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Characterization of a High-level Telithromycin-resistant Clinical Isolate of *Streptococcus pneumoniae* Collected from the PROTEKT US Surveillance Study Years 1-6

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Knight, D.R[1]., J. Blackman Northwood[1], I. Morrissey[1], S. Brown[2], C. Couturier[3]

[1]Quotient Bioresearch Ltd, Microbiology, Cambridgeshire, UK. [2]Clinical Microbiology Institute, Portland, USA. [3]Sanofi aventis, Paris, France

Abstract (Modified)

Background: PROTEKT US investigated the susceptibility of key respiratory isolates collected from the USA between 2000 and 2006. Of the 55729 *Streptococcus pneumoniae* (SP) from PROTEKT US years 1-6 only 22 (0.04%) were telithromycin-resistant (TelR). Of these isolates all except one (PU6080005) had a Tel MIC of 4 or 8 mg/L. PU6080005 had a Tel MIC of ≥256 mg/L and in addition to macrolide MIC values of ≥256 mg/L the isolate was also resistant to tetracycline but susceptible to beta-lactams and fluoroquinolones. **Methods:** The presence of *erm*B was determined by PCR. Segments of the L4 & L22 riboprotein genes, the *erm*B gene and the 4 copies of the 23S rRNA gene were sequenced using previously published methods. **Results:** All TelR SP contained *ermB* with a stop codon at position 28 of their upstream control peptides. In addition, isolate PU6080005 possessed a novel U754A point mutation (*E. coli* numbering) at all four alleles of domain II in 23S rRNA. Riboprotein sequencing showed wildtype L4 and L22 alleles. **Conclusion:** We present a clinical strain of *Streptococcus pneumoniae* with a telithromycin MIC of ≥256 mg/L possessing a combination of *erm*(B) and 23S domain II mutations. The presence of ermB and the truncation of its upstream control peptide confer low level resistance to telithromycin. Riboprotein L4 and L22 were of wildtype in consequence we attribute the very high Tel MIC to a U754A point mutation in domain II of the 23S rRNA at all four alleles never before reported in SP. Indeed it has been demonstrated in *E. coli*, that the proximity of hairpin 35 (residues 738-759) within domain II of 23S rRNA is close to the peptidyl transferase centre and thus a constituent part of ribosomal function and macrolide and ketolide binding affinity. The mutation U754A identified in *E. coli* conferred low level TelR in addition to erythromycin resistance. We propose that the U754A mutation at all four alleles can cause high level TelR in SP, although this is extremely rare (1 in 55729).

Background

Telithromycin is a semi-synthetic derivative of the macrolide erythromycin A. It was the first of a new generation of ketolide antibiotics approved for clinical use. Ketolides have been designed to overcome macrolide resistance (1). They inhibit bacterial protein synthesis by interacting with the peptidyl transferase site of the 50S subunit of the bacterial ribosome (1). DNA footprinting has shown that telithromycin interacts closely with adenine residues in domain II at 752 and V at 2058 and 2059 of the 23S rRNA (2).

A loss of the domain V binding site caused by methylation by *erm*(B) is a key mechanism in macrolide resistance. Ketolides such as telithromycin bind with a stronger affinity than macrolides and in isolates with *erm*(B) ketolide susceptibility is retained (1). Resistance in laboratory generated strains has been shown to occur due to mutations in the *erm*(B) upstream control peptide and reduced susceptibility to telithromycin has been shown to occur due to mutations in the *erm*(B) gene itself, ribosomal proteins L4/L22 and domain II of 23S rRNA (3).

High level pneumococcal resistance to telithromycin in clinical isolates is extremely rare, and prior to this publication only one isolate has been recorded (3,4). This isolate had a telithromycin MIC of >256 mg/L and possessed a combination of L4, *erm*(B) and *erm*(B) upstream control peptide mutations.

PROTEKT (Prospective Resistant Organism Tracking and Epidemiology for the Ketolide Telithromycin) US was a longitudinal, multicentre surveillance study of antimicrobial resistance among respiratory tract pathogens from the United States. Its role was to investigate the susceptibility of key respiratory isolates collected between 2000 and 2006.

Of the 55729 *Streptococcus pneumoniae* from PROTEKT US years 1-6 only 22 (0.04%) were telithromycin-resistant. All but one of these isolates (PU608005) had a telithromycin MIC of 4 or 8 mg/L. PU6080005 had a telithromycin MIC of >256 mg/L and in addition to macrolide MIC values of ≥256 mg/L. The isolate was also resistant to tetracycline but susceptible to beta-lactams and fluoroquinolones.

In this study we investigated the underlying mechanisms behind this rare high level resistance.

Methods

Antibacterial MICs were determined using the broth microdilution method recommended by the Clinical Laboratory Standards Institute (CLSI) (5). CLSI breakpoints were used to determine telithromycin susceptibility, ≤1 mg/L for susceptible, 2 mg/L for intermediate, and ≥ 4 mg/L for resistance (6).

Methods cont.

The presence of *erm*(B) and *mef*(A) was determined by PCR using a previously published method (7) which utilizes the ABI Prism 7000 Sequence detection System (Applied Biosystems, Warrington, UK), in a 96 well format.

Segments of the L4 & L22 riboprotein genes, all four alleles encoding the 23S rRNA gene, the *erm*B gene and its upstream control region were amplified and sequenced using previously published methods (8,9). All sequencing was performed on an ABI PRISM 3100 Genetic Analyser (Applied Biosystems, Warrington, UK). Sequence analysis was performed using the DNASTAR analysis program (DNASTAR, Madison, WI, USA).

Results

***erm*(B) and *mef*(A)**

PU6080005 was confirmed to be *erm*(B) positive and *mef*(A) negative. The *erm*(B) gene possessed a stop codon at position 28 of the upstream control peptide resulting in a truncated control protein. Three mutations were found in the *erm*(B) gene itself: I75T, S100N, H118R and L4 and L22 riboprotein genes were of wildtype.

23s rRNA

23S rRNA sequencing revealed the presence of a novel U754A point mutation (*E. coli* numbering) at all four alleles of domain II with wildtype sequences at in all 4 alleles of domain V.

Figure 1 below shows the secondary structure of *E. coli* 23S rRNA (A). The hairpin 35 of domain II (A) is shown enlarged. The U754A mutation in hairpin 35 is shown by an arrow.

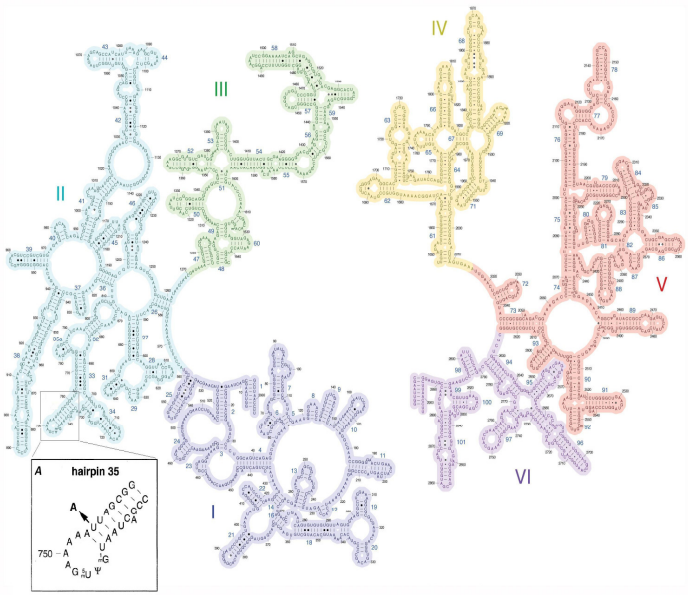

Fig. 1 Secondary structure of *E. coli* 23S rRNA, image adapted from (2,10)

Discussion

Transformation studies have shown that a combination of mutations within the *erm*(B) gene and within the upstream control peptide reduce the susceptibility of *Streptococcus pneumoniae* to telithromycin but do not confer high level resistance (3). The same study showed that a combination of L4 and *erm*(B) mutations were required for high level resistance (telithromycin MIC >256 mg/L). Although PU6080005 possesses a truncated *erm*(B) control peptide and *erm*(B) mutations, because the L4 riboprotein is wildtype the high level resistance is due to another factor.

Xiong *et al* 1999 demonstrated that telithromycin has two binding sites in 23S rRNA; one exists within domain V around residue 2058 and another within domain II at around position 738-759 (2). Residues 738-759 are located in the vicinity of the hairpin 35 loop, a region thought to be an integral part of the ribosomal peptidyl transferase centre (11,12).

These binding sites are located adjacent to the peptide exit tunnel where the nascent polypeptide emerges from the ribosome. At its narrowest point the exit tunnel structure is formed by the L4/L22 riboproteins, mutations either in the 23S rRNA or the riboproteins are likely to inhibit antibiotic binding resulting in reduced susceptibility (11,12 and 13). Xiong *et al* also demonstrated that transformants with a U754A mutation (*E. coli* numbering) and wildtype L4/ *erm*(B) genes showed reduced susceptibility to telithromycin (2)

In consideration of the above evidence, we can deduce that the presence of *erm*(B) and the truncation of its upstream control peptide confer only low level resistance to telithromycin at best and that the absence of any L4/ L22 riboprotein mutations we attribute the very high telithromycin MIC to a U754A point mutation in domain II of the 23S rRNA at all four alleles.

Due to the close proximity of hairpin 35 to the peptidyl transferase centre of the ribosome of *Streptococcus pneumoniae*, It is conceivable that mutations within the hairpin may change the overall conformation of the loop making it less favourable for interaction with telithromycin and thus reducing ketolide binding affinity.

The U754A point mutation in domain II of the 23S rRNA at all four alleles never before reported in a clinical isolate of *Streptococcus pneumoniae* results in high level resistance to telithromycin, although this is extremely rare (1 in 55729).

References

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Correspondence

Quotient Bioresearch Ltd
Microbiology
Newmarket Road
Fordham
Cambridgeshire CB7 5WW
United Kingdom
TEL: +44(0)1638 722974 | FAX: +44 (0)1638 724200
daniel.knight@quotientbioresearch.com, www.quotientbioresearch.com

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