In Vitro Activities of Telithromycin, Linezolid, and Quinupristin-Dalfopristin against *Streptococcus pneumoniae* with Macrolide Resistance Due to Ribosomal Mutations

David J. Farrell,* Ian Morrissey, Sarah Bakker, Sylvie Buckridge, and David Felmingham

GR Micro Ltd., London, United Kingdom

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To date, 86 of 7,746 macrolide-resistant *Streptococcus pneumoniae* isolates from 1999 to 2002 PROTEKT (Prospective Resistant Organism Tracking and Epidemiology for the Ketolide Telithromycin) surveillance studies were negative for methylase and efflux mechanisms. Mutations in 23S rRNA or the genes encoding riboprotein L4 or L22 were found in 77 of 86 isolates. Six isolates were resistant to quinupristin-dalfopristin and two were resistant to linezolid, while telithromycin demonstrated good activities against all isolates.

Macrolide, lincosamide, and streptogramin B (MLS_B) resistance in *Streptococcus pneumoniae* occurs predominantly by modification of the drug binding site and/or by drug efflux. Drug efflux is encoded by the *mef*(A) gene, while target modification is usually the result of methylation of the 23S rRNA, mostly by the product of the *erm*(B) gene and rarely by the *erm*(A) subclass *erm*(TR) gene. In vitro studies have demonstrated that target modification can also be achieved by mutations in domains II and V of 23S rRNA (in one to four of the four copies of this gene present in *S. pneumoniae*) and in the genes encoding riboproteins L4 and L22 (1, 11). These mutations can confer resistance to MLS_B antibacterials and, in some cases, to ketolides (1, 11). Ribosomal mutations have also been found in MLS_B-resistant clinical isolates, although reports are rare at present (2, 9, 10).

PROTEKT (Prospective Resistant Organism Tracking and Epidemiology for the Ketolide Telithromycin) is a longitudinal, multicenter surveillance study of antibacterial resistance among respiratory tract pathogens from numerous countries worldwide. PROTEKT US is a sister study involving the United States only and began in 2000. All macrolide-resistant *S. pneumoniae* strains isolated during year 1 of the PROTEKT study (1999 to 2000) were screened for the common efflux and methylase genes associated with macrolide resistance to determine the global distributions of these mechanisms. Of 1,043 macrolide-resistant isolates screened, 16 (1.5%) isolates repeatedly tested negative for the *erm* and *mef* genes, while the remaining isolates were resistant to macrolides (4, 5). All isolates were found to harbor ribosomal mutations, most commonly A2059G (12 isolates) (4).

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Of the 23,543 isolates of *S. pneumoniae* collected from 1999 to 2002 in the PROTEKT and PROTEKT US studies, 7,746

(32.9%) were macrolide resistant; 86 (0.37%) macrolide-resistant isolates (including the 16 isolates reported previously) were negative for methylase and efflux mechanisms. The ribosomal mutations associated with macrolide resistance in these isolates were determined in this study. Before the isolates were sequenced for ribosomal mutations, macrolide resistance was confirmed by repeating the MIC determinations and comparing the MIC data with the initial results. Detection of L4 and L22 riboprotein and 23S rRNA gene mutations was performed as described previously (3). Briefly, the complete L4 and L22 operons and all four 23S rRNA operons were sequenced.

In total, 23 different mutational combinations were found in the 86 isolates, with A2059G in all four copies of the 23S rRNA gene being the most prevalent mutation (29 of 86 isolates [33.7%]) (Table 1). Eight isolates had two mutations, and two isolates had three mutations. Sixty isolates had mutations that have been described previously (13 mutational combinations), while 13 isolates had mutations not described previously (10 mutational combinations) (2, 9, 10). A mutational event was not found in nine isolates, and hence, the mechanism(s) of resistance remains unknown.

Ribosomal mutations were well distributed globally, being found in the following countries: Australia (*n* = 1 mutation), Belgium (*n* = 1), Canada (*n* = 17), Germany (*n* = 6), Hong Kong (*n* = 1), Hungary (*n* = 1), Japan (*n* = 7), Poland (*n* = 1), Switzerland (*n* = 1), Turkey (*n* = 1), and the United States (*n* = 49). Care should be taken in interpreting the prevalence of mutations by country, as the majority (47 of 49 [95.9%]) of strains from the United States were obtained from the PROTEKT US (2000 to 2001) surveillance study.

All 86 isolates were susceptible to telithromycin at the published NCCLS tentative susceptibility breakpoint of ≤ 1 μ g/ml (8). If the European Union susceptibility breakpoint of ≤ 0.5 μ g/ml is applied, three isolates would be classified as having intermediate susceptibility to telithromycin (MIC = 1 μ g/ml).

Six isolates were found to be resistant to quinupristin-dalfopristin; for five isolates the quinupristin-dalfopristin MIC was 4 μ g/ml and for one isolate the quinupristin-dalfopristin MIC was 8 μ g/ml. Resistance to quinupristin-dalfopristin in clinical isolates of *S. pneumoniae* is very uncommon, with only three reports thus far in the literature (3, 6, 7). Five separate muta-

* Corresponding author. Mailing address: GR Micro Ltd., 7-9 William Rd., London NW1 3ER, United Kingdom. Phone: 44 (0)20 73887320. Fax: 44 (0)20 73887324. E-mail: D.Farrell@grmicro.co.uk.

TABLE 1. Distributions of ribosomal mutations and telithromycin, linezolid, and quinupristin-dalfopristin MICs for 86 macrolide-resistant *S. pneumoniae*

Mutation (no. of <i>rrm</i> operons) ^a	No. of isolates	MIC or MIC range (μg/ml) ^b								
		TEL	ERY	CLA	ROX	AZI	CLI	KIT	QD	LIN
A2058G (2)	2	0.06–0.12	64–>64	16–>32	>32	>64	1–8	1–16	1	1–2
A2058G (3)	4	0.03–0.12	2–>64	2–>32	8–>32	2–>64	0.06–8	1–16	0.5–1	1–2
A2058G (4)	7	0.12–0.5	1–>64	1–>32	2–>32	0.5–>64	0.12–4	0.5–32	0.25–2	1–2
A2059C (4)	2	≤0.015–0.06	8–>64	4–>32	8–>32	4–>64	0.25–16	1	1	1–2
A2059G (1)	1	≤0.015	8	2	>32	>64	0.25	32	0.5	1
A2059G (2)	3	≤0.015	16–>64	4–32	>32	8–>64	0.5	0.5–>32	0.25–1	1–2
A2059G (3)	8	≤0.015–0.06	>64	32–>32	32–>32	>64	0.25–16	0.5–32	0.5–2	1–2
A2059G (4)	29	≤0.015–0.12	2–>64	0.5–>32	0.03–>32	4–>64	0.03–8	0.06–>32	0.5–2	0.25–2
C2611A (4)	1	0.06	>64	32	>32	>64	0.25	8	2	2
C2611G (3)	1	≤0.015	0.5	0.25	1	1	0.06	0.5	4	1
L22 109RTAHIT114 tandem duplication	2	1	2–32	1–8	2–32	4–>64	0.25–0.5	0.5–8	4–8	1–2
L22 G95D + A2059G (4)	3	0.06	64–>64	32–>32	>32	>64	1–2	16–32	0.25–4	1–2
A2059G (3) + G2057A (4)	1	0.25	>64	>32	>32	>64	8	8	2	4
A2059G + A2059C (1)	1	0.25	64	8	16	>64	1	16	1	2
A2059C (4) + L4 G71R	1	0.25	>64	>32	>32	>64	32	2	1	1
A2058U (3) + L4 I78V	2	0.5	>64	32–>32	>32	>64	0.25–0.5	32–>32	1	1
L4 K68S and 12-bp deletion, 69GTGR72	1	0.12	64	32	>32	>64	1	16	4	2
L4 3-bp insertion, from 68KG69 to 68KEG69	1	≤0.015	64	16	32	>64	0.25	8	2	2
L4 6-bp deletion, from 64PWRQ67 to 64P_Q67	1	0.03	2	2	4	2	0.25	1	0.5	4
L4, from 69GT70 to 69VP70	2	0.03–0.06	>64	16–>32	>32	>64	0.25	32–>32	1–4	1–2
L4, from 69GTG71 to 69TPS71, and V88I	1	0.12	>64	32	>32	>64	1	4	1	2
L4 E30K, from 69GTG71 to 69TPS71, and V88I	2	0.12	>64	32–>32	>32	>64	0.5–1	16–32	1	2
L4 K68Q	1	1	>64	16	32	>64	2	16	2	1
Wild type (no mutations detected)	9	≤0.015–0.5	>64	32–>32	>32	>64	1–8	1–>32	0.25–1	0.5–2
Total	86	≤0.015–1	0.5–>64	0.25–>32	0.03–>32	0.5–>64	0.03–32	0.06–>32	0.25–8	0.25–4

^a Mutations not described previously are in boldface.
^b TEL, telithromycin; ERY, erythromycin; CLA, clarithromycin; ROX, roxithromycin; AZI, azithromycin; CLI, clindamycin; KIT, rokitamycin; QD, quinupristin-dalfopristin; LIN, linezolid.

tions in the 23S rRNA, L4, and L22 genes were associated with this resistance, with three of these described previously (a 109RTAHIT114 tandem duplication in L22, 23S rRNA C2611G, and G95D combined with A2059G in L22) (3, 7). Two of 8,837 (0.02%) clinical isolates collected in the SENTRY study (2001 and 2002) were found to be resistant to quinupristin-dalfopristin, with the MICs for both isolates being 4 µg/ml and with both isolates having a similar L22 mutation, 109RTAHIT113 (6).

Two isolates were found to be resistant to linezolid (MICs = 4 µg/ml). This resistance was associated with two separate novel mutations (A2059G in three copies of the 23S rRNA gene in combination with G2057A in four copies of the 23S rRNA gene and a 6-bp deletion in the L4 riboprotein gene, ₆₄PWRQ₆₇ to ₆₄P__Q₆₇). This is the first report of linezolid resistance in clinical isolates of *S. pneumoniae*.

It was previously suggested (3) that resistance might be dependent upon the number of 23S rRNA genes mutated in a dose-response manner. The data from this larger population of isolates argues against this, as the MIC ranges overlap for isolates with A2059G mutations in one to four alleles (Table 1). Differential expression of mutated and nonmutated copies of the gene in response to environmental pressure, particularly antimicrobial exposure, is a possibility that requires investigation.

In summary, telithromycin retained good activity against all 86 isolates, demonstrating that so far this antibacterial is refractory to resistance associated with a wide variety of ribosomal mutations and ribosomal mutation combinations, including mutations associated with linezolid and quinupristin-dalfopristin resistance; however, causality needs to be confirmed by mutational analysis with isogenic strains. A mutational event was not found in 9 of the 86 isolates, and hence, the mechanism(s) of macrolide resistance remains to be elucidated.

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