

Paradoxical high-level spiramycin resistance and erythromycin susceptibility in a *Streptococcus constellatus* clinical isolate

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INTRODUCTION

METHODS

- ✓ *Streptococcus constellatus* is a Gram positive bacteria belonging to the *Streptococcus milleri* group, usually recovered in the oral cavity flora and also in urinary and intestinal tracts.
- ✓ Several mechanisms of resistance to antibiotics belonging to macrolides, lincosamides or streptogramins (MLS) have been described including target modifications (*erm* class genes), active efflux and enzymatic inactivation.
- ✓ MLS resistance in *S. milleri* group is not frequent, mainly due to Erm proteins while uncommon resistance mechanisms have been exceptionally reported.

Bacterial strain and identification

Clinical isolate UCN96 was collected in 2014 at the Gustave Roussy Institute (Villejuif, France) from a radionecrosis wound. Identification was performed using the Maldi-Tof technology (Microflex, Bruker Daltonics) and confirmed by partial sequencing of the *sodA* gene.

Antimicrobial susceptibility testing (AST)

AST was carried out by the disc diffusion method using Mueller-Hinton agar plates supplemented with lysed horse blood (5%) and β-NAD (20 mg/L) according to the CA-SFM recommendations. MICs of MLS antibiotics were determined by the broth microdilution technique. *Staphylococcus aureus* ATCC 29213 and *Streptococcus pneumoniae* ATCC 49619 were used as AST controls while a MLS-susceptible *S. constellatus* was also tested.

OBJECTIVES

The aim of the study was to characterize phenotypically and genotypically an atypical MLS resistance phenotype in a clinical isolate belonging to the *S. milleri* group.

PCR analysis for MLS resistance genes

PCR screening for resistance genes was performed for *erm(A)*, *erm(TR)*, *erm(B)*, *erm(C)*, *msr(A)*, *mef(A)*, *erm(F)*, *erm(T)*, *erm(X)* as well as for all variants of *vat*, *vga*, *vgb*, *lsa* and *lnu* genes. Specific mutations in L3-, L4- and L22-encoding genes were also screened as well as those in domain V of the 23S rRNA gene.

RESULTS

- The strain UCN96 was identified as *S. milleri* group by MALDI-TOF and species-level identification (*S. constellatus*) was obtained by *sodA* sequencing.
- UCN96 was susceptible to all ML antibiotics except for spiramycin (MIC ≥256 mg/L). It was also resistant to streptogramins A and B individually or combined (Table 1)
- Screening for all acquired resistance genes was negative while none mutation was found in genes coding for L3, L4 and L22 ribosomal proteins. Interestingly, a single mutation, A2062C (according to *Escherichia coli* numbering), was detected in the domain V of 23S rRNA (Figure 1) and confirmed this uncommon resistance phenotype as previously described in *S. pneumoniae*¹.

Table 1. MICs of MLS antibiotics against studied strains

Strains	MIC (μg/ml) ^b									
	Macrolides				Lincosamides		Streptogramins			
	ERY (14)	TEL (14)	AZI (15)	SPI (16)	LIN	CLI	DAL (A)	QUI (B)	Q-D	PRI
<i>S. constellatus</i> 13422	≤0.01	≤0.01	0.06	0.06	0.5	0.03	2	1	0.5	0.12
<i>S. constellatus</i> UCN96	0.03 (2)	≤0.01(1)	0.12 (2)	≥256 (4,096)	1 (2)	0.06 (2)	≥64 (32)	8 (8)	4 (8)	8 (64)
<i>S. aureus</i> 29213	0.25	0.06	1	8	1	0.25	4	2	0.5	0.25
<i>S. pneumoniae</i> 49619	0.06	≤0.008	0.12	0.25	0.5	0.06	16	1	0.25	0.12

^a ERY, erythromycin (14-membered macrolide); TEL, telithromycin (14-membered macrolide); AZI, azithromycin (14-membered macrolide); SPI, spiramycin (14-membered macrolide); LIN, lincomycin; CLI, clindamycin; DAL, dalfopristin (streptogramin A); QUI, quinupristin (streptogramin B); Q-D, quinupristin-dalfopristin; PRI, pristinamycin.

Figure 1. Alignment of portion of the nucleotide sequence of 23S rRNA (*rrl*) encoding gene.

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S. constellatus UCN96 CGGAAAGCCCCATGGAGCTTTACTGCAGTTTGATATTGAGTGTCTGTGTA 150
S. constellatus 13422 CGGAAAGACCCCATGGAGCTTTACTGCAGTTTGATATTGAGTGTCTGTG 2102
S. pneumoniae CGGAAAGACCCCATGGAGCTTTACTGCAGTTTGATATTGAGTGTCTGTGTA 2106
E. coli CGGAAAGACCCCGTGAACCTTTACTATAGCTTGACACTGAACATTGAGC 2103
    
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2062

CONCLUSION

- ✓ This is the first report of such uncommon phenotype of resistance to macrolides and streptogramins in an isolate of *S. milleri* group.
- ✓ Mutations at the position 2062 has been detected once in *S. pneumoniae*, and not yet in other *Streptococcus* spp. This mechanism is very likely uncommon in Gram-positive bacteria since the different copies of 23S rRNA operons (4 in *S. constellatus*) should be mutated for the development of resistance.