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Emergence of a *Streptococcus pneumoniae* Clinical Isolate Highly Resistant to Telithromycin and Fluoroquinolones

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***Streptococcus pneumoniae* is a major pathogen causing community-acquired pneumonia and acute bronchitis. Macrolides, fluoroquinolones (FQs), and, recently, telithromycin (TEL) constitute primary therapeutic options, and rare cases of resistance have been reported. In this report, we describe the emergence of an *S. pneumoniae* clinical isolate with high-level TEL resistance (MIC, 256 μg/ml) and simultaneous resistance to FQs. Ongoing studies are oriented to elucidate the precise mechanism of resistance to TEL.**

Macrolides, fluoroquinolones (FQs), and, more recently, telithromycin (TEL) have appeared as attractive therapeutic options for the treatment of community-acquired pneumonia because of their activities against the most important respiratory pathogens and the infrequent resistance reported (3, 8, 12). *Streptococcus pneumoniae* is a major pathogen causing community-acquired pneumonia and acute exacerbations of chronic bronchitis. During the last decade, the rates of antimicrobial resistance among this species have been increasing worldwide, making the selection of adequate empirical antimicrobial therapy difficult (3, 4, 5, 9, 10). The aim of the present study is to alert the international scientific community about the emergence of an *S. pneumoniae* clinical isolate that is highly resistant to both FQs and TEL.

In July 2002, a 29-year-old woman was admitted to the Instituto de Cardiología y Cirugía Cardiovascular Fundación Favaloro with symptoms of an acute exacerbation of chronic bronchitis. The patient had a history of chronic obstructive lung disease and was treated with several courses of antibiotics, including levofloxacin. *S. pneumoniae* M4256 was isolated from a sputum sample taken at the time of admission, and the patient was empirically treated with azithromycin (500 mg/day). The isolate exhibited resistance to levofloxacin and azithromycin but susceptibility to TEL. After 3 days of azithromycin treatment, the therapy was switched to 10 days of TEL (800 mg/day) (1) with a good clinical response. A month later, the patient received the same TEL scheme as prophylaxis. In October 2002, the patient was readmitted with respiratory symptoms and deteriorating clinical signs. A second *S. pneumoniae*, designated M4243, was isolated from sputum but this time displayed no zone of inhibition to TEL by disk diffusion testing. The patient received ampicillin (8 g/day) plus vancomycin (2 g/day) for 21 days, showing a good clinical outcome.

Both *S. pneumoniae* clinical isolates were identified to spe-

TABLE 1. MICs and resistance determinants of *S. pneumoniae* clinical isolates M4256 and M4243

Parameter[a]	Result for isolate (sample date)[b]:	
	M4256 (July 2002)	M4243 (Oct. 2002)
MICs (μg/ml)		
Penicillin	2	2
Cefuroxime	4	4
Cefotaxime	2	2
Imipenem	0.12	0.12
TMS	8	8
Tetracycline	16	32
Chloramphenicol	16	16
Rifampicin	0.03	0.03
Linezolid	1	1
Vancomycin	0.5	1
Ciprofloxacin	64	64
Ofloxacin	64	64
Levofloxacin	64	64
Gatifloxacin	16	8
Moxifloxacin	16	8
Erythromycin	16	1,024
Clindamycin	0.06	8
Azithromycin	32	1,024
Telithromycin		
AD, air	0.12	256
AD, CO_2	0.25	1,024
BD, air	0.12	512
BD, CO_2	0.25	1,024
Fluoroquinolone resistance mutation(s) in:		
gyrA	S81F	S81F
gyrB	E474K	E474K
parC	S79F, K137N	S79F, K137N
parE	I460V	I460V
Macrolide resistance gene carriage		
mefA	+	+
ermB	−	−
ermA	−	−
ermTR	−	−
SmaI-PFGE type	A	A

[a] TMS, trimethoprim-sulphametoxazole; AD, agar dilution; BD, broth dilution.
[b] +, PCR positive; −, PCR negative.

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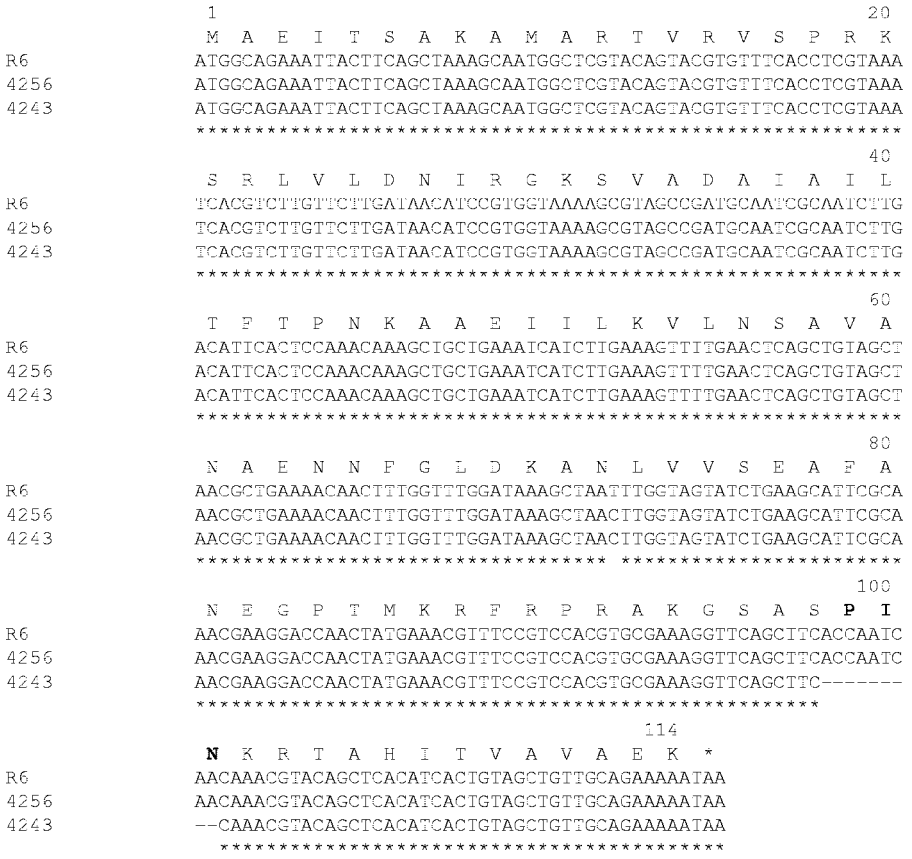

FIG. 1. Sequence alignment of L22 riboproteins of *S. pneumoniae* R6, *S. pneumoniae* M4256, and *S. pneumoniae* M4243. Numbers correspond to amino acid positions. Bold letters indicate the amino acid residues deleted in the *S. pneumoniae* M4243 strain.

cies level by their susceptibilities to optochin and solubilities to bile salts. They were serotyped as 19F by the Quellung reaction (Quellung antisera; Staten Seruminstitut, Copenhagen). MICs by the agar dilution method were determined using Mueller-Hinton agar (Difco, BD Microbiology Systems, Sparks, Md.) supplemented with 5% sheep blood, 24 h of incubation at 35°C, and a 5% CO_2 atmosphere. MICs were analyzed according to CLSI (formerly NCCLS) guidelines (17). The susceptibility profiles of M4256 and M4243 differed only in the MICs of TEL and macrolides (Table 1).

The prevalence of *S. pneumoniae* isolates that are resistant to FQs is still low worldwide (4), and the rate of resistance to levofloxacin in adults from Argentina was 2% in 2004, according to data from the National Surveillance Network, WHONET-Argentina (unpublished data). *S. pneumoniae* M4256 and M4243 showed high levels of resistance to FQs (Table 1). DNA sequence analysis of the quinolone resistance-determining regions of the *gyrA*, *gyrB*, *parC*, and *parE* genes was performed using conditions previously described (18). Both isolates showed identical mutations in the following four quinolone resistance-determining regions analyzed: Ser-81→ Phe, *gyrA*; Glu-474→ Lys, *gyrB*; Ser-79→ Phe and Lys-137→ Asn, *parC*; and Ile-460→ Val, *parE* (Table 1). The same mutations were described by Nagai et al. in an *S. pneumoniae* mutant strain using gatifloxacin as selector (16), but this is the first time that such amino acid changes have been described in a clinical isolate. MICs of FQs determined in the presence and absence of reserpine have been successfully used by Morosini et al. to recognize resistance conferred by an efflux mechanism (15). Therefore, we determined MICs of ciprofloxacin with and without 64 μg/ml of reserpine. No reduction in the ciprofloxacin MICs was observed for either isolate with the addition of reserpine, suggesting the absence of an efflux pump contributing to the FQ resistance.

S. pneumoniae M4256 displayed phenotype M, resistance to erythromycin and azithromycin and susceptibility to clindamycin and TEL (MICs of 16, 32, 0.06, and 0.12 μg/ml, respectively). The subsequent isolate (M4243) showed a constitutive macrolide-lincosamide-streptogramin B (MLS_B)-phenotype, with resistance to erythromycin, azithromycin, and clindamycin (MICs of 1,024, 1,024, and 8 μg/ml, respectively), but an alarmingly increased MIC of TEL (256 μg/ml). In order to detect *mefA*, *ermB*, *ermA*, or *ermTR* genes, PCR assays were performed (11, 21). Both isolates were positive for *mefA* and were repeatedly negative for *ermB*, *ermA*, and *ermTR* genes (Table 1). PCR-restriction fragment length polymorphism of the 348-bp *mefA* amplicon using BamHI was performed to discriminate between *mefA* and *mefE* alleles (14), and we found that both strains harbored the *mefE* allele (data not shown). Decreased susceptibility to TEL is associated with (i) mutations in the L4 and L22 riboproteins, domain II or V of the 23S rRNA, (ii) the presence of the *ermB* gene, or (iii) a combination of these mechanisms (19, 22, 24). In vitro selection of TEL-resistant *S. pneumoniae* carrying the *mefA* gene has been

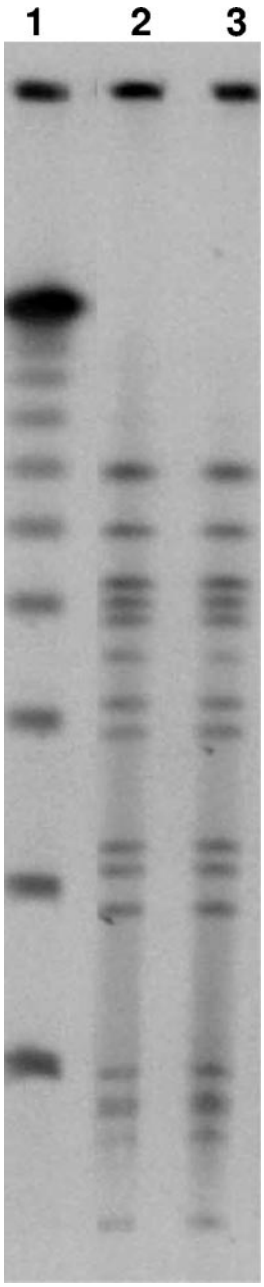

FIG. 2. SmaI restriction endonuclease patterns obtained by PFGE for both *S. pneumoniae* clinical isolates. Lane 1, lambda ladder; lane 2, *S. pneumoniae* M4256; lane 3, *S. pneumoniae* M4243.

described, but these strains displayed lower levels of resistance (MIC, 8 μg/ml) than those of M4243 (24). Sequence analysis of domains II and V of four individual *rrl* (23S rRNA) genes from M4243 strains revealed an A→T point mutation at position 2058 which was absent in parental strain M4256 (7). In addition, we identified a three amino acid deletion located at the C-teminal portion of the protein in the *rplV* (L22 riboprotein) gene of the *S. pneumoniae* M4243 strain by using standard conditions for PCR and L22-F (5′-CAT GGT AGG CCA CAA ACT TGG T-3′) and L22-R (5′-CAC GCA TAC CAA TTG GAT GT-3′) primers (Fig. 1). Interestingly, this deletion has not been previously described. Analysis results of *rplD* (L4 riboprotein), using L4-F (5′-CCT TAT CAA AGG TAA CGT ACC A-3′) and L4-R2 (5′-GAT CAA AAG TTT GTG TGC ACG-3′) primers, were identical between the *S. pneumoniae* M4243 and *S. pneumoniae* M4256 strains; however, they differed in a point mutation resulting in a Ser→Asn amino-acid change at position 20 (S20N) relative to the wild-type *S. pneumoniae* R6 strain. The implication of each ribosomal and riboproteins mutation, as well as the combination of different mechanisms of resistance, is under investigation.

Discrepancies in TEL susceptibility due to variations in the MIC methodology employed have been reported for *S. pneumoniae* (6). The atmosphere of incubation, air versus 5% CO_2 (the latter is associated with a "pH effect" due to CO_2 on the media), and the method used are the most significant variables (2, 6, 25). Therefore, MICs of TEL were determined both by agar dilution, as described above, and by broth dilution (Mueller-Hinton broth plus 5% lysed horse blood and 24 h of incubation at 35°C), in both the presence and the absence of 5% CO_2 (Table 1). For *S. pneumoniae* M4256, the MICs of TEL determined in ambient air were 0.12 μg/ml both by agar and by broth dilution, and this value increased to 0.25 μg/ml when the strain was incubated with 5% CO_2 (Table 1). For *S. pneumoniae* M4243, the MICs in air were 256 and 512 μg/ml by agar and broth dilution, respectively, and increased to 1,024 μg/ml when the strain was incubated in CO_2 (Table 1). In summary, there were no differences between TEL MICs determined by agar or broth dilution methods, but differences of 1- or 2-$\log_2$ dilutions were detected when the strains were incubated in a CO_2-enriched atmosphere.

Pulsed-field gel electrophoresis (PFGE) was performed using Sma-I enzyme and previously described conditions (5). Both isolates showed the same PFGE profile (Fig. 2), which was not related to any of the international clones already described (13). We conclude that the high level of resistance to TEL displayed in *S. pneumoniae* M4243 could probably be due to the selection of an intratreatment mutant.

TEL had demonstrated good clinical efficacy in patients with mild to moderate community-acquired pneumonia (9, 23). Moreover, Farrel and Felmingham analyzed 13,874 *S. pneumoniae* clinical isolates and reported a low rate of TEL resistance (0.2%), with 8 μg/ml being the highest MIC found in that survey (8). To the best of our knowledge, there has been only one previous report of clinical selection of TEL resistance (16 μg/ml) in *S. pneumoniae* (19). In laboratory-generated mutants of *S. pneumoniae* carrying the *ermB* gene, observed TEL MICs were 64 μg/ml (24). Current information thus suggests that the selection of isolates showing resistance to TEL is a possible but infrequent phenomenon. However, there are insufficient data to date to evaluate the role of prior therapy with azithromycin in the selection of the M4243 TEL-resistant isolate.

TEL has been available for clinical use for the past few years, and resistance to this drug is extremely rare at present (20). However, the emergence of the clinical isolate of *S. pneumoniae* with very high-level TEL resistance (MIC 256 μg/ml) described in this report and simultaneous resistance to FQs constitutes a public health concern that requires worldwide attention. Continuous antimicrobial resistance surveillance of this pathogen and conscientious use of macrolides, FQs, and

ketolides are urgently needed to preserve the scarce therapeutic alternatives.

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REFERENCES

1. **Balfour, J. A., and D. P. Figgitt.**2001. Telithromycin. Drugs **61:**815–830.
2. **Batard, E., M. E. Juvin, C. Jacqueline, D. Bugnon, J. Caillon, G. Potel, and H. B. Drugeon.** 2005. Influence of carbon dioxide on the MIC of telithromycin for *Streptococcus pneumoniae*: an in vitro-in vivo study. Antimicrob. Agents Chemother. **49:**464–466.
3. **Beekmann, S. E., K. P. Heilmann, S. S. Richter, J. García-de-Lomas, G. V. Doern, and the GRASP Study Group.** 2005. Antimicrobial resistance in *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* and group A β-haemolytic streptococci in 2002–2003 results of the multinational GRASP surveillance program. Int. J. Antimicrob. Agents **25:**148–156.
4. **Canton, R., M. Morosini, M. C. Enright, and I. Morrissey.** 2003. Worldwide incidence, molecular epidemiology and mutations implicated in fluoroquinolone-resistant *Streptococcus pneumoniae*: data from the global PROTEKT surveillance programme. J. Antimicrob. Chemother. **52:**944–952.
5. **Corso, A., E. P. Severina, V. F. Petruk, Y. R. Mauriz, and A. Tomasz.** 1998. Molecular characterization of penicillin-resistant *Streptococcus pneumoniae* isolates causing respiratory disease in the United States. Microb. Drug Resist. **4:**325–337.
6. **Davies, T. A., L. M. Kelly, M. R. Jacobs, and P. C. Appelbaum.** 2000. Antipneumococcal activity of telithromycin by agar dilution, microdilution, E test, and disk diffusion methodologies. J. Clin. Microbiol. **38:**1444–1448.
7. **Farrel, D. J., S. Douthwaite, I. Morrissey, S. Bakker, J. Poehlsgaard, L. Jakobsen, and D. Felmingham.** 2003. Macrolide resistance by ribosomal mutation in clinical isolates of *Streptococcus pneumoniae* from the PROTEKT 1999–2000 study. Antimicrob. Agents Chemother **47:**1777–1783.
8. **Farrel, D. J., and D. Felmingham.** 2004. Activities of TEL against 13,874 *Streptococcus pneumoniae* isolates collected between 1999 and 2003. Antimicrob. Agents Chemother. **48:**1882–1884.
9. **Felmingham, D., R. R. Reinert, Y. Hirakata, and A. Rodloff.** 2002. Increasing prevalence of antimicrobial resistance among isolates of *Streptococcus pneumoniae* from the PROTEKT surveillance study, and comparative *in vitro* activity of the ketolide, telithromycin. J. Antimicrob. Chemother. **50**(Suppl. 1)**:**25–37.
10. **Hortal, M., M. Lovgren, F. de la Hoz, C. I. Agudelo, M. C. Brandileone, T. Camou, S. Casagrande, E. Catañeda, A. Corso, G. Echaniz, J. C. Hormazabal, J. Pace, R. Palacio, G. Perez-Giffoni, R. Ruvinsky, J. L. Di Fabio, and the PAHO SIREVA-Vigia Study Group.** 2001. Antibiotic resistance in *Streptococcus pneumoniae* in six Latin American countries: 1993–1999 surveillance. Microb. Drug Resist. **7:**391–401.
11. **Kataja, J., H. Seppala, M. Skurnik, H. Sarkkinen, and P. Houvinen.** 1998. Different erythromycin resistance mechanisms in group C and group G streptococci. Antimicrob. Agents Chemother. **42:**1493–1494.
12. **Low, D. E., D. Felmingham, S. D. Brown, M. Rangaraju, and R. Nusrat.** 2004. Activity of telithromycin against key pathogens associated with community-acquired respiratory tract infections. J. Infection **49:**115–125.
13. **McGee, L., L. McDougal, J. Zhou, B. G. Spratt, F. C. Tenover, R. George, R. Hakenbeck, W. Hryniewicz, J. C. Lefévre, A. Tomasz, and K. P. Klugman.** 2001. Nomenclature of major antimicrobial-resistant clones of *Streptococcus pneumoniae* defined by the Pneumococcal Molecular Epidemiology Network. J. Clin. Microbiol. **39:**2565–2571.
14. **Montanari, M. P., M. Mingoia, I. Cochetti, and P. E. Varaldo.** 2003. Phenotypes and genotypes of erythromycin-resistant pneumococci in Italy. J. Clin. Microbiol. **41:**428–431.
15. **Morosini, M. I., E. Loza, R. del Campo, F. Almaraz, F. Baquero, and R. Cantón.** 2003. Fluoroquinolone-resistant *Streptococcus pneumoniae* in Spain: activities of garenoxacin against clinical isolates including strains with altered topoisomerases. Antimicrob. Agents Chemother. **47:**2692–2695.
16. **Nagai, K., T. A. Davies, B. E. Dewasse, M. R. Jacobs, and P. C. Appelbaum.** 2001. Single- and multi-step resistance selection study of gemifloxacin compared with trovafloxacin, ciprofloxacin, gatifloxacin and moxifloxacin in *Streptococcus pneumoniae*. J. Antimicrob. Chemother. **48:**365–374.
17. **National Committee for Clinical Laboratory Standards.** 2004. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 6th ed. Approved standard M7-A6. National Committee for Clinical Laboratory Standards. Wayne, Pa.
18. **Pan, X., J. Ambler, S. Mehtar, and L. M. Fisher.** 1996. Involvement of topoisomerase IV and DNA gyrase as ciprofloxacin targets in *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **40:**2321–2326.
19. **Pérez-Trallero, E., J. M. Marimon, L. Iglesias, and J. Larruskain.** 2003. Fluoroquinolone and macrolide treatment failure in pneumococcal pneumonia and selection of multidrug-resistant isolates. Emerg. Infect. Dis. **9:**1159–1162.
20. **Reinert, R. R.** 2004. Clinical efficacy of ketolides in the treatment of respiratory tract infections. J. Antimicrob. Chemother. **53:**918–927.
21. **Sutclife, J., T. Grebe, A. Tait-Kamradt, and L. Wondrack.** 1996. Detection of erythromycin-resistant determinants by PCR. Antimicrob. Agents Chemother. **40:**2562–2566.
22. **Tait-Kamradt, A., T. Davies, P. C. Appelbaum, F. Depardieu, P. Courvalin, J. Petitpas, L. Wondrack, A. Walker, M. R. Jacobs, and J. Sutcliffe.** 2000. Two new mechanisms of macrolide resistance in clinical strains of *Streptococcus pneumoniae* from Eastern Europe and North America. Antimicrob. Agents Chemother. **44:**3395–3401.
23. **Tellier, G., M. S. Niederman, R. Nusrat, M. Patel, and B. Lavin.** 2004. Clinical and bacteriological efficacy and safety of 5 and 7 day regimens of telithromycin once daily compared with a 10 day regimen of clarithromycin twice daily in patients with mild to moderate community-acquired pneumonia. J. Antimicrob. Chemother. **54:**515–523.
24. **Walsh, F., J. Willcock, and S. Amyes.** 2003. High-level telithromycin resistance in laboratory-generated mutants of *Streptococcus pneumoniae*. J. Antimicrob. Chemother. **52:**345–353.
25. **Walsh, F., F. Carnegy, J. Willcock, and S. Amyes.** 2004. Comparative in vitro activity of telithromycin against macrolide-resistant and -susceptible *Streptococcus pneumoniae*, Moraxella catarrhalis and *Haemophilus influenzae*. J. Antimicrob. Chemother. **53:**793–796.