.12	0.12	99.4
Pen-I	2	4	40.0	0.25	1	85.7	0.25	2	67.4	0.25	2	76.3	0.25	2	72.2
Pen-R	4	8	0	2	4	0	4	4	0	4	4	0	4	8	0
Erythromycin A															
Pen-S	4	>64	44.9	0.06	0.5	89.5	0.06	4	88.8	0.06	0.06	95.0	0.06	0.12	93.4
Pen-I	64	>64	14.7	0.06	0.06	90.4	>64	>64	43.8	0.06	>64	66.2	0.06	>64	58.3
Pen-R	64	>64	6.9	4	>64	40.0	>64	>64	31.9	0.06	8	77.2	4	>64	31.3
Levofloxacin															
Pen-S	1	1	95.2	1	1	100	1	1	99.7	1	1	99.3	1	1	98.1
Pen-I	0.5	1	98.7	1	1	100	1	1	100	1	1	100	1	1	98.6
Pen-R	1	1	94.5	1	1	100	1	1	100	1	1	97.5	1	1	97.3
Co-trimoxazole															
Pen-S	0.5	2	70.3	0.25	2	80	0.25	2	82.1	1	8	49.3	0.25	0.5	81.4
Pen-I	1	8	42.7	1	4	42.9	1	8	42.6	4	>16	25.2	1	16	45.8
Pen-R	4	>16	11.6	8	16	0	8	16	8.8	8	>16	7.6	8	>16	6.1
Tetracycline															
Pen-S	16	>16	37.0	0.25	0.5	90.5	0.25	8	89.2	0.25	16	81.0	0.25	1	97.7
Pen-I	>16	>16	14.7	0.25	>16	81.0	>16	>16	37.9	0.25	>16	71.2	0.25	>16	68.1
Pen-R	>16	>16	8.4	>16	>16	40.0	>16	>16	40.3	0.5	>16	59.5	0.5	>16	58.5

Pen-S, penicillin G susceptible (MIC ≤ 0.06 mg/L); Pen-I, penicillin G intermediate (MIC 0.12–1 mg/L); Pen-R, penicillin G resistant (MIC ≥2 mg/L).
[a]For susceptibility criteria see Table 1.
[b]Inhibited by ≤1 mg/L (tentative NCCLS susceptibility breakpoint).

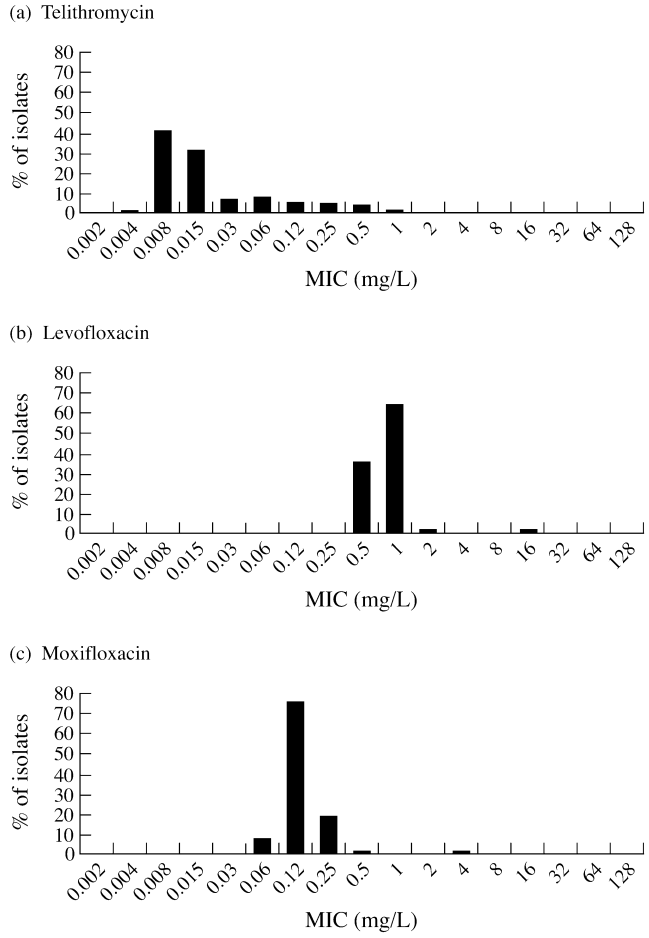

Figure 4. MIC distributions for telithromycin and fluoroquinolones against 3362 isolates of *S. pneumoniae* collected in total in the PROTEKT study (1999–2000). For key see Figure 2.

cross-resistant to clindamycin and tetracycline, respectively, but they remained susceptible to levofloxacin, moxifloxacin, telithromycin and linezolid (data not shown). Those isolates that were resistant to levofloxacin ($n = 35$) were also highly resistant to the other oral agents, with the exception of telithromycin and linezolid (all fluoroquinolone-resistant *S. pneumoniae* remained fully susceptible to these two oral agents). The *in vitro* activity of telithromycin by penicillin G, erythromycin A or fluoroquinolone susceptibility/resistance phenotype is summarized in Table 7.

Discussion

Over the last three decades, antimicrobial resistance among isolates of *S. pneumoniae* has spread across the globe at an alarming rate, and now threatens to compromise the clinical usefulness of the current portfolio of agents for treating infections associated with this pathogen. International antimicrobial surveillance studies play a critical role in defining the nature and extent of the resistance problem, as well as in

Table 6. Multiple resistance among 3362 isolates of *S. pneumoniae* collected in the PROTEKT study (1999–2000) (the percentages quoted in the table are the percentage of isolates resistant to Agent 1 that are also resistant to Agent 2)

		No. (%) of isolates also resistant to:				
Phenotype	No. of isolates	penicillin G	clindamycin	erythromycin A	levofloxacin	tetracycline	co-trimoxazole
Penicillin G resistant	744		329 (44.2%)	539 (72.4%)	18 (2.4%)	486 (65.3%)	552 (74.2%)
Clindamycin resistant	661	329 (49.8%)		659 (99.7%)	19 (2.9%)	586 (88.7%)	366 (55.4%)
Erythromycin A resistant	1043	539 (51.7%)	659 (63.2%)		27 (2.6%)	798 (76.5%)	527 (50.5%)
Levofloxacin resistant	35	18 (51.4%)	19 (54.2%)	27 (77.1%)		21 (60.0%)	20 (57.1%)
Tetracycline resistant	997	486 (48.7%)	586 (58.8%)	798 (80.0%)	21 (2.1%)		491 (49.2%)
Co-trimoxazole resistant	960	552 (57.5%)	366 (38.1%)	527 (54.9%)	20 (2.1%)	491 (51.1%)	

Table 7. *In vitro* activity of telithromycin against *S. pneumoniae* isolates collected in PROTEKT, by antimicrobial resistance phenotype

Phenotype	*n*	MIC (mg/L)				% susceptibility[a]
		Mode	MIC_{50}	MIC_{90}	Range	
Penicillin G						
Susceptible	2142	0.008	0.008	0.03	0.002–0.5	100
Intermediate	476	0.008	0.015	0.12	0.004–1	100
Resistant	744	0.06	0.06	0.5	0.004–8	99.6
Erythromycin A						
Susceptible	2312	0.008	0.008	0.015	0.002–0.12	100
Intermediate	7	0.015	0.015	0.03	0.008–0.03	100
Resistant	1043	0.06	0.06	0.5	0.008–8	99.7
Levofloxacin						
Susceptible	3317	0.008	0.015	0.12	0.002–8	99.9
Intermediate	10	0.008	0.03	0.12	0.008–0.12	100
Resistant	35	0.03	0.03	0.12	0.008–0.5	100
Combined	3362	0.008	0.015	0.12	0.002–8	99.9

[a]Inhibited by ≤1 mg/L (tentative NCCLS susceptibility breakpoint).

guiding development of antimicrobial policies and usage.[23] Furthermore, by monitoring changes in resistance patterns and the spread of significant resistance phenotypes across the globe, these studies enable authorities to implement intervention strategies to halt the spread of such strains. The PROTEKT study was initiated in 1999 to monitor longitudinally the global spread of resistance among bacterial respiratory pathogens associated with CARTIs. Testing of all isolates at a central laboratory using validated identification techniques and NCCLS-recommended methodologies has helped to ensure the quality and consistency of the data generated.

Consistent with previous international surveillance studies,[10,11,24] PROTEKT reported large differences between countries in the penicillin G resistance profile of *S. pneumoniae*, the highest rates of resistance being noted in clinical isolates from patients in the Asian region. In all three of the Asian countries studied, the resistant phenotype (44.5–71.6% of strains) dominated over the intermediate phenotype (1.4–19.8%). For example, within Hong Kong, the prevalence of penicillin G intermediate and penicillin G-resistant strains was 1.4% and 57.1%, respectively, which is consistent with that reported by Felmingham *et al.*[10] Furthermore, our data suggest a large upward shift in the penicillin G MICs among *S. pneumoniae* from Japan: a multicentre study of 1997–1998 pneumococcal isolates reported penicillin G intermediate and penicillin G resistant rates in Japan of 44% and 10%, respectively,[11] while in the present 1999–2000 study, the percentages were 20% and 45%, respectively.

Outside of Asia, penicillin G resistance rates in excess of 40% were recorded in France and Spain, while in the neighbouring countries of Germany and Switzerland, resistance to this agent averaged 2.2% and 4.5%, respectively. The data also suggest that the prevalence of penicillin G resistance in the USA has risen substantially from the 9.5% reported in a 1994–1995 study by Doern *et al*.[7] For 1997–1998 US isolates, Thornsberry *et al.* found 13% penicillin G resistance,[25] Hoban *et al.* reported 15% resistance among 1999 isolates,[24] and in 1999–2000 isolates we have found 33% resistance. Although the penicillin G resistance prevalence in the USA seems exceptionally high in this study, preliminary data from a subsequent sister surveillance study, PROTEKT US, reported a rate of 26% among 10 103 isolates of *S. pneumoniae*, with individual rates as high as 36.5% being observed in the southeastern region of the country.[26]

The increasing prevalence of penicillin G resistance among *S. pneumoniae* is a cause for concern, not only because of the impact that it may have on the clinical usefulness of this agent, but also because resistance to penicillin G has been found to be associated with resistance to other β-lactams, such as the cephalosporins, as well as to several non-β-lactam classes. In *S. pneumoniae*, reduced susceptibility to β-lactams, such as penicillin, arises through alterations in the penicillin-binding proteins (PBPs)—1A, 1B, 2A, 2B, 2X and 3—via acquisition of genetic material from other *Streptococcus* spp coexisting in close proximity, e.g. *S. mitis*. The resistance phenotype of the resulting strain depends upon which particular PBP is modified. For example, while many of the penicillin-resistant strains first identified remained susceptible to third-generation cephalosporins, resistance to these agents is now well established among penicillin-resistant pneumococci, reflecting alterations in PBPs, particularly 1A and 2X.

As reported in other studies,[7,24,25,27] co-resistance to erythromycin A, clindamycin, tetracycline and co-trimoxazole was higher among strains with intermediate susceptibility to penicillin G than among penicillin G-susceptible strains, with an even higher prevalence being noted among strains highly resistant to penicillin G. In the absence of any known common mechanism of resistance between penicillin G and these non-β-lactam antimicrobials, this relationship most probably reflects increased selective pressure among *S. pneumoniae* created by broad-based antimicrobial usage.[24] This hypothesis is supported by the fact that in some regions, elevated resistance to these antimicrobials was also noted in the penicillin G-susceptible population, particularly in Asia (macrolides, co-trimoxazole and tetracycline), Europe (macrolides) and Latin America (co-trimoxazole and tetracycline), where these agents are widely prescribed.

Overall, resistance to erythromycin A among *S. pneumoniae* averaged 31%, and in many countries was more prevalent than resistance to penicillin G. There were no important differences in the activity of the newer agents, e.g. clarithromycin and azithromycin, and pneumococci resistant to erythromycin A were invariably resistant to these second-generation macrolides. As reported in the 1996–1997 Alexander Project,[10] we found France, Belgium, Spain and Italy to be the foci of macrolide resistance in Western Europe. However, the 58% and 43% macrolide resistance rates reported in our study for 1999–2000 isolates from France and Italy, respectively, are substantially higher than those reported by Felmingham *et al.* for 1997 isolates in the Alexander Project (46% and 30%, respectively).[10] Within Western Europe, we also found substantial increases in the prevalence of macrolide resistance in Germany (16% versus 7%), Portugal (16% versus 4%) and Eire (26% versus 14%), compared with the prevalences reported in the Alexander Project for 1997 isolates. Outside Western Europe, high rates of resistance were found in all Asian countries participating in the study, as well as in Hungary, Mexico and the USA. Hoban and colleagues have recently reported a significant rise in macrolide resistance among US pneumococci isolated in the SENTRY study, rising from 17% in 1998 to 23% in 1999.[24] The 31% macrolide resistance rate observed in our study for 1999–2000 *S. pneumoniae* US isolates, suggests that in the USA, resistance to this class has risen from the 1999 data reported in SENTRY. This is supported by the preliminary data from PROTEKT US, which found that 31% of pneumococci collected during 2000–2001 from 206 centres across the USA were resistant to erythromycin A.[26] A recent analysis conducted in Spain has shown a clear correlation between erythromycin A resistance and overall macrolide consumption, the relationship appearing to be due mainly to consumption of those macrolides dosed once or twice daily.[28] While this analysis is not able to demonstrate cause and effect, it does suggest that increased use of long-acting macrolides may be linked to the increase in prevalence of erythromycin A-resistant pneumococci observed in recent years.

Two major forms of macrolide resistance exist in *Streptococcus* spp: (i) modification of the 23S ribosomal RNA target site by methylation (encoded by *erm* genes); and (ii) antimicrobial efflux by proton-motive force or utilizing ATP (encoded by *mef* genes).[29,30] Modification of the ribosomal target site confers a high-level resistance not only to macrolides, but also to lincosamides and streptogramin$_B$ (so-called MLS$_B$ phenotype), while the resistance conferred by efflux abnormality (M phenotype) is usually of a moderate level and confined to 14- and 15-membered ring macrolides only (clindamycin usually remains active). In a genotypic analysis of the resistance mechanism in 1043 macrolide-resistant pneumococcal isolates from the PROTEKT 1999–2000 study, 56.2% of isolates expressed *erm*(B) (predominantly in Europe) and 35.3% expressed *mef*(A) (predominantly in North America).[31] In our study, 63% of erythromycin A-resistant pneumococci were also resistant to clindamycin, consistent with *erm* (MLS$_B$) resistance. Reduced susceptibility to clindamycin was particularly prevalent in Asia and in Europe. Relative to *mef*-mediated resistance, these strains may be more difficult to eradicate, with a subsequent risk of clinical failure.

In the present study, the prevalence of resistance to the newer anti-pneumococcal fluoroquinolones, e.g. levofloxacin and moxifloxacin, remained low (~1%). There were, however, pockets of resistance to these agents, particularly in Hong Kong (14%) and South Korea (3%). The situation in Hong Kong is particularly alarming since all fluoroquinolone-resistant pneumococci were also highly resistant to β-lactams, macrolides, co-trimoxazole, cephalosporins and tetracycline—the only two oral agents that retained full susceptibility against these isolates were the ketolide, telithromycin and linezolid.[32] Molecular characterization has shown that the vast majority of the fluoroquinolone resistance detected in Hong Kong was due to the spread of the 23F Spanish multidrug-resistant clone.[32] Goldsmith *et al.* have reported an increased prevalence of fluoroquinolone resistance among penicillin G-resistant pneumococci in Northern Ireland,[33] although subsequent surveillance studies have not been able to confirm this relationship for other regions.[11,24,25] In our study, levofloxacin-resistant and/or moxifloxacin-resistant strains were isolates from countries with relatively low rates of penicillin G resistance (i.e. Germany, Switzerland and Canada), as well as from those countries in which reduced penicillin G susceptibility is more prevalent (e.g. Asia). Half of the 35 levofloxacin-resistant pneumococci identified in this study were also resistant to penicillin G, and approximately three-quarters were co-resistant to erythromycin A. No glycopeptide-resistant pneumococci were identified in the study.

One of the objectives of the PROTEKT study was to provide current surveillance data for telithromycin at its time of

launch into clinical practice. The study has shown this new ketolide to be the most potent of the oral antimicrobials currently available against *S. pneumoniae*. Moreover, the data confirm previously published findings that telithromycin maintains its anti-pneumococcal activity against strains that are resistant to β-lactams, macrolides and fluoroquinolones.[18] Using the proposed NCCLS interpretative criteria of ≤1 mg/L to define susceptibility to telithromycin gives an overall susceptibility of 99.9% for *S. pneumoniae*. Applying the slightly lower breakpoint (≤0.5 mg/L) adopted by the European authorities to the PROTEKT MIC data has negligible effect on the overall susceptibility to telithromycin, reducing it to 99.3%. Results from subsequent years of the PROTEKT study will help to establish the impact that the introduction of telithromycin will have on susceptibility of *S. pneumoniae* not only to this agent, but also to non-ketolide antibacterials. *In vitro* studies and preliminary data from human studies suggest that telithromycin has a favourable ecological profile, with a low potential to select for resistant mutants, and, in contrast to the macrolides, telithromycin does not induce MLS resistance *in vitro*.[20] The low potential for telithromycin to select for pneumococcal resistance has been confirmed recently by Davies and colleagues,[34] who performed a series of *in vitro* serial passage experiments in which five macrolide-susceptible and six macrolide-resistant [three *erm*(B), three *mef*(A)] strains of *S. pneumoniae* were repeatedly exposed to sub-inhibitory concentrations of telithromycin or other MLS_B antibacterials. They found that telithromycin selected for resistant mutants significantly less often than the other agents. Indeed, of the 54 mutants isolated with raised MICs of at least one of the test agents, only three were resistant to telithromycin compared with 36, 37, 36, 45 and 15 mutants resistant to azithromycin, clarithromycin, erythromycin A, roxithromycin and clindamycin, respectively. Furthermore, studies in man have shown that telithromycin selects for resistance among oropharyngeal and intestinal bacterial microflora significantly less than clarithromycin.[21]

In conclusion, the 1999–2000 data from PROTEKT confirm the widespread and increasing prevalence of antimicrobial resistance among *S. pneumoniae*. The large intercountry variation reported in the prevalence of resistance, not only to penicillin G but also to other antimicrobials, highlights the need for continuing surveillance of resistance at both the national and international level, with the data being analysed and disseminated rapidly so that changes in susceptibility patterns can be quickly detected allowing appropriate control measures to be put in place. In this era of increasing pneumococcal resistance, the ketolide telithromycin has emerged as a promising new candidate for the treatment of CARTIs, possessing potent anti-pneumococcal activity even against strains with resistance to other commonly used antimicrobials.

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Increasing prevalence of antimicrobial resistance among isolates of *Streptococcus pneumoniae* from the PROTEKT surveillance study, and comparative *in vitro* activity of the ketolide, telithromycin

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The prevalence of resistance to a range of antimicrobials was determined for isolates of *Streptococcus pneumoniae* examined in the PROTEKT (Prospective Resistant Organism Tracking and Epidemiology for the Ketolide Telithromycin) surveillance study (1999–2000) using NCCLS testing methods and interpretative criteria. Of 3362 pneumococcal isolates collected from 69 centres in 25 countries, 22.1% overall were resistant to penicillin G, with the highest rates of resistance found among isolates from Asia (53.4%), France (46.2%) and Spain (42.1%). Erythromycin A resistance occurred in 31.1% of isolates overall with the highest rates found in Asia (79.6%), France (57.6%), Hungary (55.6%) and Italy (42.9%). Marked geographical differences in the prevalence of both penicillin G (the Netherlands 0%; South Korea 71.5%) and erythromycin A (Sweden 4.7%; South Korea 87.6%) resistance were observed. Asia was characterized by the highest prevalence of resistance, overall, with only eight of 19 antimicrobials (co-amoxiclav, linezolid, vancomycin, teicoplanin, quinupristin/dalfopristin, levofloxacin, moxifloxacin and telithromycin) retaining high activity against isolates of *S. pneumoniae* from this region. Notable rates of resistance to clarithromycin, azithromycin, co-trimoxazole and tetracycline were observed in the majority of countries submitting isolates of *S. pneumoniae* to the PROTEKT surveillance study. Fluoroquinolone resistance was low (1%), overall, although 14.3% of 70 isolates from Hong Kong were resistant to levofloxacin and moxifloxacin, all but one of these isolates belonging to a single clone of the 23F serotype. Although, at present, apparently limited to pockets of clonal spread, continued vigilance with regard to the evolution of fluoroquinolone resistance is indicated. Telithromycin (MIC_{90} 0.12 mg/L; 99.9% of isolates susceptible) and linezolid (MIC_{90} 2 mg/L; 100% of isolates susceptible) were the two most active oral agents tested, both compounds retaining activity against isolates of fluoroquinolone-resistant *S. pneumoniae*. The results of the PROTEKT surveillance study 1999–2000 emphasize the widespread evolution of resistance to a variety of antimicrobials amongst isolates of *S. pneumoniae* and demonstrate the potential of telithromycin as a therapeutic option for the treatment of community-acquired respiratory tract infections caused by this organism.

Introduction

Although a common component of the normal human respiratory flora, *Streptococcus pneumoniae* is also one of the most important bacterial pathogens. Infection with this organism is among the most frequent causes of community-acquired respiratory tract infections (CARTIs), including community-acquired pneumonia (45% of cases), otitis media (30–40%) and sinusitis (20–35%), as well as being one of the leading causes of meningitis and bacteraemia.[1–3] Indeed, it has been estimated that each year in the USA, *S. pneumoniae* infections account for 500 000 cases of pneumonia, 55 000 cases of bacteraemia and 6000 cases of meningitis.[4,5]

While β-lactams, most notably penicillin G, have been the mainstay of antimicrobial treatment for CARTIs, resistance to these agents has reached alarming levels in many regions. Isolates of *S. pneumoniae* with reduced susceptibility to penicillin G were first reported during the 1960s in Australia and Papua New Guinea.[3,6] By the early 1970s penicillin G-intermediate and -resistant strains had been detected in

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Poland and Germany, with Japan, South Africa, Switzerland and the UK also reporting the presence of such strains during the mid to late 1970s.[6] Subsequently, penicillin G-resistant strains of *S. pneumoniae* have rapidly spread worldwide and their prevalence continues to increase.[7–12] Of even more concern is the degree to which these isolates are cross-resistant, not only to other β-lactam drugs, such as the cephalosporins, but also to non-β-lactam classes, e.g. the macrolides.[10] Resistance to fluoroquinolones is also emerging among *S. pneumoniae*, albeit currently at relatively low levels.[13]

In this era of increasing antimicrobial resistance, treatment of CARTIs should ideally be pathogen directed, based on identification and susceptibility testing of the causative organism. In reality, however, an aetiologically specific diagnosis, let alone susceptibility results, is rarely available to assist in the selection of therapy, which is consequently largely empirical. In view of the importance of *S. pneumoniae* in CARTIs and the traditionally empirical approach to the treatment of these infections, knowledge of local antimicrobial resistance patterns is essential to ensure rational prescribing.[14] These issues highlight the need for continuing surveillance of antimicrobial resistance at local, national and international levels, as well as the need for research into new antibacterial agents with the ability to overcome *S. pneumoniae* resistance mechanisms and which do not induce or select further resistance.

The ketolides are a new family of antibacterial agents belonging to the macrolide–lincosamide–streptogramin class.[15,16] Telithromycin, the first ketolide antibacterial, has demonstrated clinical efficacy across a range of CARTIs, and potent *in vitro* activity against the pathogens commonly implicated in these infections, including drug-resistant *S. pneumoniae*.[17–19] Data from both *in vitro* and human studies suggest that this agent also has a low potential for the selection or induction of resistance, and has minimal effect on normal human gut flora.[20,21] In 1999, the PROTEKT (Prospective Resistant Organism Tracking and Epidemiology for the Ketolide Telithromycin) surveillance study was established to track longitudinally the susceptibility of common respiratory pathogens from patients with CARTIs to current and new antimicrobials, including the new ketolide, telithromycin. This paper describes the widespread prevalence of resistance and the comparative activity of common and newly developed antimicrobial agents against isolates of *S. pneumoniae* collected during the first year (1999–2000) of the PROTEKT study.

Materials and methods

Collecting centres

During the 1999–2000 respiratory season, 69 centres in 25 countries participated in PROTEKT: Canada (7), USA (8), Mexico (4), Brazil (7), Argentina (2), Germany (7), the Netherlands (1), Sweden (1), UK (2), Eire (1), Belgium (1), France (4), Portugal (2), Spain (2), Switzerland (2), Italy (2), Austria (1), Turkey (1), Hungary (1), Poland (1), Hong Kong (1), Japan (6), South Korea (2), Australia (2) and Indonesia (1).

Bacterial isolates

Each centre recruited into the PROTEKT study was requested to collect a minimum of 60 isolates of *S. pneumoniae* from patients with any of six types of community-acquired infection, namely sinusitis, otitis media, pharyngitis, pneumonia, acute bacterial exacerbations of chronic bronchitis and acute exacerbation of chronic obstructive airways disease. Sources for isolation of RTI pathogens were cultures from blood, sputum, bronchoalveolar lavage, middle ear fluid, nasopharyngeal swab or aspirate, and sinus aspirate. Patients with nosocomial RTIs and those with cystic fibrosis were excluded, and duplicate strains or strains originating from existing collections were not included in the study. Demographic data collected included the age and sex of the patient, infection, culture source, in/outpatient status, specimen accession number and date of sample collection. Criteria for isolate storage, transportation and identification, and confirmation of isolate identification have been described in detail previously.[22]

Antimicrobial susceptibility testing

MICs were determined at a central laboratory (GR Micro Ltd, London, UK) for a panel of existing and new antimicrobials, using previously described methods.[22] In brief, we used the NCCLS broth microdilution method with lyophilized microtitre plates (Sensititre system; Trek Diagnostics) and an inoculum of $3–7 \times 10^4$ cfu in 100 μL medium. MIC endpoints were read as the lowest concentration of antimicrobial that totally inhibited macroscopically visible growth of the inoculum.

Isolates were tested against the following antimicrobials: penicillin G, co-amoxiclav (2:1 ratio), cefaclor, cefixime, cefpodoxime, cefuroxime, azithromycin, clarithromycin, erythromycin A, telithromycin, clindamycin, levofloxacin, ciprofloxacin, moxifloxacin, co-trimoxazole (1:19 ratio), linezolid, quinupristin–dalfopristin (30:70 ratio), teicoplanin, tetracycline and vancomycin. Percentage susceptibilities were calculated based on NCCLS breakpoints, although it should be noted that NCCLS breakpoints applied to telithromycin are tentative and not yet approved (Table 1).

Results

A total of 3362 isolates of *S. pneumoniae* were collected from 25 countries within Western Europe (n = 1322), North America (n = 687), Latin America (n = 518), Asia (n = 515), Eastern Europe (n = 199) and Australasia (n = 121). Informa-

Table 1. Interpretative categories for *S. pneumoniae*[a]

Antimicrobial agent	Interpretative categories (mg/L)		
	susceptible	intermediate	resistant
Co-amoxiclav	≤2	4	≥8
Azithromycin	≤0.5	1	≥2
Cefaclor	≤1	2	≥4
Cefixime	susceptibility reported as for penicillin G		
Cefpodoxime	≤0.5	1	≥2
Cefuroxime	≤1	2	≥4
Clarithromycin	≤0.25	0.5	≥1
Clindamycin	≤0.25	0.5	≥1
Co-trimoxazole	≤0.5	1–2	≥4
Erythromycin A	≤0.25	0.5	≥1
Levofloxacin	≤2	4	≥8
Linezolid	≤2		≥8
Moxifloxacin	≤1	2	≥4
Penicillin G	≤0.06	0.12–1	≥2
Quinupristin/dalfopristin	≤1	2	≥4
Teicoplanin	≤8	16	≥32
Telithromycin	≤1	2	≥4
Tetracycline	≤2	4	≥8
Vancomycin	≤1	–	–

[a]All breakpoints given are NCCLS, with the exception of telithromycin for which NCCLS-approved breakpoints are not yet available (tentative breakpoints given).

tion on culture source, patient age and gender, and treatment setting were available for approximately 45–87% of the isolates collected, and this is summarized in Table 2.

Prevalence of antimicrobial resistance

Susceptibility of *S. pneumoniae* to penicillin G and to erythromycin A is shown in Table 3 by region and by country.

Strains of *S. pneumoniae* with reduced susceptibility to penicillin G were evident in all of the participating countries, at an overall prevalence of 36.2%. There was, however, marked variation between countries in the prevalence of such isolates, ranging from 3.9% in the Netherlands to 81% in South Korea. Of those isolates with reduced susceptibility to penicillin G, the resistant phenotype was found to dominate over the intermediate phenotype in 12 of the 25 countries, with particularly high rates of penicillin G resistance being reported in all three countries within Asia (44.5–71.5%), as well as in France (46.2%) and Spain (42.1%). The Netherlands and Indonesia were the only countries not to report any isolates with full resistance to penicillin G, although the number of isolates submitted by the latter is too small to draw any meaningful conclusions. Overall, 22.1% of isolates were resistant to penicillin G, with 7.9% of isolates having a penicillin G MIC ≥ 4 mg/L.

Erythromyin A resistance rates were also very high (31.0% overall), and in most countries (19/25) they exceeded rates of penicillin G resistance. Again, Asian countries had a very high prevalence of erythromycin A resistance, averaging 79.6%, while for isolates collected from North America, Western Europe, Latin America, Eastern Europe and Australasia, the prevalence of erythromycin A resistance ranged from 11.6 to 29.1%. Across Europe, rates of resistance of *S. pneumoniae* isolates to erythromycin A reached 55.6% in Hungary and 57.6% in France. Other European countries with high levels of resistance included Belgium (32.1%), Eire (26.4%), Italy (42.9%) and Spain (28.6%).

Fluoroquinolone resistance (defined as levofloxacin MIC ≥ 8 mg/L) was detected in nine of the countries participating in PROTEKT in 1999–2000, reaching rates of 14.3% in Hong Kong (Figure 1). However, the overall prevalence of fluoroquinolone resistance was low, averaging 1.0%. Similar resistance rates were noted for moxifloxacin.

Comparative in vitro activity of the test agents

MIC and percentage susceptibility data for the 19 antimicrobials tested against *S. pneumoniae* are reported by region in Table 4. These data highlight striking differences between regions in terms of susceptibility of *S. pneumoniae* not only to

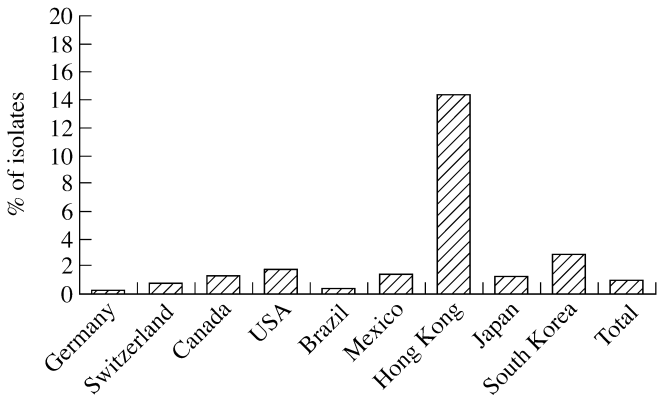

Figure 1. Prevalence of fluoroquinolone resistance among *S. pneumoniae* ($n = 3362$) collected in the PROTEKT study (1999–2000). (Only those countries where fluoroquinolone-resistant isolates were detected are shown.)

penicillin G and erythromycin A, but also to other commonly used agents. In particular, Asian countries had a very high prevalence of resistance to many of the antimicrobials tested, with only eight of the 19 agents being active against >50% of all 515 *S. pneumoniae* isolates: co-amoxiclav (87.6%), linezolid (100%), teicoplanin (100%), vancomycin (100%), quinupristin/dalfopristin (96.3%), moxifloxacin (96.7%), levofloxacin (95.3%) and telithromycin (100%).

The pattern of resistance to the cephalosporins tended to parallel that to penicillin G, the highest and lowest susceptibilities being recorded for isolates collected from Australasia and Asia, respectively. Based on MIC and susceptibility data, co-amoxiclav proved the most active of the β-lactams tested, although susceptibility to this agent was reduced somewhat in both Asia and North America (87.6% and 90.5%, respectively) compared with the other regions (97.5–100%).

Table 2. Patient demographics and culture source of 3362 isolates of *S. pneumoniae* collected in the PROTEKT study (1999–2000)

Parameter	Group	Number	Percentage of total with data	Percentage of total collected
Age	0–2	440	15.30	13.09
	3–14	362	12.60	10.77
	>14–65	1261	43.90	37.51
	>65	809	28.20	24.06
	NR	490	–	14.57
Gender	male	1831	63.10	54.46
	female	1073	36.90	31.92
	NR	458	–	13.62
Source	blood	502	17.30	14.93
	BAL	258	8.90	7.67
	sputum	1482	51.10	44.08
	sinus	137	4.70	4.07
	ear	203	7.00	6.04
	MEF	30	1.00	0.89
	nasopharyngeal	234	8.10	6.96
	throat	54	1.90	1.61
	NR	462	–	13.74
Infection site	AECB	448	24.40	13.33
	COAD	141	7.70	4.19
	pneumonia	742	40.40	22.07
	sinusitis	158	8.60	4.70
	otitis media	265	14.40	7.88
	tonsillitis/pharyngitis	81	4.40	2.41
	NR	1527	–	45.42
In/outpatient	in	1514	51.50	45.03
	out	1425	48.50	42.39
	NR	423	–	12.58

NR, not recorded; AECB, acute exacerbation of chronic bronchitis; COAD, chronic obstructive airways disease; BAL, bronchoalveolar lavage; MEF, middle ear fluid.

Table 3. Number (%) of *S. pneumoniae* strains by penicillin G and erythromycin A susceptibility interpretative category in each continent and country

Region/country	Total no. of isolates tested	Penicillin G susceptibility[a] [no. (%) of isolates]			Erythromycin A susceptibility[b] [no. (%) of isolates]		
		susceptible	intermediate	resistant	susceptible	intermediate	resistant
Western Europe	1322	996 (75.3%)	115 (8.7)	211 (16.0)	998 (75.5)	2 (0.2)	322 (24.4)
Austria	57	53 (93.0%)	1 (1.8)	3 (5.3)	49 (86.0)	1 (1.8%)	7 (12.3)
Belgium	28	23 (82.1%)	1 (3.6)	4 (14.3)	19 (67.9)	0 (0)	9 (32.1)
Eire	53	31 (58.5)	4 (7.5)	18 (34.0)	39 (73.6)	0 (0)	14 (26.4)
France	184	70 (38.0)	29 (15.8)	85 (46.2)	77 (41.8)	1 (0.5)	106 (57.6)
Germany	325	298 (91.7)	20 (6.2)	7 (2.2)	274 (84.3)	0 (0)	51 (15.7)
Italy	119	101 (84.9)	6 (5.0)	12 (10.1)	68 (57.1)	0 (0)	51 (42.9)
The Netherlands	51	49 (96.1)	2 (3.9)	0 (0)	47 (92.2)	0 (0)	4 (7.8)
Portugal	106	76 (71.7)	19 (17.9)	11 (10.4)	89 (84.0)	0 (0)	17 (16.0)
Spain	133	62 (46.6)	15 (11.3)	56 (42.1)	95 (71.4)	0 (0)	38 (28.6)
Sweden	64	58 (90.6)	1 (1.6)	5 (7.8)	61 (95.3)	0 (0)	3 (4.7)
Switzerland	111	97 (87.4)	9 (8.1)	5 (4.5)	101 (91.0)	0 (0)	10 (9.0)
UK	91	78 (85.7)	8 (8.8)	5 (5.5)	79 (86.8)	0 (0)	12 (13.2)
Eastern Europe	199	118 (59.3)	54 (27.1)	27 (13.6)	141 (70.9)	0 (0)	58 (29.1)
Hungary	54	19 (35.2)	28 (51.9)	7 (13.0)	24 (44.4)	0 (0)	30 (55.6)
Poland	68	50 (73.5)	9 (13.2)	9 (13.2)	52 (76.5)	0 (0)	16 (23.5)
Turkey	77	49 (63.6)	17 (22.1)	11 (14.3)	65 (84.4)	0 (0)	12 (15.6)
North America	687	468 (68.1)	72 (10.5)	147 (21.4)	525 (76.4)	2 (0.3)	160 (23.3)
Canada	350	276 (78.9)	37 (10.6)	37 (10.6)	292 (83.4)	2 (0.6)	56 (16.0)
USA	337	192 (57.0)	35 (10.4)	110 (32.6)	233 (69.1)	0 (0)	104 (30.9)
Latin America	518	300 (57.9)	139 (26.8)	79 (15.3)	438 (84.6)	1 (0.2)	79 (15.3)
Argentina	55	40 (72.7)	6 (10.9)	9 (16.4)	49 (89.1)	0 (0)	6 (10.9)
Brazil	260	172 (66.2)	67 (25.8)	21 (8.1)	242 (93.1)	1 (0.4)	17 (6.5)
Mexico	203	88 (43.3)	66 (32.5)	49 (24.1)	147 (72.4)	0 (0)	56 (27.6)
Australasia	121	95 (78.5)	21 (17.4)	5 (4.1)	106 (87.6)	1 (0.8)	14 (11.6)
Australia	114	91 (79.8)	18 (15.8)	5 (4.4)	99 (86.8)	1 (0.9)	14 (12.3)
Indonesia	7	4 (57.1)	3 (42.9)	0 (0)	7 (100)	0 (0)	0 (0)
Asia	515	165 (32.0)	75 (14.6)	275 (53.4)	104 (20.2)	1 (0.2)	410 (79.6)
Hong Kong	70	29 (41.4)	1 (1.4)	40 (57.1)	20 (28.6)	0 (0)	50 (71.4)
Japan	308	110 (35.7)	61 (19.8)	137 (44.5)	67 (21.8)	1 (0.3)	240 (77.9)
South Korea	137	26 (19.0)	13 (9.5)	98 (71.5)	17 (12.4)	0 (0)	120 (87.6)
Total	3362	2142 (63.7)	476 (14.2)	744 (22.1)	2312 (68.8)	7 (0.2)	1043 (31.0)

[a]Penicillin G susceptibility interpretative criteria: susceptible, ≤0.06 mg/L; intermediate, 0.12–1 mg/L; resistant, ≥2 mg/L.
[b]Erythromycin A susceptibility interpretative criteria: susceptible, ≤0.25 mg/L; intermediate, 0.5 mg/L; resistant, ≥1 mg/L.

Of the macrolide–lincosamide–streptogramin antimicrobials, telithromycin proved the most active overall (99.9% susceptibility). In contrast, high rates of resistance were reported for both clarithromycin (30.6%) and azithromycin (30.7%); the activity of these agents and patterns of resistance being essentially equivalent to those of erythromycin A across the regions studied. Resistance to clindamycin averaged 19.7%. Clindamycin resistance was reported most frequently for isolates collected from Asia and from Europe, where resistance rates were three-fold and seven-fold higher, respectively, than for the other regions. All regions reported a high prevalence of resistance to co-trimoxazole, ranging from 15.7% in Australasia to 45.6% in Latin America (28.6% overall). Overall, resistance to tetracycline averaged 29.7%, the highest prevalence being reported in Asia (81.2%). All isolates collected were susceptible to linezolid, teicoplanin and vancomycin.

Of the oral agents tested, telithromycin and linezolid proved to have the most potent anti-pneumococcal activity, based on MIC and percentage susceptibilities. *S. pneumoniae* MIC distributions for telithromycin compared with β-lactams, macrolides and fluoroquinolones are shown in Figures 2–4.

Table 4. *In vitro* activity of 19 antimicrobial agents against 3362 isolates of *S. pneumoniae* collected from across five continents in the PROTEKT study (1999–2000)[a]

	Asia ($n = 515$)			Australasia ($n = 121$)			Europe ($n = 1521$)			Latin America ($n = 518$)			North America ($n = 687$)		
Antimicrobial	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S
Telithromycin[b]	0.06	0.5	100	0.008	0.015	100	0.015	0.06	99.9	0.008	0.06	100	0.015	0.25	100
Penicillin G	2	4	32.0	0.015	0.25	78.5	0.015	2	73.2	0.06	2	57.9	0.015	2	68.1
Co-amoxiclav	1	4	87.6	0.03	0.25	100	0.03	2	97.5	0.03	2	98.7	0.03	2	90.5
Cefaclor	64	>64	21.6	1	8	81.0	1	>64	70.2	1	64	63.7	1	>64	63.5
Cefixime	32	64	32.0	0.25	4	78.5	0.25	32	73.2	0.5	32	57.9	0.25	32	68.1
Cefpodoxime	2	4	36.3	0.12	0.25	93.4	0.12	2	80.6	0.12	v2	78.4	0.12	4	75.3
Cefuroxime	4	8	36.7	0.03	0.5	92.6	0.06	8	80.6	0.12	4	77.0	0.06	8	75.3
Azithromycin	64	>64	20.2	0.12	4	87.6	0.12	>64	75.1	0.12	8	84.8	0.12	16	76.4
Clarithromycin	32	>32	20.4	0.03	1	88.4	0.03	>32	75.0	0.03	4	84.8	0.06	8	76.4
Clindamycin	2	>4	49.5	0.06	0.12	94.2	0.06	>4	80.1	0.06	0.12	93.1	0.06	0.12	91.1
Erythromycin A	64	>64	20.2	0.06	2	87.6	0.06	>64	74.9	0.06	4	84.6	0.06	16	76.4
Levofloxacin	1	1	95.3	1	1	100	1	1	99.8	1	1	99.2	1	1	98.0
Moxifloxacin	0.12	0.25	96.7	0.12	0.25	100	0.12	0.25	99.9	0.12	0.25	99.2	0.12	0.25	98.7
Co-trimoxazole	1	16	35.0	0.25	4	70.2	48.8	11.2	66.3	2	16	36.5	0.5	16	61.6
Tetracycline	>16	>16	18.4	0.25	>16	86.6	60.6	32	75.9	0.25	>16	75.1	0.25	>16	86.2
Quinupristin/dalfopristin	0.5	1	96.3	0.5	1	99.2	0.5	1	98.9	0.5	0.5	99.8	0.5	1	99.6
Linezolid	1	1	100	1	1	100	1	1	100	1	2	100	1	2	100
Teicoplanin	0.06	0.12	100	0.06	0.12	100	0.06	0.12	100	0.06	0.12	100	0.06	0.12	100
Vancomycin	0.5	0.5	100	0.5	0.5	100	0.5	0.5	100	0.5	v0.5	100	0.5	0.5	100

[a]For susceptibility criteria see Table 1.
[b]Inhibited by ≤1 mg/L (tentative NCCLS susceptibility breakpoint).

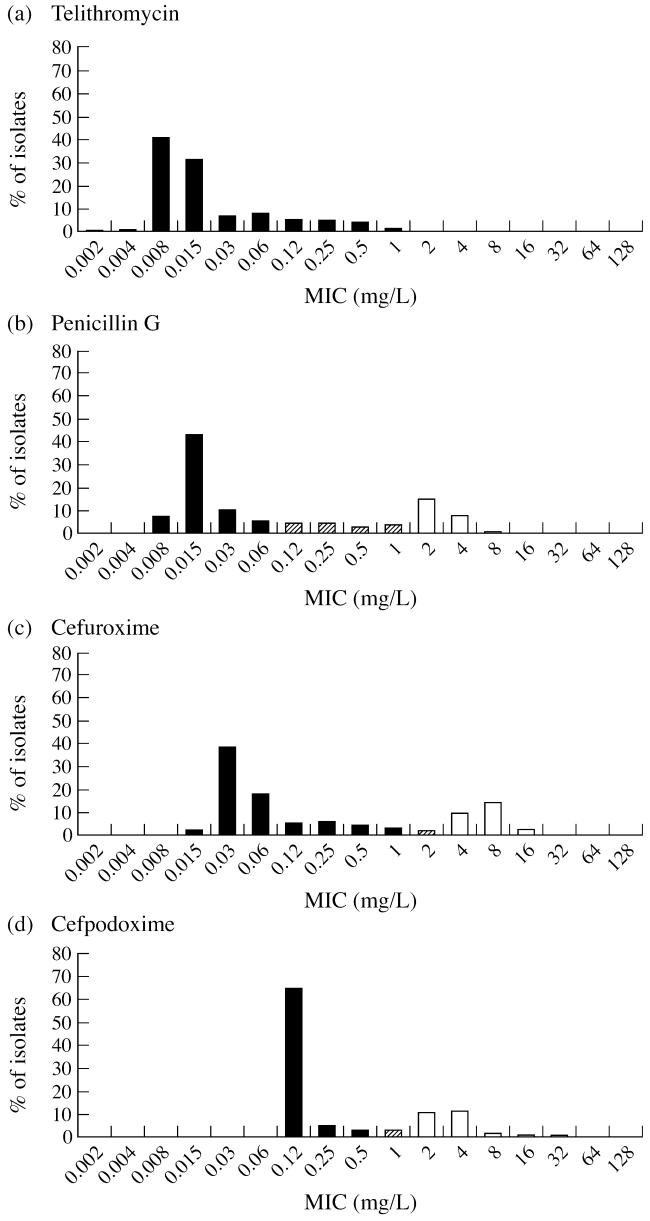

Figure 2. MIC distributions for telithromycin and β-lactams against 3362 isolates of *S. pneumoniae* collected in total in the PROTEKT study (1999–2000). Key: black bars, susceptible; shaded bars, intermediate; white bars, resistant

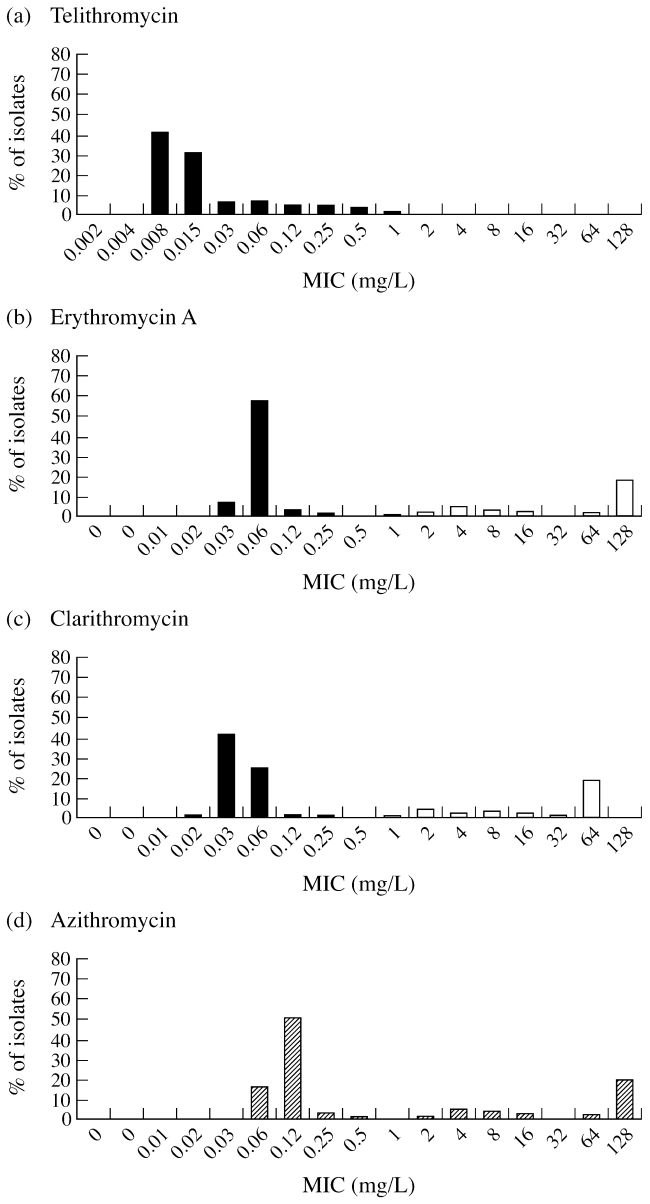

Figure 3. MIC distributions for telithromycin and macrolides against 3362 isolates of *S. pneumoniae* collected in the PROTEKT study (1999–2000). For key see Figure 2.

Cross- and parallel-resistance

In total, 12.6% of penicillin G-susceptible *S. pneumoniae* isolates were found to be resistant to erythromycin A, with rates rising to 49.2% and 72.4% for penicillin G-intermediate and -resistant isolates, respectively. Table 5 summarizes the activity of representatives of the major groups of antimicrobials against *S. pneumoniae* isolates collected in PROTEKT according to the penicillin G susceptibility phenotype, and by region. With the exception of levofloxacin and telithromycin, resistance to the drugs listed in Table 5 was notably higher among penicillin G-non-susceptible isolates than among penicillin G-susceptible isolates. A similar cross-resistance profile was observed between penicillin G and other β-lactams, and between penicillin G and macrolides (data not shown). Again there was marked geographical variation in the prevalence of antimicrobial resistance among the penicillin G resistance phenotypes.

Table 6 shows the extent to which *S. pneumoniae* isolates were multi-resistant to commonly used oral antimicrobials. Approximately 50% of all erythromycin A-resistant strains were also resistant to penicillin G and co-trimoxazole, while 63.2% and 76.5% of erythromycin A-resistant strains were

Table 5. *In vitro* activity of selected antimicrobial agents against 3362 isolates of *S. pneumoniae* collected in the PROTEKT study (1999–2000), according to penicillin G susceptibility and continent[a]

	Asia ($n = 515$)			Australasia ($n = 121$)			Europe ($n = 1521$)			Latin America ($n = 518$)			North America ($n = 687$)		
Antimicrobial	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S
Telithromycin[b]															
Pen-S	0.03	0.25	100	0.008	0.015	100	0.008	0.015	100	0.008	0.015	100	0.008	0.015	100
Pen-I	0.06	0.5	100	0.008	0.015	100	0.015	0.06	100	0.015	0.25	100	0.015	0.12	100
Pen-R	0.06	0.5	100	0.015	0.03	100	0.015	0.5	99.2	0.008	0.12	100	0.06	0.5	100
Co-amoxiclav															
Pen-S	0.03	0.06	100	0.03	0.03	100	0.03	0.03	100	0.03	0.03	100	0.03	0.03	100
Pen-I	0.25	1	98.7	0.25	0.5	100	0.12	1	100	0.12	1	100	0.25	2	100
Pen-R	2	4	77.0	2	2	100	2	8	84.0	2	2	91.4	2	>4	55.8
Cefpodoxime															
Pen-S	0.12	0.5	95.2	0.12	0.12	100	0.12	0.12	100	0.12	0.12	100	0.12	0.12	99.4
Pen-I	2	4	40.0	0.25	1	85.7	0.25	2	67.4	0.25	2	76.3	0.25	2	72.2
Pen-R	4	8	0	2	4	0	4	4	0	4	4	0	4	8	0
Erythromycin A															
Pen-S	4	>64	44.9	0.06	0.5	89.5	0.06	4	88.8	0.06	0.06	95.0	0.06	0.12	93.4
Pen-I	64	>64	14.7	0.06	0.06	90.4	>64	>64	43.8	0.06	>64	66.2	0.06	>64	58.3
Pen-R	64	>64	6.9	4	>64	40.0	>64	>64	31.9	0.06	8	77.2	4	>64	31.3
Levofloxacin															
Pen-S	1	1	95.2	1	1	100	1	1	99.7	1	1	99.3	1	1	98.1
Pen-I	0.5	1	98.7	1	1	100	1	1	100	1	1	100	1	1	98.6
Pen-R	1	1	94.5	1	1	100	1	1	100	1	1	97.5	1	1	97.3
Co-trimoxazole															
Pen-S	0.5	2	70.3	0.25	2	80	0.25	2	82.1	1	8	49.3	0.25	0.5	81.4
Pen-I	1	8	42.7	1	4	42.9	1	8	42.6	4	>16	25.2	1	16	45.8
Pen-R	4	>16	11.6	8	16	0	8	16	8.8	8	>16	7.6	8	>16	6.1
Tetracycline															
Pen-S	16	>16	37.0	0.25	0.5	90.5	0.25	8	89.2	0.25	16	81.0	0.25	1	97.7
Pen-I	>16	>16	14.7	0.25	>16	81.0	>16	>16	37.9	0.25	>16	71.2	0.25	>16	68.1
Pen-R	>16	>16	8.4	>16	>16	40.0	>16	>16	40.3	0.5	>16	59.5	0.5	>16	58.5

Pen-S, penicillin G susceptible (MIC ≤ 0.06 mg/L); Pen-I, penicillin G intermediate (MIC 0.12–1 mg/L); Pen-R, penicillin G resistant (MIC ≥2 mg/L).
[a]For susceptibility criteria see Table 1.
[b]Inhibited by ≤1 mg/L (tentative NCCLS susceptibility breakpoint).

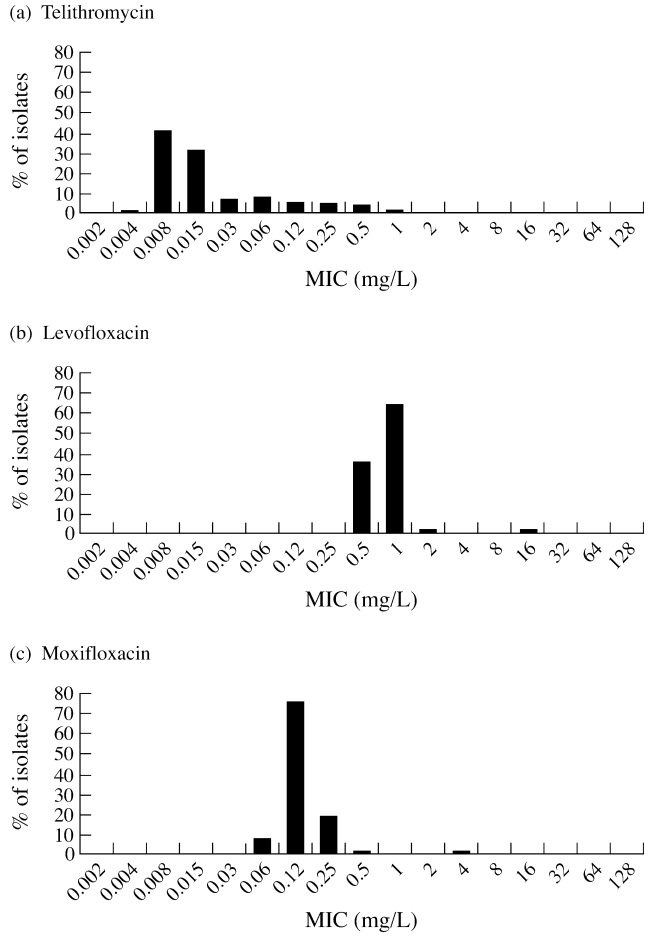

Figure 4. MIC distributions for telithromycin and fluoroquinolones against 3362 isolates of *S. pneumoniae* collected in total in the PROTEKT study (1999–2000). For key see Figure 2.

cross-resistant to clindamycin and tetracycline, respectively, but they remained susceptible to levofloxacin, moxifloxacin, telithromycin and linezolid (data not shown). Those isolates that were resistant to levofloxacin ($n = 35$) were also highly resistant to the other oral agents, with the exception of telithromycin and linezolid (all fluoroquinolone-resistant *S. pneumoniae* remained fully susceptible to these two oral agents). The *in vitro* activity of telithromycin by penicillin G, erythromycin A or fluoroquinolone susceptibility/resistance phenotype is summarized in Table 7.

Discussion

Over the last three decades, antimicrobial resistance among isolates of *S. pneumoniae* has spread across the globe at an alarming rate, and now threatens to compromise the clinical usefulness of the current portfolio of agents for treating infections associated with this pathogen. International antimicrobial surveillance studies play a critical role in defining the nature and extent of the resistance problem, as well as in

Table 6. Multiple resistance among 3362 isolates of *S. pneumoniae* collected in the PROTEKT study (1999–2000) (the percentages quoted in the table are the percentage of isolates resistant to Agent 1 that are also resistant to Agent 2)

		No. (%) of isolates also resistant to:				
Phenotype	No. of isolates	penicillin G	clindamycin	erythromycin A	levofloxacin	tetracycline	co-trimoxazole
Penicillin G resistant	744		329 (44.2%)	539 (72.4%)	18 (2.4%)	486 (65.3%)	552 (74.2%)
Clindamycin resistant	661	329 (49.8%)		659 (99.7%)	19 (2.9%)	586 (88.7%)	366 (55.4%)
Erythromycin A resistant	1043	539 (51.7%)	659 (63.2%)		27 (2.6%)	798 (76.5%)	527 (50.5%)
Levofloxacin resistant	35	18 (51.4%)	19 (54.2%)	27 (77.1%)		21 (60.0%)	20 (57.1%)
Tetracycline resistant	997	486 (48.7%)	586 (58.8%)	798 (80.0%)	21 (2.1%)		491 (49.2%)
Co-trimoxazole resistant	960	552 (57.5%)	366 (38.1%)	527 (54.9%)	20 (2.1%)	491 (51.1%)	

Table 7. *In vitro* activity of telithromycin against *S. pneumoniae* isolates collected in PROTEKT, by antimicrobial resistance phenotype

Phenotype	*n*	MIC (mg/L)				% susceptibility[a]
		Mode	MIC_{50}	MIC_{90}	Range	
Penicillin G						
Susceptible	2142	0.008	0.008	0.03	0.002–0.5	100
Intermediate	476	0.008	0.015	0.12	0.004–1	100
Resistant	744	0.06	0.06	0.5	0.004–8	99.6
Erythromycin A						
Susceptible	2312	0.008	0.008	0.015	0.002–0.12	100
Intermediate	7	0.015	0.015	0.03	0.008–0.03	100
Resistant	1043	0.06	0.06	0.5	0.008–8	99.7
Levofloxacin						
Susceptible	3317	0.008	0.015	0.12	0.002–8	99.9
Intermediate	10	0.008	0.03	0.12	0.008–0.12	100
Resistant	35	0.03	0.03	0.12	0.008–0.5	100
Combined	3362	0.008	0.015	0.12	0.002–8	99.9

[a]Inhibited by ≤1 mg/L (tentative NCCLS susceptibility breakpoint).

guiding development of antimicrobial policies and usage.[23] Furthermore, by monitoring changes in resistance patterns and the spread of significant resistance phenotypes across the globe, these studies enable authorities to implement intervention strategies to halt the spread of such strains. The PROTEKT study was initiated in 1999 to monitor longitudinally the global spread of resistance among bacterial respiratory pathogens associated with CARTIs. Testing of all isolates at a central laboratory using validated identification techniques and NCCLS-recommended methodologies has helped to ensure the quality and consistency of the data generated.

Consistent with previous international surveillance studies,[10,11,24] PROTEKT reported large differences between countries in the penicillin G resistance profile of *S. pneumoniae*, the highest rates of resistance being noted in clinical isolates from patients in the Asian region. In all three of the Asian countries studied, the resistant phenotype (44.5–71.6% of strains) dominated over the intermediate phenotype (1.4–19.8%). For example, within Hong Kong, the prevalence of penicillin G intermediate and penicillin G-resistant strains was 1.4% and 57.1%, respectively, which is consistent with that reported by Felmingham *et al.*[10] Furthermore, our data suggest a large upward shift in the penicillin G MICs among *S. pneumoniae* from Japan: a multicentre study of 1997–1998 pneumococcal isolates reported penicillin G intermediate and penicillin G resistant rates in Japan of 44% and 10%, respectively,[11] while in the present 1999–2000 study, the percentages were 20% and 45%, respectively.

Outside of Asia, penicillin G resistance rates in excess of 40% were recorded in France and Spain, while in the neighbouring countries of Germany and Switzerland, resistance to this agent averaged 2.2% and 4.5%, respectively. The data also suggest that the prevalence of penicillin G resistance in the USA has risen substantially from the 9.5% reported in a 1994–1995 study by Doern *et al*.[7] For 1997–1998 US isolates, Thornsberry *et al.* found 13% penicillin G resistance,[25] Hoban *et al.* reported 15% resistance among 1999 isolates,[24] and in 1999–2000 isolates we have found 33% resistance. Although the penicillin G resistance prevalence in the USA seems exceptionally high in this study, preliminary data from a subsequent sister surveillance study, PROTEKT US, reported a rate of 26% among 10 103 isolates of *S. pneumoniae*, with individual rates as high as 36.5% being observed in the southeastern region of the country.[26]

The increasing prevalence of penicillin G resistance among *S. pneumoniae* is a cause for concern, not only because of the impact that it may have on the clinical usefulness of this agent, but also because resistance to penicillin G has been found to be associated with resistance to other β-lactams, such as the cephalosporins, as well as to several non-β-lactam classes. In *S. pneumoniae*, reduced susceptibility to β-lactams, such as penicillin, arises through alterations in the penicillin-binding proteins (PBPs)—1A, 1B, 2A, 2B, 2X and 3—via acquisition of genetic material from other *Streptococcus* spp coexisting in close proximity, e.g. *S. mitis*. The resistance phenotype of the resulting strain depends upon which particular PBP is modified. For example, while many of the penicillin-resistant strains first identified remained susceptible to third-generation cephalosporins, resistance to these agents is now well established among penicillin-resistant pneumococci, reflecting alterations in PBPs, particularly 1A and 2X.

As reported in other studies,[7,24,25,27] co-resistance to erythromycin A, clindamycin, tetracycline and co-trimoxazole was higher among strains with intermediate susceptibility to penicillin G than among penicillin G-susceptible strains, with an even higher prevalence being noted among strains highly resistant to penicillin G. In the absence of any known common mechanism of resistance between penicillin G and these non-β-lactam antimicrobials, this relationship most probably reflects increased selective pressure among *S. pneumoniae* created by broad-based antimicrobial usage.[24] This hypothesis is supported by the fact that in some regions, elevated resistance to these antimicrobials was also noted in the penicillin G-susceptible population, particularly in Asia (macrolides, co-trimoxazole and tetracycline), Europe (macrolides) and Latin America (co-trimoxazole and tetracycline), where these agents are widely prescribed.

Overall, resistance to erythromycin A among *S. pneumoniae* averaged 31%, and in many countries was more prevalent than resistance to penicillin G. There were no important differences in the activity of the newer agents, e.g. clarithromycin and azithromycin, and pneumococci resistant to erythromycin A were invariably resistant to these second-generation macrolides. As reported in the 1996–1997 Alexander Project,[10] we found France, Belgium, Spain and Italy to be the foci of macrolide resistance in Western Europe. However, the 58% and 43% macrolide resistance rates reported in our study for 1999–2000 isolates from France and Italy, respectively, are substantially higher than those reported by Felmingham *et al.* for 1997 isolates in the Alexander Project (46% and 30%, respectively).[10] Within Western Europe, we also found substantial increases in the prevalence of macrolide resistance in Germany (16% versus 7%), Portugal (16% versus 4%) and Eire (26% versus 14%), compared with the prevalences reported in the Alexander Project for 1997 isolates. Outside Western Europe, high rates of resistance were found in all Asian countries participating in the study, as well as in Hungary, Mexico and the USA. Hoban and colleagues have recently reported a significant rise in macrolide resistance among US pneumococci isolated in the SENTRY study, rising from 17% in 1998 to 23% in 1999.[24] The 31% macrolide resistance rate observed in our study for 1999–2000 *S. pneumoniae* US isolates, suggests that in the USA, resistance to this class has risen from the 1999 data reported in SENTRY. This is supported by the preliminary data from PROTEKT US, which found that 31% of pneumococci collected during 2000–2001 from 206 centres across the USA were resistant to erythromycin A.[26] A recent analysis conducted in Spain has shown a clear correlation between erythromycin A resistance and overall macrolide consumption, the relationship appearing to be due mainly to consumption of those macrolides dosed once or twice daily.[28] While this analysis is not able to demonstrate cause and effect, it does suggest that increased use of long-acting macrolides may be linked to the increase in prevalence of erythromycin A-resistant pneumococci observed in recent years.

Two major forms of macrolide resistance exist in *Streptococcus* spp: (i) modification of the 23S ribosomal RNA target site by methylation (encoded by *erm* genes); and (ii) antimicrobial efflux by proton-motive force or utilizing ATP (encoded by *mef* genes).[29,30] Modification of the ribosomal target site confers a high-level resistance not only to macrolides, but also to lincosamides and streptogramin$_B$ (so-called MLS$_B$ phenotype), while the resistance conferred by efflux abnormality (M phenotype) is usually of a moderate level and confined to 14- and 15-membered ring macrolides only (clindamycin usually remains active). In a genotypic analysis of the resistance mechanism in 1043 macrolide-resistant pneumococcal isolates from the PROTEKT 1999–2000 study, 56.2% of isolates expressed *erm*(B) (predominantly in Europe) and 35.3% expressed *mef*(A) (predominantly in North America).[31] In our study, 63% of erythromycin A-resistant pneumococci were also resistant to clindamycin, consistent with *erm* (MLS$_B$) resistance. Reduced susceptibility to clindamycin was particularly prevalent in Asia and in Europe. Relative to *mef*-mediated resistance, these strains may be more difficult to eradicate, with a subsequent risk of clinical failure.

In the present study, the prevalence of resistance to the newer anti-pneumococcal fluoroquinolones, e.g. levofloxacin and moxifloxacin, remained low (~1%). There were, however, pockets of resistance to these agents, particularly in Hong Kong (14%) and South Korea (3%). The situation in Hong Kong is particularly alarming since all fluoroquinolone-resistant pneumococci were also highly resistant to β-lactams, macrolides, co-trimoxazole, cephalosporins and tetracycline—the only two oral agents that retained full susceptibility against these isolates were the ketolide, telithromycin and linezolid.[32] Molecular characterization has shown that the vast majority of the fluoroquinolone resistance detected in Hong Kong was due to the spread of the 23F Spanish multidrug-resistant clone.[32] Goldsmith *et al.* have reported an increased prevalence of fluoroquinolone resistance among penicillin G-resistant pneumococci in Northern Ireland,[33] although subsequent surveillance studies have not been able to confirm this relationship for other regions.[11,24,25] In our study, levofloxacin-resistant and/or moxifloxacin-resistant strains were isolates from countries with relatively low rates of penicillin G resistance (i.e. Germany, Switzerland and Canada), as well as from those countries in which reduced penicillin G susceptibility is more prevalent (e.g. Asia). Half of the 35 levofloxacin-resistant pneumococci identified in this study were also resistant to penicillin G, and approximately three-quarters were co-resistant to erythromycin A. No glycopeptide-resistant pneumococci were identified in the study.

One of the objectives of the PROTEKT study was to provide current surveillance data for telithromycin at its time of

launch into clinical practice. The study has shown this new ketolide to be the most potent of the oral antimicrobials currently available against *S. pneumoniae*. Moreover, the data confirm previously published findings that telithromycin maintains its anti-pneumococcal activity against strains that are resistant to β-lactams, macrolides and fluoroquinolones.[18] Using the proposed NCCLS interpretative criteria of ≤1 mg/L to define susceptibility to telithromycin gives an overall susceptibility of 99.9% for *S. pneumoniae*. Applying the slightly lower breakpoint (≤0.5 mg/L) adopted by the European authorities to the PROTEKT MIC data has negligible effect on the overall susceptibility to telithromycin, reducing it to 99.3%. Results from subsequent years of the PROTEKT study will help to establish the impact that the introduction of telithromycin will have on susceptibility of *S. pneumoniae* not only to this agent, but also to non-ketolide antibacterials. *In vitro* studies and preliminary data from human studies suggest that telithromycin has a favourable ecological profile, with a low potential to select for resistant mutants, and, in contrast to the macrolides, telithromycin does not induce MLS resistance *in vitro*.[20] The low potential for telithromycin to select for pneumococcal resistance has been confirmed recently by Davies and colleagues,[34] who performed a series of *in vitro* serial passage experiments in which five macrolide-susceptible and six macrolide-resistant [three *erm*(B), three *mef*(A)] strains of *S. pneumoniae* were repeatedly exposed to sub-inhibitory concentrations of telithromycin or other MLS_B antibacterials. They found that telithromycin selected for resistant mutants significantly less often than the other agents. Indeed, of the 54 mutants isolated with raised MICs of at least one of the test agents, only three were resistant to telithromycin compared with 36, 37, 36, 45 and 15 mutants resistant to azithromycin, clarithromycin, erythromycin A, roxithromycin and clindamycin, respectively. Furthermore, studies in man have shown that telithromycin selects for resistance among oropharyngeal and intestinal bacterial microflora significantly less than clarithromycin.[21]

In conclusion, the 1999–2000 data from PROTEKT confirm the widespread and increasing prevalence of antimicrobial resistance among *S. pneumoniae*. The large intercountry variation reported in the prevalence of resistance, not only to penicillin G but also to other antimicrobials, highlights the need for continuing surveillance of resistance at both the national and international level, with the data being analysed and disseminated rapidly so that changes in susceptibility patterns can be quickly detected allowing appropriate control measures to be put in place. In this era of increasing pneumococcal resistance, the ketolide telithromycin has emerged as a promising new candidate for the treatment of CARTIs, possessing potent anti-pneumococcal activity even against strains with resistance to other commonly used antimicrobials.

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Increasing prevalence of antimicrobial resistance among isolates of *Streptococcus pneumoniae* from the PROTEKT surveillance study, and comparative *in vitro* activity of the ketolide, telithromycin

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The prevalence of resistance to a range of antimicrobials was determined for isolates of *Streptococcus pneumoniae* examined in the PROTEKT (Prospective Resistant Organism Tracking and Epidemiology for the Ketolide Telithromycin) surveillance study (1999–2000) using NCCLS testing methods and interpretative criteria. Of 3362 pneumococcal isolates collected from 69 centres in 25 countries, 22.1% overall were resistant to penicillin G, with the highest rates of resistance found among isolates from Asia (53.4%), France (46.2%) and Spain (42.1%). Erythromycin A resistance occurred in 31.1% of isolates overall with the highest rates found in Asia (79.6%), France (57.6%), Hungary (55.6%) and Italy (42.9%). Marked geographical differences in the prevalence of both penicillin G (the Netherlands 0%; South Korea 71.5%) and erythromycin A (Sweden 4.7%; South Korea 87.6%) resistance were observed. Asia was characterized by the highest prevalence of resistance, overall, with only eight of 19 antimicrobials (co-amoxiclav, linezolid, vancomycin, teicoplanin, quinupristin/dalfopristin, levofloxacin, moxifloxacin and telithromycin) retaining high activity against isolates of *S. pneumoniae* from this region. Notable rates of resistance to clarithromycin, azithromycin, co-trimoxazole and tetracycline were observed in the majority of countries submitting isolates of *S. pneumoniae* to the PROTEKT surveillance study. Fluoroquinolone resistance was low (1%), overall, although 14.3% of 70 isolates from Hong Kong were resistant to levofloxacin and moxifloxacin, all but one of these isolates belonging to a single clone of the 23F serotype. Although, at present, apparently limited to pockets of clonal spread, continued vigilance with regard to the evolution of fluoroquinolone resistance is indicated. Telithromycin (MIC_{90} 0.12 mg/L; 99.9% of isolates susceptible) and linezolid (MIC_{90} 2 mg/L; 100% of isolates susceptible) were the two most active oral agents tested, both compounds retaining activity against isolates of fluoroquinolone-resistant *S. pneumoniae*. The results of the PROTEKT surveillance study 1999–2000 emphasize the widespread evolution of resistance to a variety of antimicrobials amongst isolates of *S. pneumoniae* and demonstrate the potential of telithromycin as a therapeutic option for the treatment of community-acquired respiratory tract infections caused by this organism.

Introduction

Although a common component of the normal human respiratory flora, *Streptococcus pneumoniae* is also one of the most important bacterial pathogens. Infection with this organism is among the most frequent causes of community-acquired respiratory tract infections (CARTIs), including community-acquired pneumonia (45% of cases), otitis media (30–40%) and sinusitis (20–35%), as well as being one of the leading causes of meningitis and bacteraemia.[1–3] Indeed, it has been estimated that each year in the USA, *S. pneumoniae* infections account for 500 000 cases of pneumonia, 55 000 cases of bacteraemia and 6000 cases of meningitis.[4,5]

While β-lactams, most notably penicillin G, have been the mainstay of antimicrobial treatment for CARTIs, resistance to these agents has reached alarming levels in many regions. Isolates of *S. pneumoniae* with reduced susceptibility to penicillin G were first reported during the 1960s in Australia and Papua New Guinea.[3,6] By the early 1970s penicillin G-intermediate and -resistant strains had been detected in

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Poland and Germany, with Japan, South Africa, Switzerland and the UK also reporting the presence of such strains during the mid to late 1970s.[6] Subsequently, penicillin G-resistant strains of *S. pneumoniae* have rapidly spread worldwide and their prevalence continues to increase.[7–12] Of even more concern is the degree to which these isolates are cross-resistant, not only to other β-lactam drugs, such as the cephalosporins, but also to non-β-lactam classes, e.g. the macrolides.[10] Resistance to fluoroquinolones is also emerging among *S. pneumoniae*, albeit currently at relatively low levels.[13]

In this era of increasing antimicrobial resistance, treatment of CARTIs should ideally be pathogen directed, based on identification and susceptibility testing of the causative organism. In reality, however, an aetiologically specific diagnosis, let alone susceptibility results, is rarely available to assist in the selection of therapy, which is consequently largely empirical. In view of the importance of *S. pneumoniae* in CARTIs and the traditionally empirical approach to the treatment of these infections, knowledge of local antimicrobial resistance patterns is essential to ensure rational prescribing.[14] These issues highlight the need for continuing surveillance of antimicrobial resistance at local, national and international levels, as well as the need for research into new antibacterial agents with the ability to overcome *S. pneumoniae* resistance mechanisms and which do not induce or select further resistance.

The ketolides are a new family of antibacterial agents belonging to the macrolide–lincosamide–streptogramin class.[15,16] Telithromycin, the first ketolide antibacterial, has demonstrated clinical efficacy across a range of CARTIs, and potent *in vitro* activity against the pathogens commonly implicated in these infections, including drug-resistant *S. pneumoniae*.[17–19] Data from both *in vitro* and human studies suggest that this agent also has a low potential for the selection or induction of resistance, and has minimal effect on normal human gut flora.[20,21] In 1999, the PROTEKT (Prospective Resistant Organism Tracking and Epidemiology for the Ketolide Telithromycin) surveillance study was established to track longitudinally the susceptibility of common respiratory pathogens from patients with CARTIs to current and new antimicrobials, including the new ketolide, telithromycin. This paper describes the widespread prevalence of resistance and the comparative activity of common and newly developed antimicrobial agents against isolates of *S. pneumoniae* collected during the first year (1999–2000) of the PROTEKT study.

Materials and methods

Collecting centres

During the 1999–2000 respiratory season, 69 centres in 25 countries participated in PROTEKT: Canada (7), USA (8), Mexico (4), Brazil (7), Argentina (2), Germany (7), the Netherlands (1), Sweden (1), UK (2), Eire (1), Belgium (1), France (4), Portugal (2), Spain (2), Switzerland (2), Italy (2), Austria (1), Turkey (1), Hungary (1), Poland (1), Hong Kong (1), Japan (6), South Korea (2), Australia (2) and Indonesia (1).

Bacterial isolates

Each centre recruited into the PROTEKT study was requested to collect a minimum of 60 isolates of *S. pneumoniae* from patients with any of six types of community-acquired infection, namely sinusitis, otitis media, pharyngitis, pneumonia, acute bacterial exacerbations of chronic bronchitis and acute exacerbation of chronic obstructive airways disease. Sources for isolation of RTI pathogens were cultures from blood, sputum, bronchoalveolar lavage, middle ear fluid, nasopharyngeal swab or aspirate, and sinus aspirate. Patients with nosocomial RTIs and those with cystic fibrosis were excluded, and duplicate strains or strains originating from existing collections were not included in the study. Demographic data collected included the age and sex of the patient, infection, culture source, in/outpatient status, specimen accession number and date of sample collection. Criteria for isolate storage, transportation and identification, and confirmation of isolate identification have been described in detail previously.[22]

Antimicrobial susceptibility testing

MICs were determined at a central laboratory (GR Micro Ltd, London, UK) for a panel of existing and new antimicrobials, using previously described methods.[22] In brief, we used the NCCLS broth microdilution method with lyophilized microtitre plates (Sensititre system; Trek Diagnostics) and an inoculum of $3–7 \times 10^4$ cfu in 100 μL medium. MIC endpoints were read as the lowest concentration of antimicrobial that totally inhibited macroscopically visible growth of the inoculum.

Isolates were tested against the following antimicrobials: penicillin G, co-amoxiclav (2:1 ratio), cefaclor, cefixime, cefpodoxime, cefuroxime, azithromycin, clarithromycin, erythromycin A, telithromycin, clindamycin, levofloxacin, ciprofloxacin, moxifloxacin, co-trimoxazole (1:19 ratio), linezolid, quinupristin–dalfopristin (30:70 ratio), teicoplanin, tetracycline and vancomycin. Percentage susceptibilities were calculated based on NCCLS breakpoints, although it should be noted that NCCLS breakpoints applied to telithromycin are tentative and not yet approved (Table 1).

Results

A total of 3362 isolates of *S. pneumoniae* were collected from 25 countries within Western Europe (n = 1322), North America (n = 687), Latin America (n = 518), Asia (n = 515), Eastern Europe (n = 199) and Australasia (n = 121). Informa-

Table 1. Interpretative categories for *S. pneumoniae*[a]

Antimicrobial agent	Interpretative categories (mg/L)		
	susceptible	intermediate	resistant
Co-amoxiclav	≤2	4	≥8
Azithromycin	≤0.5	1	≥2
Cefaclor	≤1	2	≥4
Cefixime	susceptibility reported as for penicillin G		
Cefpodoxime	≤0.5	1	≥2
Cefuroxime	≤1	2	≥4
Clarithromycin	≤0.25	0.5	≥1
Clindamycin	≤0.25	0.5	≥1
Co-trimoxazole	≤0.5	1–2	≥4
Erythromycin A	≤0.25	0.5	≥1
Levofloxacin	≤2	4	≥8
Linezolid	≤2		≥8
Moxifloxacin	≤1	2	≥4
Penicillin G	≤0.06	0.12–1	≥2
Quinupristin/dalfopristin	≤1	2	≥4
Teicoplanin	≤8	16	≥32
Telithromycin	≤1	2	≥4
Tetracycline	≤2	4	≥8
Vancomycin	≤1	–	–

[a]All breakpoints given are NCCLS, with the exception of telithromycin for which NCCLS-approved breakpoints are not yet available (tentative breakpoints given).

tion on culture source, patient age and gender, and treatment setting were available for approximately 45–87% of the isolates collected, and this is summarized in Table 2.

Prevalence of antimicrobial resistance

Susceptibility of *S. pneumoniae* to penicillin G and to erythromycin A is shown in Table 3 by region and by country.

Strains of *S. pneumoniae* with reduced susceptibility to penicillin G were evident in all of the participating countries, at an overall prevalence of 36.2%. There was, however, marked variation between countries in the prevalence of such isolates, ranging from 3.9% in the Netherlands to 81% in South Korea. Of those isolates with reduced susceptibility to penicillin G, the resistant phenotype was found to dominate over the intermediate phenotype in 12 of the 25 countries, with particularly high rates of penicillin G resistance being reported in all three countries within Asia (44.5–71.5%), as well as in France (46.2%) and Spain (42.1%). The Netherlands and Indonesia were the only countries not to report any isolates with full resistance to penicillin G, although the number of isolates submitted by the latter is too small to draw any meaningful conclusions. Overall, 22.1% of isolates were resistant to penicillin G, with 7.9% of isolates having a penicillin G MIC ≥ 4 mg/L.

Erythromyin A resistance rates were also very high (31.0% overall), and in most countries (19/25) they exceeded rates of penicillin G resistance. Again, Asian countries had a very high prevalence of erythromycin A resistance, averaging 79.6%, while for isolates collected from North America, Western Europe, Latin America, Eastern Europe and Australasia, the prevalence of erythromycin A resistance ranged from 11.6 to 29.1%. Across Europe, rates of resistance of *S. pneumoniae* isolates to erythromycin A reached 55.6% in Hungary and 57.6% in France. Other European countries with high levels of resistance included Belgium (32.1%), Eire (26.4%), Italy (42.9%) and Spain (28.6%).

Fluoroquinolone resistance (defined as levofloxacin MIC ≥ 8 mg/L) was detected in nine of the countries participating in PROTEKT in 1999–2000, reaching rates of 14.3% in Hong Kong (Figure 1). However, the overall prevalence of fluoroquinolone resistance was low, averaging 1.0%. Similar resistance rates were noted for moxifloxacin.

Comparative in vitro activity of the test agents

MIC and percentage susceptibility data for the 19 antimicrobials tested against *S. pneumoniae* are reported by region in Table 4. These data highlight striking differences between regions in terms of susceptibility of *S. pneumoniae* not only to

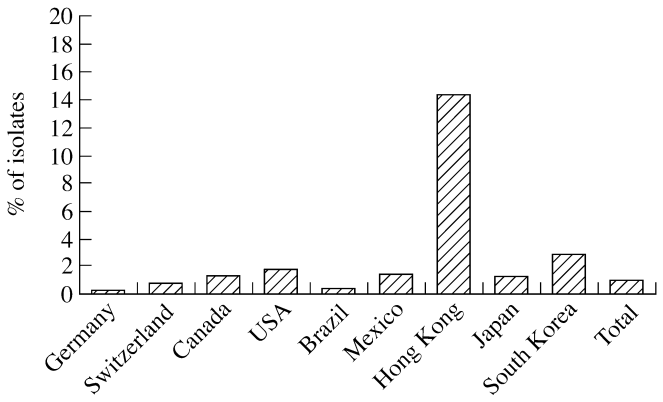

Figure 1. Prevalence of fluoroquinolone resistance among *S. pneumoniae* ($n = 3362$) collected in the PROTEKT study (1999–2000). (Only those countries where fluoroquinolone-resistant isolates were detected are shown.)

penicillin G and erythromycin A, but also to other commonly used agents. In particular, Asian countries had a very high prevalence of resistance to many of the antimicrobials tested, with only eight of the 19 agents being active against >50% of all 515 *S. pneumoniae* isolates: co-amoxiclav (87.6%), linezolid (100%), teicoplanin (100%), vancomycin (100%), quinupristin/dalfopristin (96.3%), moxifloxacin (96.7%), levofloxacin (95.3%) and telithromycin (100%).

The pattern of resistance to the cephalosporins tended to parallel that to penicillin G, the highest and lowest susceptibilities being recorded for isolates collected from Australasia and Asia, respectively. Based on MIC and susceptibility data, co-amoxiclav proved the most active of the β-lactams tested, although susceptibility to this agent was reduced somewhat in both Asia and North America (87.6% and 90.5%, respectively) compared with the other regions (97.5–100%).

Table 2. Patient demographics and culture source of 3362 isolates of *S. pneumoniae* collected in the PROTEKT study (1999–2000)

Parameter	Group	Number	Percentage of total with data	Percentage of total collected
Age	0–2	440	15.30	13.09
	3–14	362	12.60	10.77
	>14–65	1261	43.90	37.51
	>65	809	28.20	24.06
	NR	490	–	14.57
Gender	male	1831	63.10	54.46
	female	1073	36.90	31.92
	NR	458	–	13.62
Source	blood	502	17.30	14.93
	BAL	258	8.90	7.67
	sputum	1482	51.10	44.08
	sinus	137	4.70	4.07
	ear	203	7.00	6.04
	MEF	30	1.00	0.89
	nasopharyngeal	234	8.10	6.96
	throat	54	1.90	1.61
	NR	462	–	13.74
Infection site	AECB	448	24.40	13.33
	COAD	141	7.70	4.19
	pneumonia	742	40.40	22.07
	sinusitis	158	8.60	4.70
	otitis media	265	14.40	7.88
	tonsillitis/pharyngitis	81	4.40	2.41
	NR	1527	–	45.42
In/outpatient	in	1514	51.50	45.03
	out	1425	48.50	42.39
	NR	423	–	12.58

NR, not recorded; AECB, acute exacerbation of chronic bronchitis; COAD, chronic obstructive airways disease; BAL, bronchoalveolar lavage; MEF, middle ear fluid.

Table 3. Number (%) of *S. pneumoniae* strains by penicillin G and erythromycin A susceptibility interpretative category in each continent and country

Region/country	Total no. of isolates tested	Penicillin G susceptibility[a] [no. (%) of isolates]			Erythromycin A susceptibility[b] [no. (%) of isolates]		
		susceptible	intermediate	resistant	susceptible	intermediate	resistant
Western Europe	1322	996 (75.3%)	115 (8.7)	211 (16.0)	998 (75.5)	2 (0.2)	322 (24.4)
Austria	57	53 (93.0%)	1 (1.8)	3 (5.3)	49 (86.0)	1 (1.8%)	7 (12.3)
Belgium	28	23 (82.1%)	1 (3.6)	4 (14.3)	19 (67.9)	0 (0)	9 (32.1)
Eire	53	31 (58.5)	4 (7.5)	18 (34.0)	39 (73.6)	0 (0)	14 (26.4)
France	184	70 (38.0)	29 (15.8)	85 (46.2)	77 (41.8)	1 (0.5)	106 (57.6)
Germany	325	298 (91.7)	20 (6.2)	7 (2.2)	274 (84.3)	0 (0)	51 (15.7)
Italy	119	101 (84.9)	6 (5.0)	12 (10.1)	68 (57.1)	0 (0)	51 (42.9)
The Netherlands	51	49 (96.1)	2 (3.9)	0 (0)	47 (92.2)	0 (0)	4 (7.8)
Portugal	106	76 (71.7)	19 (17.9)	11 (10.4)	89 (84.0)	0 (0)	17 (16.0)
Spain	133	62 (46.6)	15 (11.3)	56 (42.1)	95 (71.4)	0 (0)	38 (28.6)
Sweden	64	58 (90.6)	1 (1.6)	5 (7.8)	61 (95.3)	0 (0)	3 (4.7)
Switzerland	111	97 (87.4)	9 (8.1)	5 (4.5)	101 (91.0)	0 (0)	10 (9.0)
UK	91	78 (85.7)	8 (8.8)	5 (5.5)	79 (86.8)	0 (0)	12 (13.2)
Eastern Europe	199	118 (59.3)	54 (27.1)	27 (13.6)	141 (70.9)	0 (0)	58 (29.1)
Hungary	54	19 (35.2)	28 (51.9)	7 (13.0)	24 (44.4)	0 (0)	30 (55.6)
Poland	68	50 (73.5)	9 (13.2)	9 (13.2)	52 (76.5)	0 (0)	16 (23.5)
Turkey	77	49 (63.6)	17 (22.1)	11 (14.3)	65 (84.4)	0 (0)	12 (15.6)
North America	687	468 (68.1)	72 (10.5)	147 (21.4)	525 (76.4)	2 (0.3)	160 (23.3)
Canada	350	276 (78.9)	37 (10.6)	37 (10.6)	292 (83.4)	2 (0.6)	56 (16.0)
USA	337	192 (57.0)	35 (10.4)	110 (32.6)	233 (69.1)	0 (0)	104 (30.9)
Latin America	518	300 (57.9)	139 (26.8)	79 (15.3)	438 (84.6)	1 (0.2)	79 (15.3)
Argentina	55	40 (72.7)	6 (10.9)	9 (16.4)	49 (89.1)	0 (0)	6 (10.9)
Brazil	260	172 (66.2)	67 (25.8)	21 (8.1)	242 (93.1)	1 (0.4)	17 (6.5)
Mexico	203	88 (43.3)	66 (32.5)	49 (24.1)	147 (72.4)	0 (0)	56 (27.6)
Australasia	121	95 (78.5)	21 (17.4)	5 (4.1)	106 (87.6)	1 (0.8)	14 (11.6)
Australia	114	91 (79.8)	18 (15.8)	5 (4.4)	99 (86.8)	1 (0.9)	14 (12.3)
Indonesia	7	4 (57.1)	3 (42.9)	0 (0)	7 (100)	0 (0)	0 (0)
Asia	515	165 (32.0)	75 (14.6)	275 (53.4)	104 (20.2)	1 (0.2)	410 (79.6)
Hong Kong	70	29 (41.4)	1 (1.4)	40 (57.1)	20 (28.6)	0 (0)	50 (71.4)
Japan	308	110 (35.7)	61 (19.8)	137 (44.5)	67 (21.8)	1 (0.3)	240 (77.9)
South Korea	137	26 (19.0)	13 (9.5)	98 (71.5)	17 (12.4)	0 (0)	120 (87.6)
Total	3362	2142 (63.7)	476 (14.2)	744 (22.1)	2312 (68.8)	7 (0.2)	1043 (31.0)

[a]Penicillin G susceptibility interpretative criteria: susceptible, ≤0.06 mg/L; intermediate, 0.12–1 mg/L; resistant, ≥2 mg/L.
[b]Erythromycin A susceptibility interpretative criteria: susceptible, ≤0.25 mg/L; intermediate, 0.5 mg/L; resistant, ≥1 mg/L.

Of the macrolide–lincosamide–streptogramin antimicrobials, telithromycin proved the most active overall (99.9% susceptibility). In contrast, high rates of resistance were reported for both clarithromycin (30.6%) and azithromycin (30.7%); the activity of these agents and patterns of resistance being essentially equivalent to those of erythromycin A across the regions studied. Resistance to clindamycin averaged 19.7%. Clindamycin resistance was reported most frequently for isolates collected from Asia and from Europe, where resistance rates were three-fold and seven-fold higher, respectively, than for the other regions. All regions reported a high prevalence of resistance to co-trimoxazole, ranging from 15.7% in Australasia to 45.6% in Latin America (28.6% overall). Overall, resistance to tetracycline averaged 29.7%, the highest prevalence being reported in Asia (81.2%). All isolates collected were susceptible to linezolid, teicoplanin and vancomycin.

Of the oral agents tested, telithromycin and linezolid proved to have the most potent anti-pneumococcal activity, based on MIC and percentage susceptibilities. *S. pneumoniae* MIC distributions for telithromycin compared with β-lactams, macrolides and fluoroquinolones are shown in Figures 2–4.

Table 4. *In vitro* activity of 19 antimicrobial agents against 3362 isolates of *S. pneumoniae* collected from across five continents in the PROTEKT study (1999–2000)[a]

	Asia ($n = 515$)			Australasia ($n = 121$)			Europe ($n = 1521$)			Latin America ($n = 518$)			North America ($n = 687$)		
Antimicrobial	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S
Telithromycin[b]	0.06	0.5	100	0.008	0.015	100	0.015	0.06	99.9	0.008	0.06	100	0.015	0.25	100
Penicillin G	2	4	32.0	0.015	0.25	78.5	0.015	2	73.2	0.06	2	57.9	0.015	2	68.1
Co-amoxiclav	1	4	87.6	0.03	0.25	100	0.03	2	97.5	0.03	2	98.7	0.03	2	90.5
Cefaclor	64	>64	21.6	1	8	81.0	1	>64	70.2	1	64	63.7	1	>64	63.5
Cefixime	32	64	32.0	0.25	4	78.5	0.25	32	73.2	0.5	32	57.9	0.25	32	68.1
Cefpodoxime	2	4	36.3	0.12	0.25	93.4	0.12	2	80.6	0.12	v2	78.4	0.12	4	75.3
Cefuroxime	4	8	36.7	0.03	0.5	92.6	0.06	8	80.6	0.12	4	77.0	0.06	8	75.3
Azithromycin	64	>64	20.2	0.12	4	87.6	0.12	>64	75.1	0.12	8	84.8	0.12	16	76.4
Clarithromycin	32	>32	20.4	0.03	1	88.4	0.03	>32	75.0	0.03	4	84.8	0.06	8	76.4
Clindamycin	2	>4	49.5	0.06	0.12	94.2	0.06	>4	80.1	0.06	0.12	93.1	0.06	0.12	91.1
Erythromycin A	64	>64	20.2	0.06	2	87.6	0.06	>64	74.9	0.06	4	84.6	0.06	16	76.4
Levofloxacin	1	1	95.3	1	1	100	1	1	99.8	1	1	99.2	1	1	98.0
Moxifloxacin	0.12	0.25	96.7	0.12	0.25	100	0.12	0.25	99.9	0.12	0.25	99.2	0.12	0.25	98.7
Co-trimoxazole	1	16	35.0	0.25	4	70.2	48.8	11.2	66.3	2	16	36.5	0.5	16	61.6
Tetracycline	>16	>16	18.4	0.25	>16	86.6	60.6	32	75.9	0.25	>16	75.1	0.25	>16	86.2
Quinupristin/dalfopristin	0.5	1	96.3	0.5	1	99.2	0.5	1	98.9	0.5	0.5	99.8	0.5	1	99.6
Linezolid	1	1	100	1	1	100	1	1	100	1	2	100	1	2	100
Teicoplanin	0.06	0.12	100	0.06	0.12	100	0.06	0.12	100	0.06	0.12	100	0.06	0.12	100
Vancomycin	0.5	0.5	100	0.5	0.5	100	0.5	0.5	100	0.5	v0.5	100	0.5	0.5	100

[a]For susceptibility criteria see Table 1.
[b]Inhibited by ≤1 mg/L (tentative NCCLS susceptibility breakpoint).

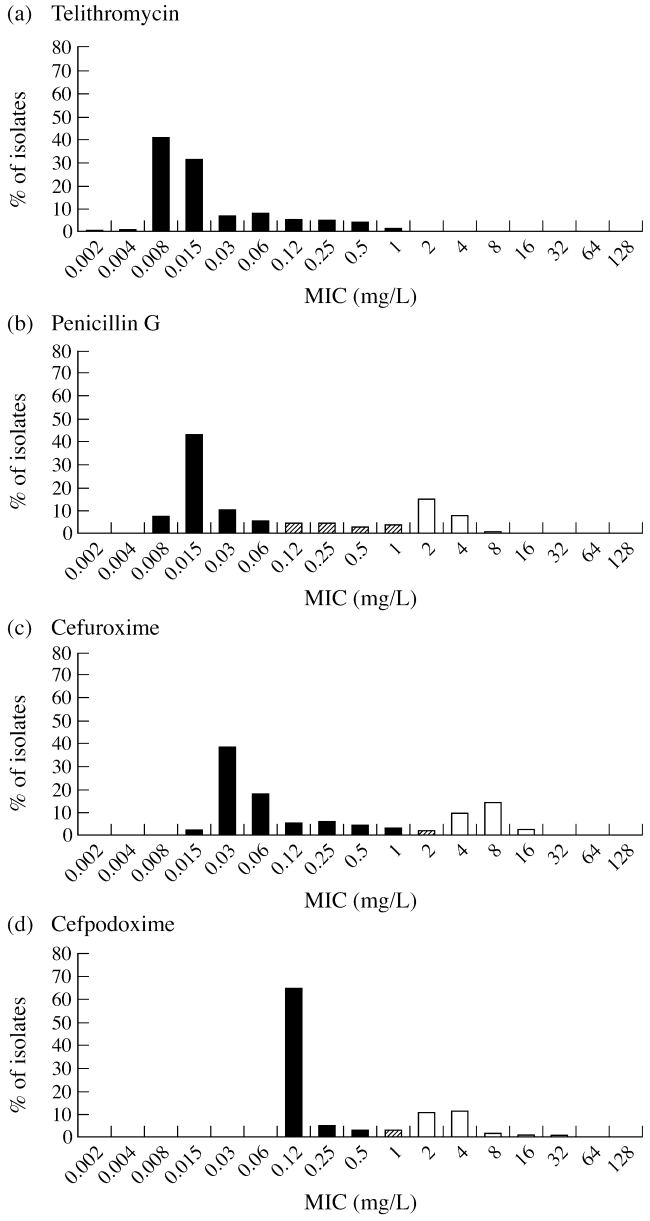

Figure 2. MIC distributions for telithromycin and β-lactams against 3362 isolates of *S. pneumoniae* collected in total in the PROTEKT study (1999–2000). Key: black bars, susceptible; shaded bars, intermediate; white bars, resistant

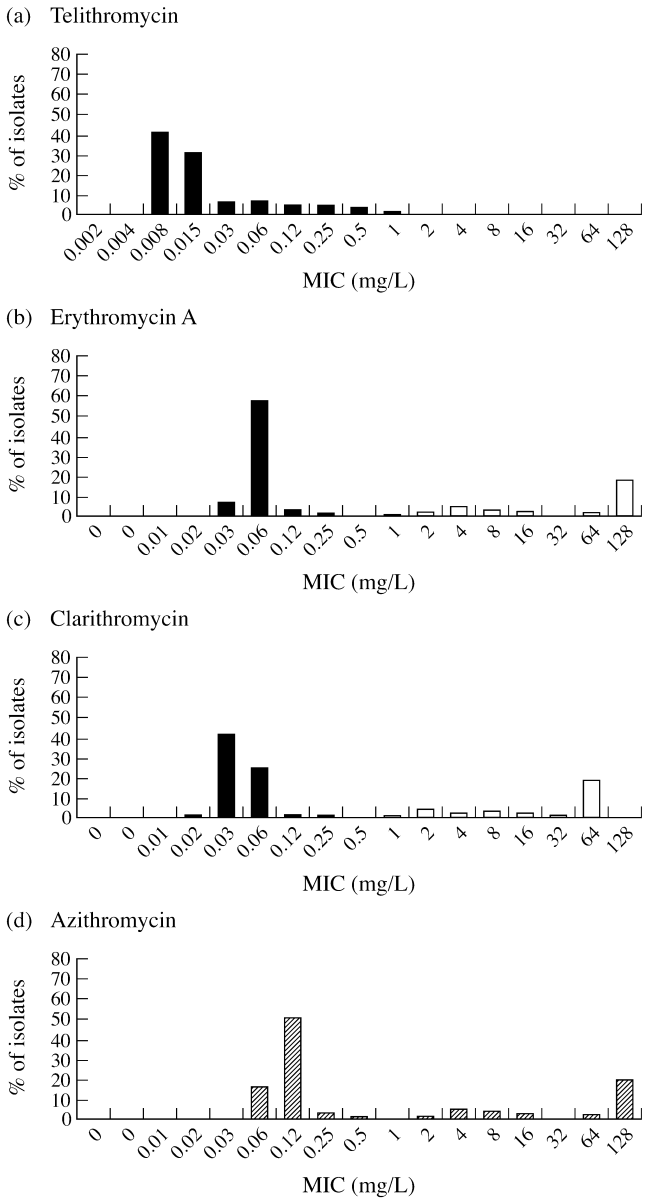

Figure 3. MIC distributions for telithromycin and macrolides against 3362 isolates of *S. pneumoniae* collected in the PROTEKT study (1999–2000). For key see Figure 2.

Cross- and parallel-resistance

In total, 12.6% of penicillin G-susceptible *S. pneumoniae* isolates were found to be resistant to erythromycin A, with rates rising to 49.2% and 72.4% for penicillin G-intermediate and -resistant isolates, respectively. Table 5 summarizes the activity of representatives of the major groups of antimicrobials against *S. pneumoniae* isolates collected in PROTEKT according to the penicillin G susceptibility phenotype, and by region. With the exception of levofloxacin and telithromycin, resistance to the drugs listed in Table 5 was notably higher among penicillin G-non-susceptible isolates than among penicillin G-susceptible isolates. A similar cross-resistance profile was observed between penicillin G and other β-lactams, and between penicillin G and macrolides (data not shown). Again there was marked geographical variation in the prevalence of antimicrobial resistance among the penicillin G resistance phenotypes.

Table 6 shows the extent to which *S. pneumoniae* isolates were multi-resistant to commonly used oral antimicrobials. Approximately 50% of all erythromycin A-resistant strains were also resistant to penicillin G and co-trimoxazole, while 63.2% and 76.5% of erythromycin A-resistant strains were

Table 5. *In vitro* activity of selected antimicrobial agents against 3362 isolates of *S. pneumoniae* collected in the PROTEKT study (1999–2000), according to penicillin G susceptibility and continent[a]

	Asia ($n = 515$)			Australasia ($n = 121$)			Europe ($n = 1521$)			Latin America ($n = 518$)			North America ($n = 687$)		
Antimicrobial	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}	%S
Telithromycin[b]															
Pen-S	0.03	0.25	100	0.008	0.015	100	0.008	0.015	100	0.008	0.015	100	0.008	0.015	100
Pen-I	0.06	0.5	100	0.008	0.015	100	0.015	0.06	100	0.015	0.25	100	0.015	0.12	100
Pen-R	0.06	0.5	100	0.015	0.03	100	0.015	0.5	99.2	0.008	0.12	100	0.06	0.5	100
Co-amoxiclav															
Pen-S	0.03	0.06	100	0.03	0.03	100	0.03	0.03	100	0.03	0.03	100	0.03	0.03	100
Pen-I	0.25	1	98.7	0.25	0.5	100	0.12	1	100	0.12	1	100	0.25	2	100
Pen-R	2	4	77.0	2	2	100	2	8	84.0	2	2	91.4	2	>4	55.8
Cefpodoxime															
Pen-S	0.12	0.5	95.2	0.12	0.12	100	0.12	0.12	100	0.12	0.12	100	0.12	0.12	99.4
Pen-I	2	4	40.0	0.25	1	85.7	0.25	2	67.4	0.25	2	76.3	0.25	2	72.2
Pen-R	4	8	0	2	4	0	4	4	0	4	4	0	4	8	0
Erythromycin A															
Pen-S	4	>64	44.9	0.06	0.5	89.5	0.06	4	88.8	0.06	0.06	95.0	0.06	0.12	93.4
Pen-I	64	>64	14.7	0.06	0.06	90.4	>64	>64	43.8	0.06	>64	66.2	0.06	>64	58.3
Pen-R	64	>64	6.9	4	>64	40.0	>64	>64	31.9	0.06	8	77.2	4	>64	31.3
Levofloxacin															
Pen-S	1	1	95.2	1	1	100	1	1	99.7	1	1	99.3	1	1	98.1
Pen-I	0.5	1	98.7	1	1	100	1	1	100	1	1	100	1	1	98.6
Pen-R	1	1	94.5	1	1	100	1	1	100	1	1	97.5	1	1	97.3
Co-trimoxazole															
Pen-S	0.5	2	70.3	0.25	2	80	0.25	2	82.1	1	8	49.3	0.25	0.5	81.4
Pen-I	1	8	42.7	1	4	42.9	1	8	42.6	4	>16	25.2	1	16	45.8
Pen-R	4	>16	11.6	8	16	0	8	16	8.8	8	>16	7.6	8	>16	6.1
Tetracycline															
Pen-S	16	>16	37.0	0.25	0.5	90.5	0.25	8	89.2	0.25	16	81.0	0.25	1	97.7
Pen-I	>16	>16	14.7	0.25	>16	81.0	>16	>16	37.9	0.25	>16	71.2	0.25	>16	68.1
Pen-R	>16	>16	8.4	>16	>16	40.0	>16	>16	40.3	0.5	>16	59.5	0.5	>16	58.5

Pen-S, penicillin G susceptible (MIC ≤ 0.06 mg/L); Pen-I, penicillin G intermediate (MIC 0.12–1 mg/L); Pen-R, penicillin G resistant (MIC ≥2 mg/L).
[a]For susceptibility criteria see Table 1.
[b]Inhibited by ≤1 mg/L (tentative NCCLS susceptibility breakpoint).

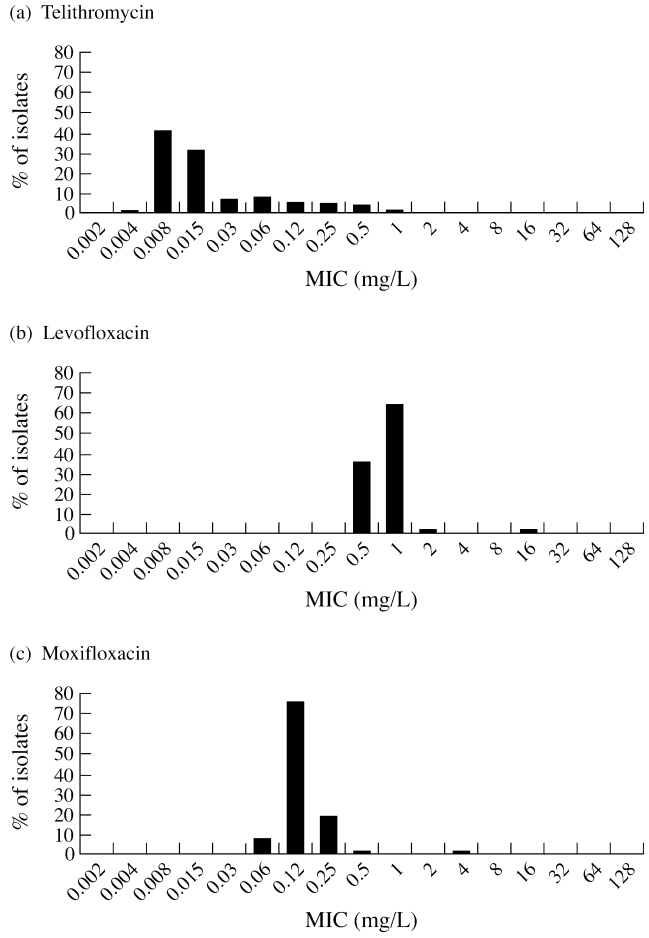

Figure 4. MIC distributions for telithromycin and fluoroquinolones against 3362 isolates of *S. pneumoniae* collected in total in the PROTEKT study (1999–2000). For key see Figure 2.

cross-resistant to clindamycin and tetracycline, respectively, but they remained susceptible to levofloxacin, moxifloxacin, telithromycin and linezolid (data not shown). Those isolates that were resistant to levofloxacin ($n = 35$) were also highly resistant to the other oral agents, with the exception of telithromycin and linezolid (all fluoroquinolone-resistant *S. pneumoniae* remained fully susceptible to these two oral agents). The *in vitro* activity of telithromycin by penicillin G, erythromycin A or fluoroquinolone susceptibility/resistance phenotype is summarized in Table 7.

Discussion

Over the last three decades, antimicrobial resistance among isolates of *S. pneumoniae* has spread across the globe at an alarming rate, and now threatens to compromise the clinical usefulness of the current portfolio of agents for treating infections associated with this pathogen. International antimicrobial surveillance studies play a critical role in defining the nature and extent of the resistance problem, as well as in

Table 6. Multiple resistance among 3362 isolates of *S. pneumoniae* collected in the PROTEKT study (1999–2000) (the percentages quoted in the table are the percentage of isolates resistant to Agent 1 that are also resistant to Agent 2)

		No. (%) of isolates also resistant to:				
Phenotype	No. of isolates	penicillin G	clindamycin	erythromycin A	levofloxacin	tetracycline	co-trimoxazole
Penicillin G resistant	744		329 (44.2%)	539 (72.4%)	18 (2.4%)	486 (65.3%)	552 (74.2%)
Clindamycin resistant	661	329 (49.8%)		659 (99.7%)	19 (2.9%)	586 (88.7%)	366 (55.4%)
Erythromycin A resistant	1043	539 (51.7%)	659 (63.2%)		27 (2.6%)	798 (76.5%)	527 (50.5%)
Levofloxacin resistant	35	18 (51.4%)	19 (54.2%)	27 (77.1%)		21 (60.0%)	20 (57.1%)
Tetracycline resistant	997	486 (48.7%)	586 (58.8%)	798 (80.0%)	21 (2.1%)		491 (49.2%)
Co-trimoxazole resistant	960	552 (57.5%)	366 (38.1%)	527 (54.9%)	20 (2.1%)	491 (51.1%)	

Table 7. *In vitro* activity of telithromycin against *S. pneumoniae* isolates collected in PROTEKT, by antimicrobial resistance phenotype

Phenotype	*n*	MIC (mg/L)				% susceptibility[a]
		Mode	MIC_{50}	MIC_{90}	Range	
Penicillin G						
Susceptible	2142	0.008	0.008	0.03	0.002–0.5	100
Intermediate	476	0.008	0.015	0.12	0.004–1	100
Resistant	744	0.06	0.06	0.5	0.004–8	99.6
Erythromycin A						
Susceptible	2312	0.008	0.008	0.015	0.002–0.12	100
Intermediate	7	0.015	0.015	0.03	0.008–0.03	100
Resistant	1043	0.06	0.06	0.5	0.008–8	99.7
Levofloxacin						
Susceptible	3317	0.008	0.015	0.12	0.002–8	99.9
Intermediate	10	0.008	0.03	0.12	0.008–0.12	100
Resistant	35	0.03	0.03	0.12	0.008–0.5	100
Combined	3362	0.008	0.015	0.12	0.002–8	99.9

[a]Inhibited by ≤1 mg/L (tentative NCCLS susceptibility breakpoint).

guiding development of antimicrobial policies and usage.[23] Furthermore, by monitoring changes in resistance patterns and the spread of significant resistance phenotypes across the globe, these studies enable authorities to implement intervention strategies to halt the spread of such strains. The PROTEKT study was initiated in 1999 to monitor longitudinally the global spread of resistance among bacterial respiratory pathogens associated with CARTIs. Testing of all isolates at a central laboratory using validated identification techniques and NCCLS-recommended methodologies has helped to ensure the quality and consistency of the data generated.

Consistent with previous international surveillance studies,[10,11,24] PROTEKT reported large differences between countries in the penicillin G resistance profile of *S. pneumoniae*, the highest rates of resistance being noted in clinical isolates from patients in the Asian region. In all three of the Asian countries studied, the resistant phenotype (44.5–71.6% of strains) dominated over the intermediate phenotype (1.4–19.8%). For example, within Hong Kong, the prevalence of penicillin G intermediate and penicillin G-resistant strains was 1.4% and 57.1%, respectively, which is consistent with that reported by Felmingham *et al.*[10] Furthermore, our data suggest a large upward shift in the penicillin G MICs among *S. pneumoniae* from Japan: a multicentre study of 1997–1998 pneumococcal isolates reported penicillin G intermediate and penicillin G resistant rates in Japan of 44% and 10%, respectively,[11] while in the present 1999–2000 study, the percentages were 20% and 45%, respectively.

Outside of Asia, penicillin G resistance rates in excess of 40% were recorded in France and Spain, while in the neighbouring countries of Germany and Switzerland, resistance to this agent averaged 2.2% and 4.5%, respectively. The data also suggest that the prevalence of penicillin G resistance in the USA has risen substantially from the 9.5% reported in a 1994–1995 study by Doern *et al*.[7] For 1997–1998 US isolates, Thornsberry *et al.* found 13% penicillin G resistance,[25] Hoban *et al.* reported 15% resistance among 1999 isolates,[24] and in 1999–2000 isolates we have found 33% resistance. Although the penicillin G resistance prevalence in the USA seems exceptionally high in this study, preliminary data from a subsequent sister surveillance study, PROTEKT US, reported a rate of 26% among 10 103 isolates of *S. pneumoniae*, with individual rates as high as 36.5% being observed in the southeastern region of the country.[26]

The increasing prevalence of penicillin G resistance among *S. pneumoniae* is a cause for concern, not only because of the impact that it may have on the clinical usefulness of this agent, but also because resistance to penicillin G has been found to be associated with resistance to other β-lactams, such as the cephalosporins, as well as to several non-β-lactam classes. In *S. pneumoniae*, reduced susceptibility to β-lactams, such as penicillin, arises through alterations in the penicillin-binding proteins (PBPs)—1A, 1B, 2A, 2B, 2X and 3—via acquisition of genetic material from other *Streptococcus* spp coexisting in close proximity, e.g. *S. mitis*. The resistance phenotype of the resulting strain depends upon which particular PBP is modified. For example, while many of the penicillin-resistant strains first identified remained susceptible to third-generation cephalosporins, resistance to these agents is now well established among penicillin-resistant pneumococci, reflecting alterations in PBPs, particularly 1A and 2X.

As reported in other studies,[7,24,25,27] co-resistance to erythromycin A, clindamycin, tetracycline and co-trimoxazole was higher among strains with intermediate susceptibility to penicillin G than among penicillin G-susceptible strains, with an even higher prevalence being noted among strains highly resistant to penicillin G. In the absence of any known common mechanism of resistance between penicillin G and these non-β-lactam antimicrobials, this relationship most probably reflects increased selective pressure among *S. pneumoniae* created by broad-based antimicrobial usage.[24] This hypothesis is supported by the fact that in some regions, elevated resistance to these antimicrobials was also noted in the penicillin G-susceptible population, particularly in Asia (macrolides, co-trimoxazole and tetracycline), Europe (macrolides) and Latin America (co-trimoxazole and tetracycline), where these agents are widely prescribed.

Overall, resistance to erythromycin A among *S. pneumoniae* averaged 31%, and in many countries was more prevalent than resistance to penicillin G. There were no important differences in the activity of the newer agents, e.g. clarithromycin and azithromycin, and pneumococci resistant to erythromycin A were invariably resistant to these second-generation macrolides. As reported in the 1996–1997 Alexander Project,[10] we found France, Belgium, Spain and Italy to be the foci of macrolide resistance in Western Europe. However, the 58% and 43% macrolide resistance rates reported in our study for 1999–2000 isolates from France and Italy, respectively, are substantially higher than those reported by Felmingham *et al.* for 1997 isolates in the Alexander Project (46% and 30%, respectively).[10] Within Western Europe, we also found substantial increases in the prevalence of macrolide resistance in Germany (16% versus 7%), Portugal (16% versus 4%) and Eire (26% versus 14%), compared with the prevalences reported in the Alexander Project for 1997 isolates. Outside Western Europe, high rates of resistance were found in all Asian countries participating in the study, as well as in Hungary, Mexico and the USA. Hoban and colleagues have recently reported a significant rise in macrolide resistance among US pneumococci isolated in the SENTRY study, rising from 17% in 1998 to 23% in 1999.[24] The 31% macrolide resistance rate observed in our study for 1999–2000 *S. pneumoniae* US isolates, suggests that in the USA, resistance to this class has risen from the 1999 data reported in SENTRY. This is supported by the preliminary data from PROTEKT US, which found that 31% of pneumococci collected during 2000–2001 from 206 centres across the USA were resistant to erythromycin A.[26] A recent analysis conducted in Spain has shown a clear correlation between erythromycin A resistance and overall macrolide consumption, the relationship appearing to be due mainly to consumption of those macrolides dosed once or twice daily.[28] While this analysis is not able to demonstrate cause and effect, it does suggest that increased use of long-acting macrolides may be linked to the increase in prevalence of erythromycin A-resistant pneumococci observed in recent years.

Two major forms of macrolide resistance exist in *Streptococcus* spp: (i) modification of the 23S ribosomal RNA target site by methylation (encoded by *erm* genes); and (ii) antimicrobial efflux by proton-motive force or utilizing ATP (encoded by *mef* genes).[29,30] Modification of the ribosomal target site confers a high-level resistance not only to macrolides, but also to lincosamides and streptogramin$_B$ (so-called MLS$_B$ phenotype), while the resistance conferred by efflux abnormality (M phenotype) is usually of a moderate level and confined to 14- and 15-membered ring macrolides only (clindamycin usually remains active). In a genotypic analysis of the resistance mechanism in 1043 macrolide-resistant pneumococcal isolates from the PROTEKT 1999–2000 study, 56.2% of isolates expressed *erm*(B) (predominantly in Europe) and 35.3% expressed *mef*(A) (predominantly in North America).[31] In our study, 63% of erythromycin A-resistant pneumococci were also resistant to clindamycin, consistent with *erm* (MLS$_B$) resistance. Reduced susceptibility to clindamycin was particularly prevalent in Asia and in Europe. Relative to *mef*-mediated resistance, these strains may be more difficult to eradicate, with a subsequent risk of clinical failure.

In the present study, the prevalence of resistance to the newer anti-pneumococcal fluoroquinolones, e.g. levofloxacin and moxifloxacin, remained low (~1%). There were, however, pockets of resistance to these agents, particularly in Hong Kong (14%) and South Korea (3%). The situation in Hong Kong is particularly alarming since all fluoroquinolone-resistant pneumococci were also highly resistant to β-lactams, macrolides, co-trimoxazole, cephalosporins and tetracycline—the only two oral agents that retained full susceptibility against these isolates were the ketolide, telithromycin and linezolid.[32] Molecular characterization has shown that the vast majority of the fluoroquinolone resistance detected in Hong Kong was due to the spread of the 23F Spanish multidrug-resistant clone.[32] Goldsmith *et al.* have reported an increased prevalence of fluoroquinolone resistance among penicillin G-resistant pneumococci in Northern Ireland,[33] although subsequent surveillance studies have not been able to confirm this relationship for other regions.[11,24,25] In our study, levofloxacin-resistant and/or moxifloxacin-resistant strains were isolates from countries with relatively low rates of penicillin G resistance (i.e. Germany, Switzerland and Canada), as well as from those countries in which reduced penicillin G susceptibility is more prevalent (e.g. Asia). Half of the 35 levofloxacin-resistant pneumococci identified in this study were also resistant to penicillin G, and approximately three-quarters were co-resistant to erythromycin A. No glycopeptide-resistant pneumococci were identified in the study.

One of the objectives of the PROTEKT study was to provide current surveillance data for telithromycin at its time of

launch into clinical practice. The study has shown this new ketolide to be the most potent of the oral antimicrobials currently available against *S. pneumoniae*. Moreover, the data confirm previously published findings that telithromycin maintains its anti-pneumococcal activity against strains that are resistant to β-lactams, macrolides and fluoroquinolones.[18] Using the proposed NCCLS interpretative criteria of ≤1 mg/L to define susceptibility to telithromycin gives an overall susceptibility of 99.9% for *S. pneumoniae*. Applying the slightly lower breakpoint (≤0.5 mg/L) adopted by the European authorities to the PROTEKT MIC data has negligible effect on the overall susceptibility to telithromycin, reducing it to 99.3%. Results from subsequent years of the PROTEKT study will help to establish the impact that the introduction of telithromycin will have on susceptibility of *S. pneumoniae* not only to this agent, but also to non-ketolide antibacterials. *In vitro* studies and preliminary data from human studies suggest that telithromycin has a favourable ecological profile, with a low potential to select for resistant mutants, and, in contrast to the macrolides, telithromycin does not induce MLS resistance *in vitro*.[20] The low potential for telithromycin to select for pneumococcal resistance has been confirmed recently by Davies and colleagues,[34] who performed a series of *in vitro* serial passage experiments in which five macrolide-susceptible and six macrolide-resistant [three *erm*(B), three *mef*(A)] strains of *S. pneumoniae* were repeatedly exposed to sub-inhibitory concentrations of telithromycin or other MLS_B antibacterials. They found that telithromycin selected for resistant mutants significantly less often than the other agents. Indeed, of the 54 mutants isolated with raised MICs of at least one of the test agents, only three were resistant to telithromycin compared with 36, 37, 36, 45 and 15 mutants resistant to azithromycin, clarithromycin, erythromycin A, roxithromycin and clindamycin, respectively. Furthermore, studies in man have shown that telithromycin selects for resistance among oropharyngeal and intestinal bacterial microflora significantly less than clarithromycin.[21]

In conclusion, the 1999–2000 data from PROTEKT confirm the widespread and increasing prevalence of antimicrobial resistance among *S. pneumoniae*. The large intercountry variation reported in the prevalence of resistance, not only to penicillin G but also to other antimicrobials, highlights the need for continuing surveillance of resistance at both the national and international level, with the data being analysed and disseminated rapidly so that changes in susceptibility patterns can be quickly detected allowing appropriate control measures to be put in place. In this era of increasing pneumococcal resistance, the ketolide telithromycin has emerged as a promising new candidate for the treatment of CARTIs, possessing potent anti-pneumococcal activity even against strains with resistance to other commonly used antimicrobials.

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