**In vitro** activity of tylosin and tilmicosin against cocci isolated from bovine mastitis.

M. BONNIER\(^1\), C. DORÉ\(^1\), J. AMÉDÉO\(^2\) and V. GUÉRIN-FAUBLÉE\(^3\)*

\(^1\)Laboratoire Départemental d’Analyses, 24 rue Antoine Joly, 35031 Rennes cedex, France  
\(^2\)Elanco Santé Animale, 13 rue Pagès, 92158 Suresnes cedex, France  
\(^3\)Département de Santé Publique Vétérinaire, Ecole Nationale Vétérinaire de Lyon, 69280 Marcy l’étoile, France

*Corresponding author: Tel: +33 478 872592 - E-mail: v.guerin@vet-lyon.fr

**SUMMARY**

Tylosin and tilmicosin Minimum Inhibitory Concentrations (MIC) were determined by the reference agar dilution technique for *Staphylococcus aureus* and *Streptococcus uberis* strains isolated from cow mastitis in Ille-et-Vilaine (France) between 2000 and 2003. Overall, 4.4 % and 12.5 % of the *S. aureus* and *S. uberis* strains tested were resistant to tylosin, respectively. Resistance frequencies were invariable over the observation period. Further studies, in particular pharmacokinetic and clinical data, are needed to evaluate the potential usefulness of tilmicosin to treat bovine mastitis.

**Key words: Tylosin, tilmicosin, MIC, cocci, cow , mastitis.**

**RÉSUMÉ**

Activité *in vitro* de la tylosine et de la tilmicosine sur des coques isolés de mammites bovines.

Les Concentrations Minimales Inhibitrices (CMI) de tylosine et de tilmicosine ont été mesurées par la technique de référence de dilution en milieu gélosé sur des souches de *Staphylococcus aureus* et de *Streptococcus uberis* isolées, en Ille-et-Vilaine (France), de 2000 à 2003, de laits de mammites chez la vache. Globalement, 4,4 % des souches de *S. aureus* étaient résistantes à la tylosine et 12,5 % des souches de *S. uberis*. Les fréquences de résistance observées étaient stables dans le temps. Des études complémentaires, pharmacocinétiques et cliniques, seraient nécessaires pour préciser l’intérêt de l’utilisation de la tilmicosine dans le traitement des mammites chez la vache.

**Mots-clés : Tylosine, tilmicosine, CMI, coques, vache, mammite.**

**Introduction**

Bovine mastitis is recognised as the most costly disease affecting dairy cattle worldwide, accounting for 38 % of the total direct costs incurred by the dairy industry [4]. Losses are associated with clinical mastitis as well as sub-clinical mastitis. Five bacterial species account for almost 80 % of all cases of bovine mastitis. *Escherichia coli* together with *Streptococcus uberis*, and *Staphylococcus aureus* are regarded as the commonest causes of clinical and sub-clinical mastitis, respectively [8, 15]. Implementing effective mastitis control programmes partly relies on antibiotic treatment of clinical mastitis during lactation, and treatment of intramammary infections at drying-off. Many antimicrobial compounds, including β-lactams, aminoglycosides, lincosamides, spiramycin, tetracycline, colistin, and bacitracin, are available for infusion by the intramammary route. Systemic administration of antibacterials has received attention as an alternative or additional therapy to intramammary treatment [7]. It is typically indicated for pathogens such as *S. uberis* and *S. aureus* that are invasive or create abscesses. In fact, the site of infection, namely the deep tissue of the mammary gland, would be more appropriately targeted. Given high bioavailability from the injection site, lipid solubility, long half-life, low protein binding and intracellular concentration in phagocytes, macrolides are of considerable interest for parenteral mastitis therapy [2]. In Europe, spiramycin [22, 24] and tylosin are labelled for treatment of bovine mastitis via parenteral routes. Moreover, the efficacy of tilmicosin – a semi-synthetic macrolide commonly approved for the treatment of bovine respiratory disease – to cure intramammary infections at drying-off has recently been investigated [19, 25]. The choice of an antibiotic must be based not only on pharmacological considerations, but also on bacteriological data. Macrolide antibiotics have a narrow spectrum of activity, limited mainly to Gram-positive pathogens. Acquired resistance to macrolides in Gram-positive cocci is primarily related to target-site modification through acquisition of an *erm* gene encoding a 23S rRNA methylase. Methylation of the target blocks binding of macrolides, lincosamides, and streptogramins B to the ribosome, leading to cross-resistance, the so-called MLSB phenotype [12]. Expression of MLSB resistance in staphylococci and streptococci can be constitutive or inducible. According to data of the Agence Française de Sécurité Sanitaire des Aliments [1], 86 % and 74-75 % of *S. aureus* and *S. uberis* strains isolated in France in 1999 from mastitis milk samples were susceptible to erythromycin, respectively. Erythromycin resistance frequencies ranging from 0.7 % to
25 % [5, 6, 9, 11, 14, 23], and from 18 % to 48 % [6, 10, 21] have been recently reported from various countries for *S. aureus* and *S. uberis*, respectively.

The present study was undertaken in order to compare *in vitro* activity of two macrolides - tylosin and tilmicosin - against *S. aureus* and *S. uberis* strains isolated from 2000 to 2003 from cases of bovine clinical mastitis in France.

### Materials and methods

#### BACTERIAL STRAINS

Forty *S. aureus* subsp *aureus* and 40 *S. uberis* strains were tested per year. All of them originated from Ille-et-Vilaine and have been isolated from quarter mastitis foremilk samples. For each bacterial species, only one strain per year from each herd sampled was included in the study, in order to test epidemiologically unrelated isolates. Bacterial identification was undertaken only for samples which grew one type of colony with more than 20 colony-forming units (cfu) per mL. Preliminary identification was achieved on the basis of morphological and biochemical characteristics of the strains. Further confirmation was carried out by means of the Slidex-Staph kit (bioMérieux, Marcy l'étoile, France) and the API32Strep gallery (bioMérieux) for *S. aureus* and *S. uberis*, respectively.

#### ANTIMICROBIAL SUSCEPTIBILITY TESTING

The reference agar dilution method was used to determine Minimum Inhibitory Concentrations (MIC), following the guidelines of the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards) [17, 18]. Tylosin was purchased from Sigma (St Louis, USA), and tilmicosin was provided by Elanco (Suresnes, France). Mueller-Hinton agar (Bio-Rad, Marnes-la-Coquette, France), supplemented with 5 per cent defibrinated sheep blood for streptococci, was used as the test medium. Inocula of approximately 10^4 cfu per spot were delivered with a Steers replicator. Plates were incubated at 35±1°C for 18 h under air. *S. aureus* ATCC29213 was used as reference strain for MIC quality controls [18]. There are no validated clinical categorization criteria for tylosin or tilmicosin and bovine mastitis [18].

### Results

Results are summarised in Table 1. The MIC values for the control strain (1 - 2 mg X L^-1 for tylosin and 2 mg X L^-1 for tilmicosin) were within the specified limits for each antibiotic tested. The distribution of MIC values per year showed no obvious differences (Figure 1).

For the *S. aureus* isolates, tylosin MICs were significantly higher than tilmicosin MICs (*p* < 0.0001). A bimodal distribution of tylosin MICs was observed, which allowed susceptible (MIC = 0.5 to 2 mg X L^-1) and resistant (MIC > 128 mg X L^-1) strains to be distinguished. A bimodal population distribution was also observed for tilmicosin with MICs ranging from 0.25 to 2 mg X L^-1 and > 128 mg X L^-1 for susceptible and resistant strains, respectively. The resistant isolates were both highly resistant to tylosin and tilmicosin, and might be assigned to a constitutive MLSB phenotype [12]. The frequency of *S. aureus* strains resistant to both molecules (1/40 each year) was very low except in 2000 (4/40).

### STATISTICAL ANALYSIS

Tylosin and tilmicosin MIC values for one set of strains were compared by using the Wilcoxon signed rank test with a level of significance α = 0.05.
With regard to *S. uberis*, tylosin MICs were significantly lower than tilmicosin MICs (p < 0.0001). Tylosin MICs were scattered, and a resistant population was not clearly discernible. Resistance mechanisms to macrolides in streptococci might account for this observation. In contrast to staphylococci, most members of the MLSB group are inducers of methylase production in streptococci. Yet, differences in the ability of the molecules of the MLSB group to induce rRNA methylation give rise to low- to high-level resistance to macrolides and structurally related compounds [12]. From 2001 to 2003, 12 of the 120 tested streptococcal strains had tylosin MICs > 8 mg/L (20% in 2000).

Tilmicosin MICs for streptococci were more homogeneous than tylosin MICs with MIC50 and MIC90 differing by one dilution, and only one of the 120 tested strains was highly resistant.

**Discussion**

In this study, frequency of acquired resistance to tylosin in *S. aureus* was low; in contrast, resistance was most common in *S. uberis*. These results were in agreement with most available data from different geographical localizations [1, 6, 10, 11, 14, 23].

The comparison of resistance frequencies between years was made possible as antimicrobial susceptibility tests were performed in the same laboratory on isolates that originated from the same geographic region. Tylosin resistance frequencies in Gram-positive cocci isolated from bovine mastitis remained constant over the observation period. According to ERSKINE and al. [6], resistance to erythromycin in *S. uberis* strains isolated from milk samples in USA was invariable over a seven year period, while an increase in the proportion of susceptible *S. aureus* was noted. On the contrary, a decrease in erythromycin susceptibility was reported by MAKOVEC and RUEGG [14] for *S. aureus* strains isolated at the same time in another USA state.

Measured tilmicosin MICs were in accordance with previous reports [20, 25]. More pharmacokinetic [25] and clinical [19] data are needed to evaluate the potential usefulness of tilmicosin to cure bovine intramammary infections.

According to our results, most of streptococci resistant to tylosin were susceptible to tilmicosin. This observation could be explained by the weak capacity of this molecule to induce resistance. Retained susceptibility of MLSB streptococci to telithromycin, a ketolide, has been reported [3, 13, 16]. The inducer capacity of tilmicosin would deserve further investigation.

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Conclusion

The use of an old narrow-spectrum antibiotic to treat bovine mastitis is justified from an ecological viewpoint. Tylosin remains a drug of choice for that purpose as resistance frequencies in Gram-positive cocci were low over several years.

References