

Comparative *in vitro* activity of telithromycin against macrolide-resistant and -susceptible *Streptococcus pneumoniae*, *Moraxella catarrhalis* and *Haemophilus influenzae*

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Objectives: The first objective was to investigate the *in vitro* activity of telithromycin against respiratory tract pathogens in comparison with other antimicrobial agents. The second objective was to identify the influence of the *erm(B)* and *mef(A)* genes on the susceptibility of *Streptococcus pneumoniae* to telithromycin.

Methods: The *in vitro* activity of telithromycin against *S. pneumoniae*, *Moraxella catarrhalis* and *Haemophilus influenzae*, isolated from the UK and 40 macrolide-resistant *S. pneumoniae* from four different countries was compared with a variety of antimicrobial agents. The 140 isolates were examined for the presence of the *erm(B)* and *mef(A)* genes. The impact of 5% CO₂ on susceptibility testing was also investigated.

Results: Telithromycin showed greatest activity against *S. pneumoniae*, but also had good activity against *M. catarrhalis* and *H. influenzae*, which was independent of their resistance profiles to other antibiotics. The MIC₉₀ of telithromycin for *S. pneumoniae* was 0.12 mg/L, which was 64-fold lower than the lowest macrolide MIC; 21% of the *S. pneumoniae* were macrolide resistant. Thirty-eight per cent of the macrolide-resistant strains were *erm(B)*-positive and 62% were *mef(A)*-positive, but no strain contained both genes. The activity of telithromycin was similar to that of azithromycin against both *M. catarrhalis* and *H. influenzae*, Erythromycin was slightly less active: 1% and 8% of *M. catarrhalis* and *H. influenzae*, respectively, were resistant to erythromycin, but none were resistant to telithromycin. Five per cent of the *S. pneumoniae* strains and 4% of the *H. influenzae* strains changed from telithromycin susceptible to non-susceptible entirely because of the incubation conditions. The MIC₅₀s and MIC₉₀s of *S. pneumoniae*, *M. catarrhalis* and *H. influenzae* increased by one dilution when incubated in CO₂.

Conclusions: Telithromycin has shown high *in vitro* activity against *S. pneumoniae*, including those strains that are macrolide susceptible and resistant as well as *M. catarrhalis* and *H. influenzae*. This study has also demonstrated that there is no cross-resistance between erythromycin and telithromycin. The impact of 5% CO₂ on susceptibility testing should be investigated further before providing definite guidelines on telithromycin susceptibility testing.

Keywords: resistance, *erm(B)*, ketolides

Introduction

Resistance to antimicrobial agents is a particular problem in community-acquired respiratory tract infections (CARTIs). In some countries macrolide resistance now exceeds penicillin resistance in *Streptococcus pneumoniae*.¹ Between 10% and 30% of *Haemophilus influenzae* and 85–100% of *Moraxella catarrhalis* worldwide are β-lactamase producers. In the UK, erythromycin A resistance in *S. pneumoniae* was higher than penicillin resistance by 7.7%.¹ Telithromycin is a ketolide, which represents a new generation of antimicrobial agents capable of overcoming these resistance prob-

lems. Telithromycin has activity against all three respiratory tract pathogens regardless of their resistance profiles.^{2,3}

The purpose of this study was to evaluate the *in vitro* activity of telithromycin in comparison with a variety of antimicrobial agents against macrolide-susceptible and -resistant *S. pneumoniae*, *M. catarrhalis* and *H. influenzae* isolated over the same time period as the PROTEKT study, 1999–2001, from the UK. As the number of macrolide-resistant *S. pneumoniae* identified from the Edinburgh and Leeds collections was low, a cohort of macrolide-resistant isolates from Europe and America were also tested against telithromycin to investigate the activity of telithromycin against resistant

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Table 1. Antimicrobial activities against *S. pneumoniae*, *M. catarrhalis* and *H. influenzae*

Antimicrobial agent	MIC range [MIC ₅₀ /MIC ₉₀] (mg/L)			
	Macrolide-resistant <i>S. pneumoniae</i> (40)	<i>Streptococcus pneumoniae</i> (100)	<i>Moraxella catarrhalis</i> (100)	<i>Haemophilus influenzae</i> (100)
Telithromycin CO ₂	0.016–1 [0.06/0.25]	0.016–2 [0.12/0.25]	0.032–1 [0.12/0.25]	0.008–8 [2/4]
Telithromycin air		0.008–1 [0.032/0.12]	0.032–0.5 [0.06/0.12]	0.032–4 [1/2]
Erythromycin		0.008–128 [0.06/8]	0.06–1 [0.25/0.25]	0.12–16 [4/8]
Azithromycin		0.008–16 [0.12/16]	0.032–0.25 [0.06/0.12]	0.06–8 [1/2]
Clindamycin		0.008–32 [0.06/0.12]	1–8 [2/4]	0.032–32 [4/16]
Moxifloxacin		0.032–0.5 [0.12/0.25]	0.016–0.12 [0.06/0.06]	0.004–0.12 [0.016/0.016]
Gemifloxacin		0.008–0.12 [0.032/0.06]	0.002–0.016 [0.008/0.016]	0.002–0.12 [0.002/0.004]
Linezolid		0.12–4 [1/2]	2–8 [4/4]	0.5–64 [8/16]

isolates. This ensured that a diverse group of macrolide-resistant *S. pneumoniae* isolates were included in the study.

Materials and methods

Bacterial strains

One hundred strains each of *S. pneumoniae*, *M. catarrhalis* and *H. influenzae* from the UK were investigated. The *S. pneumoniae* were isolated from Leeds and Edinburgh, the *M. catarrhalis* from Edinburgh, Leeds and Wales and the *H. influenzae* from Edinburgh and Glasgow between 1999 and 2001. Forty macrolide-resistant *S. pneumoniae* isolates were donated from the USA, Canada, Belgium and Italy. These isolates were donated by Dr Zhong (Abbott Laboratories, USA), Dr de Azavedo (University of Toronto, Canada), Dr Lagrou (Rega Institute for Medical Research, Belgium) and Dr Marchese (University of Genova, Italy), respectively. The control strains consisted of *S. pneumoniae* NCTC 13593, *Staphylococcus aureus* NCTC 6571, *H. influenzae* NCTC 11931 and a laboratory reference strain of *M. catarrhalis*.

Antimicrobial agents

The antimicrobial agents were stored and prepared according to the manufacturers' guidelines. Telithromycin (Aventis Pharma Ltd), erythromycin (David Bull Laboratories, Warwick, UK), azithromycin (Pfizer Ltd), clindamycin (Sigma), moxifloxacin (Bayer), gemifloxacin (Smith-Kline Beecham Pharmaceuticals) and linezolid (Pharmacia and Upjohn) were tested *in vitro*.

Minimum inhibitory concentrations

The MICs were determined on Columbia agar base supplemented with 5% defibrinated horse blood for *S. pneumoniae* and *M. catarrhalis* and on chocolate Columbia agar plates for *H. influenzae* according to the BSAC guidelines for susceptibility testing.⁴ The MICs were determined by the standard agar doubling dilution method. The MIC plates containing telithromycin were incubated in both air and 5% CO₂, at 37°C. All other plates were incubated in air at 37°C. The MIC tests were repeated at least once for each strain.

The antimicrobial breakpoints used were those from the BSAC guidelines except for telithromycin for which the NCCLS tentative breakpoints were used.^{5,6} Resistance to the antimicrobial agents was assigned at the following MIC values: telithromycin ≥ 4 mg/L for *S. pneumoniae* and ≥ 16 mg/L for *H. influenzae*, erythromycin ≥ 1 mg/L, azithromycin ≥ 2 mg/L and clindamycin ≥ 1 mg/L.

Characterization of macrolide resistance mechanism

S. pneumoniae strains with an erythromycin MIC ≥ 1 mg/L or an azithromycin MIC ≥ 2 mg/L isolated from the UK and the 40 donated isolates were screened for the presence of the *erm(B)* and *mef(A)* genes. *S. pneumoniae* strains were emulsified in 200 μ L of MilliQ water and boiled for 10 min in order to extract the total DNA. The supernatant was used as the DNA template in PCR experiments. The PCR conditions and primers for the detection of *erm(B)* and *mef(A)* genes are those described previously by Sutcliffe *et al.*⁷ and Tait-Kamradt *et al.*⁸ The strains were tested for both genes at the same time. The *erm(B)* and *mef(A)* primers were added to the same reaction mixture with a MgCl₂ concentration of 4 mM.

Results

Susceptibility profiles

Telithromycin had the second lowest MIC₉₀ of all the antimicrobial agents tested against *S. pneumoniae* as shown in Table 1. The telithromycin MIC₉₀ was 64-fold lower than the lowest macrolide MIC₉₀. While the MIC₉₀ of clindamycin was the same as that of telithromycin, the upper limit of the range was substantially higher. Telithromycin activity against the 40 macrolide-resistant *S. pneumoniae* was the same as that against the 100 UK isolates with varying macrolide susceptibilities. The range endpoint (1 mg/L) was lower than that for the UK isolates (2 mg/L) and the MIC₉₀s (0.25 mg/L) were identical. Thus telithromycin activity was not affected by the macrolide resistance mechanisms of these strains. Gemifloxacin and moxifloxacin both had high activity against *S. pneumoniae* with their highest MICs at 0.12 and 0.5 mg/L, respectively. Linezolid did not perform as well as the other antimicrobial agents. The fluoroquinolones had the greatest activity of the antimicrobial agents tested against *M. catarrhalis*. Their MIC₉₀s ranged from 0.016 to 0.06 mg/L. The MIC₉₀s of the macrolides were 0.12 and 0.25 mg/L. There was little difference between the performance of telithromycin and the macrolides. Clindamycin and linezolid both had low activity against *M. catarrhalis in vitro* with MIC₉₀s of 4 mg/L. Telithromycin had relatively good activity against *H. influenzae* with an MIC₉₀ of 2 mg/L. It had lower MIC₅₀ and MIC₉₀ values than erythromycin by two dilutions. For *H. influenzae*, clindamycin and linezolid had the same MIC₉₀s of 16 mg/L. Once again the fluoroquinolones performed with the highest activity and low MIC₉₀s of 0.004–0.016 mg/L.

Telithromycin susceptibility

Table 2. Comparison of telithromycin susceptibility and erythromycin and clindamycin resistance profiles of *S. pneumoniae*

Telithromycin MIC (n)	0.008 (10)	0.016 (16)	0.032 (35)	0.06 (22)	0.12 (12)	0.25 (3)	0.5 (1)	1 (1)
Erythromycin resistant % (n)	10 (1)	0 (0)	14 (5)	9 (2)	67 (8)	100 (3)	100 (1)	100 (1)
Clindamycin resistant % (n)	0 (0)	0 (0)	6 (2)	4.5 (1)	25 (3)	0 (0)	0 (0)	0 (0)
Erythromycin and clindamycin resistant % (n)	0 (0)	0 (0)	6 (2)	4.5 (1)	25 (3)	0 (0)	0 (0)	0 (0)
Erythromycin and clindamycin susceptible % (n)	90 (9)	100 (16)	86 (30)	91 (20)	33 (4)	0 (0)	0 (0)	0 (0)

n, number of isolates.

Correlation between telithromycin and erythromycin

The correlation between telithromycin MIC and that of erythromycin and clindamycin for *S. pneumoniae* are detailed in Table 2. These results indicated that the five isolates with the highest telithromycin MICs were erythromycin resistant but clindamycin susceptible. Eight of the 12 isolates with a telithromycin MIC of 0.12 mg/L were erythromycin resistant, and three of these were also clindamycin resistant. The *S. pneumoniae* isolates resistant to clindamycin had telithromycin MICs of 0.032–0.12 mg/L and were also erythromycin resistant.

Effect of CO₂ incubation

When the MIC plates containing telithromycin were incubated in 5% CO₂ (Table 1) the range and MIC₉₀ for *S. pneumoniae* increased by one dilution and the MIC₅₀ increased by two dilutions. The *S. pneumoniae* range endpoint increased from 1 to 2 mg/L when incubated in CO₂. With a telithromycin MIC of 2 mg/L a *S. pneumoniae* strain is categorized as intermediate using the BSAC guidelines and resistant using the French guidelines. Five per cent of *S. pneumoniae* were telithromycin resistant and 2% or 5% had intermediate resistance using the French guidelines and BSAC guidelines, respectively, when measured in CO₂. With regard to *M. catarrhalis* and *H. influenzae* the MIC₅₀ and MIC₉₀ both increased by one dilution when incubated in CO₂. The approved telithromycin breakpoint for *H. influenzae* is 4 mg/L for susceptible, 8 mg/L for intermediate and ≥16 mg/L for resistant. Strains with an MIC of 4 mg/L in air were categorized as susceptible but with CO₂ incubation showed an MIC of 8 mg/L and were categorized as intermediate.

Resistance levels

Telithromycin had the lowest resistance levels, considerably lower than either the macrolides or the lincosamide. There was no resistance to telithromycin in either *S. pneumoniae*, *M. catarrhalis* or *H. influenzae* using the NCCLS breakpoints (Table 3). Using the guidelines suggested by Soussy *et al.*,⁹ i.e. telithromycin susceptibility <0.5 mg/L and resistance ≥2 mg/L, telithromycin intermediate resistance of 1 mg/L for Gram-positive bacteria, the resistance levels for *S. pneumoniae* increased slightly. One per cent of *S. pneumoniae* isolates had telithromycin intermediate resistance. In comparison, 21% of the *S. pneumoniae* population was erythromycin resistant and 6% were resistant to clindamycin. In *M. catarrhalis* the entire population were found to be clindamycin resistant while one strain was also erythromycin resistant. No resistance to telithromycin

Table 3. Resistance profiles of *S. pneumoniae*, *M. catarrhalis* and *H. influenzae*

	Percentage resistance		
	<i>S. pneumoniae</i>	<i>M. catarrhalis</i>	<i>H. influenzae</i>
Telithromycin	0	0	0
Erythromycin	21	1	8
Azithromycin	14	0	1
Clindamycin	6	100	97

or azithromycin was observed. Erythromycin resistance was at 8% for *H. influenzae*, whereas azithromycin resistance was at 1%.

Mechanisms of macrolide resistance

The 21 macrolide-resistant *S. pneumoniae* strains consisted of eight *erm*(B)-positive and 13 *mef*(A)-positive strains. There were no strains containing both genes. All the strains except two Edinburgh isolates had matching phenotype and genotype, i.e. *erm*(B)-positive strains were macrolide and clindamycin resistant and *mef*(A)-positive strains were resistant only to the macrolides. The Edinburgh *erm*(B)-positive strains had clindamycin MICs of 0.12 and 0.25 mg/L. The 40 donated strains comprised 29 *erm*(B)-positive and 11 *mef*(A)-positive strains. No strains contained both genes. Within this group four strains were also *erm*(B)-positive but clindamycin susceptible with MICs of 0.12 or 0.25 mg/L.

Discussion

Susceptibility testing of respiratory tract pathogens is often performed in a CO₂ environment to ensure that the bacteria grow to the required concentration. In a CO₂ environment the pH of the test medium decreases. Macrolide activity is adversely affected by this pH decrease. In order to investigate whether this was also true for the ketolide telithromycin, susceptibility testing was also carried out in 5% CO₂. This study has shown that the MIC of telithromycin increased when incubated in 5% CO₂ in comparison with air incubation. Five per cent of the *S. pneumoniae* strains and 4% of the *H. influenzae* strains changed from telithromycin susceptible to non-susceptible entirely because of the incubation conditions. The MIC₅₀s and MIC₉₀s of *S. pneumoniae*, *M. catarrhalis* and *H. influenzae* increased by one dilution when incubated in CO₂. These results indicated that the pH of the medium also caused an increase in

telithromycin MIC. An increase in the telithromycin mode MIC and MIC range was also detected by Bemer-Melchoir *et al.*¹⁰ when the susceptibility of *Streptococcus pyogenes* was tested in 5–6% CO₂. However, unlike the results described by Bemer-Melchoir *et al.*, which suggested that the MIC of telithromycin in CO₂ increased only for erythromycin-resistant isolates, no such correlation was identified with *S. pneumoniae*, *M. catarrhalis* or *H. influenzae*. In this and previous studies macrolide resistance has been associated mainly with *S. pneumoniae* rather than *M. catarrhalis* or *H. influenzae*.¹

The French guidelines recommend testing telithromycin activity against *S. pneumoniae* in air as well as CO₂ in order to confirm resistance for clinical characterization. However, no such recommendations were presented for *H. influenzae*. The results of this study suggest that telithromycin susceptibility should be tested in air for all three organisms. Therefore, the French committee need to investigate the effect of incubation in CO₂ for *H. influenzae* also. The latest BSAC guidelines recommend that *S. pneumoniae* and *H. influenzae* are incubated in CO₂ and *M. catarrhalis* is incubated in air for susceptibility testing. Under these conditions the tentative susceptibility to telithromycin point for the three organisms is <0.5 mg/L. As incubation in CO₂ has affected each of the three organisms tested in this study it would be appropriate for the BSAC to investigate this further with these and other organisms and larger populations before providing definite guidelines on telithromycin susceptibility testing and breakpoints.¹¹

In the Alexander Project, erythromycin resistance in *S. pneumoniae* from the UK was at 13.6% in 1996 and 7.2% in 1997.¹² However, this study has shown that the resistance level in Edinburgh and Leeds for the same period was 21% in *S. pneumoniae*. The percentage of *H. influenzae* resistant to erythromycin is 8%; however, none are telithromycin resistant and almost all are azithromycin susceptible.

The results of the correlation between telithromycin, erythromycin and clindamycin identified a pattern similar to that described previously by Johnson *et al.*¹³ All the erythromycin- and clindamycin-resistant isolates were susceptible to telithromycin. The telithromycin MICs for the isolates that were erythromycin resistant alone or in combination with clindamycin resistance were higher than those for the susceptible isolates. The *S. pneumoniae* with an erythromycin-resistant and clindamycin-susceptible phenotype were less susceptible to telithromycin than those resistant to both. The isolates with the highest telithromycin MICs were erythromycin resistant and clindamycin susceptible. Therefore, in agreement with Johnson *et al.*¹³ telithromycin was less affected by the *erm*(B)-positive than by the *mef*(A)-positive isolates. Thus, while telithromycin can overcome both resistance mechanisms as all isolates were telithromycin susceptible, the macrolide efflux system and to a lesser extent the target methylation do reduce telithromycin activity.

Telithromycin has previously been shown to have activity against macrolide-resistant *S. pneumoniae*. This finding has been borne out in these results as it was tested against 61 macrolide-resistant *S. pneumoniae* isolates. Telithromycin had consistently lower MICs than erythromycin for all the bacteria tested. No resistance to telithromycin was found in *S. pneumoniae*, *M. catarrhalis* or *H. influenzae*. The 21 macrolide-resistant *S. pneumoniae* isolates from the UK comprised *erm*(B)- and *mef*(A)-positive isolates in a ratio of 1:2. Usually in Europe the *erm*(B) gene predominates and the converse is found in the USA. This study contradicts this finding in so far as there was a greater number of *mef*(A)-positive UK isolates than *erm*(B)-positive isolates. Isolates that were erythromycin and clindamycin resistant have been attributed a constitutive macrolide resistance phenotype

and those that were erythromycin resistant and clindamycin susceptible an inducible macrolide resistance phenotype.¹⁴ Therefore, the results of this study indicate that the constitutive resistance phenotype predominates in the isolates investigated in this study. Thus telithromycin has activity against inducible macrolide-resistant *S. pneumoniae* and also against the inducible macrolide-resistant *S. pneumoniae* investigated in this study. The high overall activity of telithromycin against the three respiratory pathogens and almost no resistance indicates that telithromycin has good potential for the treatment of RTIs, including those caused by macrolide-resistant strains.

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