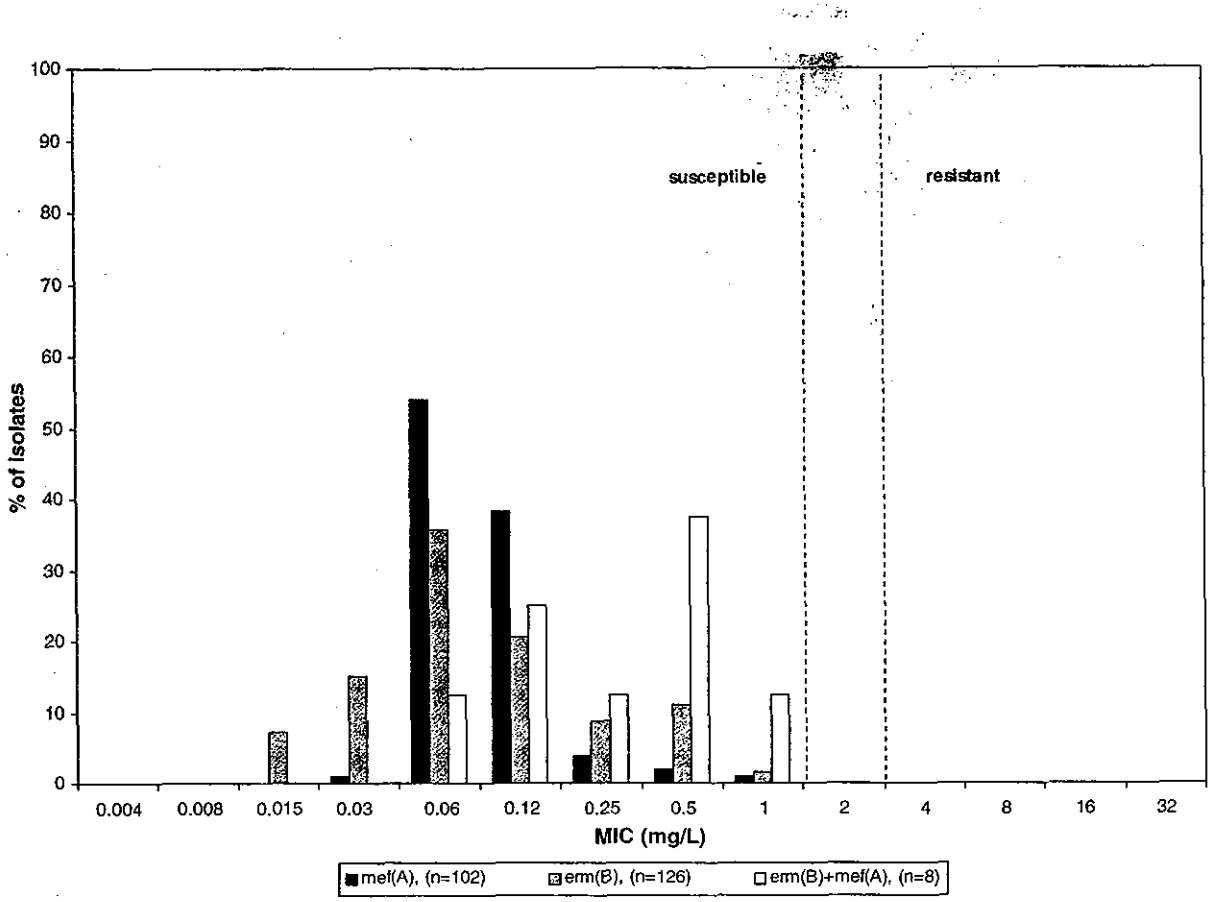

Figure 2 Telithromycin MIC distribution for macrolide-resistant genotypes of *Streptococcus pneumoniae* from Japan.

however, had ampicillin MICs of 2 mg/L (intermediate resistance according to NCCLS breakpoints).

Comparative in vitro activity of all antimicrobial compounds tested against *H. influenzae* and categorised by β-lactamase production is shown in Table 4. Of the β-lactams tested, cefditoren (MIC_{90} 0.06 mg/L; no NCCLS breakpoint) and cefixime (MIC_{90} 0.25 mg/L; 100%) were the most active.

Chloramphenicol resistance had low prevalence (3.6%), with nine of the ten nonsusceptible isolates also β-lactamase-positive. Similarly, tetracycline resistance was low (6.4%) with resistant isolates predominantly β-lactamase-positive (12/18). The MIC_{90} values for both chloramphenicol and tetracycline among β-lactamase-positive *H. influenzae* isolates (16 mg/L) were 16 times greater than for β-lactamase-negative isolates (Table 4).

The MICs of the macrolides and telithromycin to *H. influenzae* isolates followed unimodal distributions in the rank order: azithromycin (MIC_{90} 1 mg/L) > telithromycin (MIC_{90} 2 mg/L) > rokitamycin (MIC_{90} 8 mg/L) > clarithromycin and roxithromycin (MIC_{90} 16 mg/L), (Table 4). There was no correlation between ketolide/macrolide susceptibility and β-lactamase production.

Moraxella catarrhalis

Of the 122 *M. catarrhalis* isolates, 118 (96.7%) were β-lactamase-positive. With the exception of some β-lactams (ampicillin, cefaclor, cefuroxime and cefcapene), all antimicrobials tested showed good activity (MIC_{90} values of $\leq$1 mg/L) against *M. catarrhalis* isolates (Table 4). Cefixime was the most active β-lactam (MIC_{90} 0.25 mg/L), followed by cefdinir and cefditoren (both, MIC_{90} 0.5 mg/L) (Table 4). The rank order of activity of the MLS class of antimicrobials was azithromycin (MIC_{90} 0.06 mg/L) > telithromycin, clarithromycin, and rokitamycin (MIC_{90} 0.25 mg/L) > roxithromycin (MIC_{90} 0.5 mg/L). Sparfloxacin and tosufloxacin were the most potent (MIC_{90} 0.008 mg/L) fluoroquinolones.

Discussion

Streptococcus pneumoniae

Previous reports have demonstrated the increasing prevalence of penicillin resistance of both intermediate (MIC 0.12–1 mg/L) and resistant (MIC

Table 4 *Comparative in vitro activity of various antimicrobials against isolates of Haemophilus influenzae and Moraxella catarrhalis from Japan.*

Antimicrobial	*Haemophilus influenzae*									*Moraxella catarrhalis*[a]	
	All isolates (n = 281)			β-lactamase positive (n = 24)			β-lactamase negative (n = 257)			All isolates (n = 122)	
	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S[b]	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S[b]	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S[b]	MIC_{50} (mg/L)	MIC_{90} (mg/L)
Ampicillin	0.25	2	87.9	>16	>16	0	0.25	1	96.1	8	16
Amoxicillin–clavulanate	0.5	2	99.3	1	2	100	0.5	2	99.2	0.12	0.25
Cefaclor	4	16	86.5	16	32	45.8	4	8	90.3	2	16
Cefcapene	0.5	1	NA	1	16	NA	0.5	4	NA	8	16
Cefdinir	0.25	1	91.8	0.5	2	79.2	0.25	1	93.0	0.12	0.5
Cefditoren	0.015	0.06	NA	0.015	0.12	NA	0.015	0.03	NA	0.12	0.5
Cefixime	0.03	0.25	100	0.12	0.5	100	0.03	0.25	100	0.25	0.25
Cefpodoxime	0.06	0.5	99.3	0.12	1	100	0.06	0.5	99.2	0.5	1
Cefuroxime	1	4	95.4	2	4	95.8	1	4	95.3	2	4
Telithromycin	1	2	100[c]	1	2	100[c]	1	2	100[c]	0.06	0.25
Roxithromycin	8	16	NA	8	8	NA	8	16	NA	0.25	0.5
Clarithromycin	8	16	88.3	8	16	75.0	8	16	89.5	0.25	0.25
Azithromycin	1	1	100	1	2	100	1	1	100	0.06	0.06
Rokitamycin	4	8	NA	4	8	NA	4	8	NA	0.25	0.25
Minocycline	1	2	NA	1	2	NA	1	2	NA	0.06	0.06
Tetracycline	0.5	1	93.6	1	16	50.0	0.5	1	97.7	0.25	0.5
Co-trimoxazole	0.06	0.06	97.9	0.06	4	87.5	0.06	0.06	98.8	0.12	0.25
Chloramphenicol	0.5	1.0	96.4	0.5	8	62.5	0.5	0.5	99.6	0.5	0.5
Ciprofloxacin	0.015	0.015	100	0.015	0.03	100	0.015	0.015	100	0.03	0.03
Levofloxacin	0.015	0.015	100	0.015	0.03	100	0.015	0.015	100	0.03	0.03
Sparfloxacin	0.004	0.008	99.3	0.008	0.008	100	0.004	0.008	99.2	0.008	0.008
Tosufloxacin	0.004	0.008	NA	0.008	0.008	NA	0.004	0.008	NA	0.008	0.008

[a] NCCLS breakpoints not available for *M. catarrhalis*.
[b] % of isolates susceptible according to NCCLS breakpoints.
[c] NCCLS (SAST 2003) approved breakpoint for *H. influenzae*: susceptible ≤4 mg/L; NA = NCCLS breakpoints not available.

≥2 mg/L) phenotypes amongst isolates of *S. pneumoniae*.[2,9,14] During the 1999–2000 winter season, 44.5% of *S. pneumoniae* RTI isolates from Japan were penicillin resistant and 19.8% were penicillin intermediate, a pattern with small geographic variation throughout Japan (Table 1). In previous studies, Yoshida et al.[15] found that penicillin resistance increased from 4.3% in 1988 to 9.8% in 1992-and Sahm et al.[10] reported 10.1% penicillin resistance for the 1997–98 winter season. Therefore, penicillin resistance in Japan is increasing and current data strongly suggest that the trend has accelerated in recent years.

Resistance to penicillin in *S. pneumoniae* is mediated by changes in the affinity of high molecular weight penicillin binding proteins (PBPs) for their substrates. As these PBPs are also targets for other β-lactams, the activity of aminopenicillins, cephalosporins and carbapenems is also reduced against penicillin-resistant strains. This is most evident with compounds considered active only against penicillin-susceptible *S. pneumoniae*, such as cefaclor and cefixime. Cefuroxime, cefpodoxime and cefdinir retained some activity against penicillin-intermediate isolates (approximately 40%), but little or no activity against resistant isolates. This perhaps reflects the trend towards greater resistance as previous work has shown that cefuroxime, among other cephalosporins, can retain activity against many penicillin-resistant strains.[16,17] The most effective β-lactams for the 1999–2000 winter season in Japan were cefditoren and amoxicillin–clavulanate, with over 90% susceptibility among penicillin-resistant strains. The amoxicillin–clavulanate results can be extrapolated to include amoxicillin as an effective β-lactam (92% susceptibility among penicillin-resistant strains), although amoxicillin itself was not tested against *S. pneumoniae*.

Macrolides form the principal alternative to β-lactams for the treatment of lower RTIs involving *S. pneumoniae*. However, it is now clear that this class of compounds, including erythromycin, clarithromycin and azithromycin, is seriously compromised by the development of resistance not only as a result of

the increasing prevalence of penicillin-resistant pneumococci but also, in Japan, among penicillin-susceptible strains.

Typical of the Far East, *S. pneumoniae* macrolide resistance in Japan is high (77.9%) with some centre variation (67.3–86.4%). This finding of 77.9% is considerably higher than the 66.5% reported for the 1997–1998 winter season.[10] The proportion of penicillin-resistant isolates ($n = 137$) that are also macrolide-resistant has not increased over the same period (124/137, 90.5%) and is slightly lower than the previous study (1997–1998, 95.5%).

Two main mechanisms are known to account for macrolide resistance in *S. pneumoniae*. With the first, resistance is associated with specific mutation within the *erm* gene that confers resistance to most macrolides, lincosamides and streptogramin B antibiotics.[18] With the second, the so-called M phenotype, resistance is mediated by an efflux mechanism due to the presence of the *mef*(A) gene that confers resistance to 14- and 15-membered macrolides.[19] Growing macrolide resistance is of increasing concern, especially that dependent upon the *erm*(B) genotype; not only because it is the more potent macrolide resistance, but because resistance to other antimicrobial compounds appear preferentially to be associated with it. This study shows that in Japan, the distribution of *erm*(B) and *mef*(A) are similar.

Telithromycin, a synthetic ketolide derived by chemical modification of desclarithromycin, was designed to maintain potent antimicrobial activity against community-acquired respiratory tract infection (CARTI) pathogens, even macrolide-resistant pneumococci, and not to induce resistance due to *erm*(B).[20] There was, however, an upward shift in telithromycin MICs among the isolates with *erm*(B)-mediated macrolide resistance compared with *mef*(A) strains. This effect of *erm*(B) resistance on the activity of telithromycin has been reported previously although, as in this study, all the isolates were still found to be inhibited by telithromycin at ≤ 1 mg/L.

Worldwide incidence of fluoroquinolone-resistant *S. pneumoniae* (levofloxacin MIC ≥ 8 mg/L) is rare, although it tends to be concentrated in pockets of Asia (specifically Hong Kong) and North America. The four (1.3%) resistant isolates from Japan were obtained from four different centres, and would therefore suggest random distribution and independent origin.

Streptococcus pyogenes

Streptococcus pyogenes was susceptible to most of the antimicrobials tested with the notable exception of the macrolides (17.5% resistant, mostly *mef*(A)). Telithromycin was 16- to 32-fold more potent than the macrolides although penicillin remains the most potent antimicrobial.

Haemophilus influenzae

There is considerable variability worldwide in the prevalence of β-lactamase production by *H. influenzae*, with previous studies showing values of 19% for Europe, 42% for the USA and around 14% for Japan.[10,21,22] The value for Japan is slightly higher than the finding here of 8.5%. Only a single (0.36%) β-lactamase-negative ampicillin-resistant (BLNAR) (ampicillin MIC ≥ 4 mg/L) strain was isolated in Japan during the winter season 1999–2000, although 3.2% of isolates were β-lactamase-negative with low-level resistance to ampicillin (MIC 2 mg/L). These values are considerably lower that those published for Japan by Hasegawa et al.[23]

Of the β-lactams tested, cefixime (100%), cefpodoxime (99.3%), cefuroxime (95.4%), and cefdinir (91.8%) were the most active, followed by ampicillin (87.9%), cefaclor (86.5%), and amoxicillin (81.5%), (Table 4). β-lactamase production conferred resistance to ampicillin and amoxicillin for all isolates, but had little or no effect on susceptibility to cefixime, cefpodoxime, and cefuroxime. For cefdinir and cefaclor the effect was partial, susceptibility being reduced by approximately 15% and 50%, respectively.

Similar partial co-resistance was observed for chloramphenicol and tetracycline, where 99.6% and 97.7% β-lactamase-negative isolates were susceptible compared with 62.5% and 50% β-lactamase-positive isolates, respectively.

All isolates were susceptible to azithromycin, with 88.3% susceptible to clarithromycin. For the 1997–1998 winter season, Sahm et al. also found 100% susceptibility of isolates to azithromycin,[10] with 93.2% susceptible to clarithromycin, indicating a slightly increased resistance towards this macrolide. In 1999–2000, the azithromycin MICs for the Japanese isolates were all ≤ 2 mg/L. All isolates of *H. influenzae* were susceptible to the ketolide telithromycin at ≤ 4 mg/L.

Moraxella catarrhalis

β-lactamase production was observed in 96.7% of *M. catarrhalis* isolates tested in Japan, a figure almost identical to 97.5% reported by Sahm et al.[10] for the 1997–98 winter season. β-lactamase-producing strains of *M. catarrhalis* were first reported in the late 1970s and by the late 1980s, these strains were predominant, accounting for more than 80% of clinical isolates in a number of studies.[8,24,25]

The β-lactamases of *M. catarrhalis* are inhibited by clavulanic acid and the combination of amoxicillin–clavulanic acid has been shown to be highly active against this species.[25–29] Indeed, in this study, among β-lactamase-positive *M. catarrhalis* the MIC_{90} for unprotected ampicillin was high at 16 mg/L, in contrast with 0.25 mg/L for amoxicillin–clavulanate.

Summary

Despite growing public awareness Japan has witnessed increased and even accelerating resistance to the macrolides and to β-lactams. Fluoroquinolone resistance, albeit at a low level, would also appear to be endemic. This study documents the high prevalence of antimicrobial resistance and co-resistance among respiratory pathogens in Japan.

For a great proportion of respiratory infections that require antimicrobial therapy, amoxicillin remains largely effective; however, in Japan, the preference is for the use of newer drugs as first-line treatment. This study reinforces the necessity for judicious use of old and new antimicrobial compounds and, with the technical ability that is now available, to evaluate resistance at a genetic level to monitor more detailed patterns of emergence.

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