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In vitro activity of josamycin against *Streptococcus pyogenes* isolated from upper respiratory tract infections in France

M. Auzou, J. Caillon, C. Poyart, P. Weber, M-C. Ploy, , R. Leclercq, **V. Cattoir**

Service de Bactériologie-Hygiène, CHU de Nantes, Nantes, France; GH Cochin-Hôtel Dieu-Broca, AP-HP, Paris, France; BIO-VSM LAB, Marne-la-Vallée, France; Service de Bactériologie-Virologie-Hyigène, CHU de Limoges, Limoges, France; Service de Microbiologie, CHU de Caen, Caen, France

Service de Microbiologie
CHU Côte de Nacre
Av. de la Côte de Nacre
14033 Caen Cedex 9, France
Phone: +33-2-31-06-45-72
Fax: +33-2-31-06-45-73
Email: cattoir-v@chu-caen.fr

Introduction

✓ Macrolides are alternative to β-lactams for the treatment of pharyngitis due to *Streptococcus pyogenes* for patients allergic to these drugs.

✓ Macrolides are classified according to the number of atoms forming the lactone ring: 14-membered (e.g. erythromycin [ERY], clarithromycin [CLA]), 15-membered (e.g. azithromycin [AZI]) or 16-membered (e.g. josamycin [JOS]).

✓ Resistance to macrolides has been reported worldwide in *S. pyogenes* although its prevalence varies considerably. In France, erythromycin resistance reached 22% in the early 2000s even if it has recently decreased to >10% [1-3].

✓ Resistance is mainly due to the acquisition of:
- a ribosomal methylase [*erm*(B) or *erm*(A) subclass *erm*(TR)] conferring inducible or constitutive MLS_B phenotype with cross-resistance to macrolides (14-, 15- and 16-membered compounds), lincosamides and streptogramins B.
- an efflux pump gene [*mef*(A)] responsible for resistance only to 14- and 15-membered macrolides.

OBJECTIVES: The aim of this study was 1) to evaluate the in vitro activity of JOS compared to that of ERY, CLA and AZI against upper respiratory tract (URT) isolates of *S. pyogenes* collected in France and 2) to determine the molecular mechanism of macrolide resistance with assessment of the relationship between the resistance genotype and phenotype.

Methods

Bacterial strains
This multicenter study included 193 consecutive clinical isolates of *S. pyogenes* recovered from URT samples of patients in four French hospitals (Caen, Limoges, Nantes, and Cochin-Paris) and one private laboratory (Marne-la-Vallée). Identification was performed using conventional methods or MALDI-TOF mass spectrometry.

Antimicrobial susceptibility testing
Antibiotic susceptibility was determined by the disc diffusion on Mueller-Hinton agar according to the CA-SFM recommendations (www.sfm-microbiologie.org/). The presence of D-shaped zone (observed around the disc of clindamycin [CLI] facing the ERY disc) indicated a MLS_B inducible phenotype. MICs of ERY, CLA, AZI, JOS and CLI were determined by the broth microdilution recommended by EUCAST. Clinical categorization was performed using EUCAST breakpoints.

PCR detection
Screening of *erm*(B), *erm*(A)-subclass *erm*(TR) and *mef*(A) genes was performed using standard PCR methods [4].

Results

❖ According to EUCAST breakpoints, the percentage of resistance to ERY and AZI was 7.3% while that for CLA was 6.7% (**Table 1**).

❖ The modal MIC and the MIC_{50} of JOS for *S. pyogenes* were 0.12 mg/L, with 95.3% of isolates having MIC ≤0.25mg/L (**Table 1** and **Figure 1**).

Table 1. MIC values for the 193 *S. pyogenes* clinical isolates.

Antibiotic	MIC (mg/L)			Susceptible strains (%)
	Range	MIC_{50}	MIC_{90}	
ERY	0.016->128	0.03	0.06	7.3
CLA	0.008->128	0.03	0.06	6.7
AZI	0.06->128	0.06	0.12	7.3
JOS	**0.06->128**	**0.12**	**0.25**	**4.7**
CLI	0.016->128	0.03	0.06	4.7

❖ Since no susceptibility breakpoint has been recommended for JOS by any antimicrobial susceptibility testing committee (i.e. CA-SFM, EUCAST or CLSI), it was not possible to calculate the prevalence of JOS resistance. However, the distribution of MICs clearly showed two distinct populations (**Figure 1**): one with **MICs ≤0.25mg/L** (considered as the susceptible population) and another with **MICs >8 mg/L** (considered as the resistant population). Then, the percentage of JOS resistance could be estimated at 4.7%.

❖ Lincosamides (such as CLI) are considered as a surrogate marker for susceptibility to JOS [5]. Since the percentage of CLI resistance was also 4.7%, the actual percentage of JOS resistance was likely 4.7%, which lower than those observed for other macrolides tested.

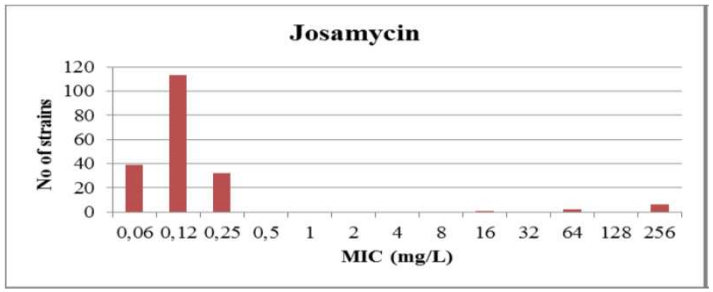

Figure 1. Distribution of MICs of josamycin.

❖ Ribosomal methylation was found to be the most common mechanism of resistance (12/14 isolates), with *erm*(B) in 9 isolates and *erm*(A) subclass *erm*(TR) in 3 isolates (**Table 2**). Two isolates harboured the *mef*(A) gene and remained susceptible to JOS (**Table 2**), as expected since it is not a substrate for this efflux pump.

Table 2. Genotypes and phenotypes of resistance.

Gene	Mechanism of resistance	MIC (mg/L)				
		ERY	CLA	AZI	**JOS**	CLI
erm(B)	Ribosomal methylation	≥128	128->128	128->128	**64->128**	4->128
erm(TR)[a]		1-8	0.5-4	2-4	**0.12-0.25**	0.03
mef(A)[b]	Active efflux	8-16	4	8	**0.12**	0.03

[a] D-shaped zone observed (image of induction).
[b] No D-shaped zone observed.

❖ The *erm*(B) gene was responsible for full MLS_B cross-resistance while *erm*(TR), which was inducibly expressed, conferred resistance only to 14- and 15-membred macrolides (i.e. ERY, CLA and AZI) and not to JOS and CLI.

❖ Concerning susceptibility to other antibiotics, none of the isolates was found to be penicillin-resistant (as expected) while tetracycline resistance was 8.3%. No resistant strain was detected for pristinamycin, vancomycin, teicoplanin, cotrimoxazole and rifampicin.

Conclusions

- Only 4.7% of *S. pyogenes* isolates were resistant to JOS, in comparison to 7.3% and 6.7% for ERY/AZI and CLA, respectively.
- This is mostly due to structural differences between JOS and 14- and 15-membered macrolides, which are well-known substrates of the efflux pump Mef(A).
- These results indicate that JOS may be a useful alternative to β-lactams for the treatment of pharyngitis.

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