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ORIGINAL ARTICLE

Prevalence and antibiotic susceptibility of coagulase-negative *Staphylococcus* species from bovine subclinical mastitis in dairy herds in the central region of Argentina

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Subclinical mastitis;
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Abstract Coagulase-negative staphylococci (CNS) are a common cause of bovine subclinical mastitis (SCM). The prevalence of CNS species causing SCM identified by genotyping varies among countries. Overall, the antimicrobial resistance in this group of organisms is increasing worldwide; however, little information exists about a CNS species resistant to antibiotics. The aim of the present study was to genotypically characterize CNS at species level and to determine the prevalence and antibiotic resistance profiles of CNS species isolated from bovine SCM in 51 dairy herds located in the central region of the province of Cordoba, Argentina. In this study, we identified 219 CNS isolates at species level by PCR-restriction fragment length polymorphism of the *groEL* gene. *Staphylococcus chromogenes* (46.6%) and *Staphylococcus haemolyticus* (32%) were the most prevalent species. A minimum of three different CNS species were present in 41.2% of the herds. *S. chromogenes* was isolated from most of the herds (86.3%), whereas *S. haemolyticus* was isolated from 66.7% of them. The broth microdilution method was used to test *in vitro* antimicrobial susceptibility. Resistance to a single compound or two related

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compounds was expressed in 43.8% of the isolates. *S. chromogenes* and *S. haemolyticus* showed a very high proportion of isolates resistant to penicillin. Resistance to two or more non-related antimicrobials was found in 30.6% of all CNS. *S. haemolyticus* exhibited a higher frequency of resistance to two or more non-related antimicrobials than *S. chromogenes*.

PALABRAS CLAVE
Mastitis subclínica;
Bovinos;
Especies de estafilococos coagulasa negativos;
Resistencia a antimicrobianos

Prevalencia y sensibilidad a antibióticos de especies de estafilococos coagulasa negativos provenientes de mastitis subclínica en bovinos de tambos de la región central de Argentina

Resumen Los estafilococos coagulasa negativos (ECN) son una causa frecuente de mastitis subclínica (MSC) en bovinos. La prevalencia de especies de ECN causantes de MSC identificadas por métodos genotípicos varía entre países. La resistencia antimicrobiana en este grupo de organismos se está incrementando en el mundo; sin embargo, existe poca información acerca de las especies de ECN resistentes a antibióticos. Los objetivos del presente estudio fueron caracterizar genotípicamente los ECN a nivel de especie y determinar la prevalencia y los perfiles de resistencia a antibióticos de las especies de ECN aisladas de MSC en bovinos de 51 rodeos situados en la provincia de Córdoba, Argentina. Mediante polimorfismos de los fragmentos de restricción del gen *groEL* identificamos 219 aislamientos de ECN a nivel de especie. *Staphylococcus chromogenes* (46,6%) y *Staphylococcus haemolyticus* (32%) fueron las especies más prevalentes. Un mínimo de 3 especies diferentes de ECN estuvieron presentes en el 41,2% de los tambos. *S. chromogenes* fue aislado en la mayoría de los tambos (86,3%), mientras que *S. haemolyticus* fue aislado en el 66,7% de aquellos. Para el análisis de sensibilidad a los antimicrobianos *in vitro* se usó el método de microdilución en caldo. La resistencia a un único compuesto o a 2 compuestos relacionados fue expresada en el 43,8% de los aislamientos. *S. chromogenes* y *S. haemolyticus* mostraron una muy elevada proporción de aislamientos resistentes a penicilina. La resistencia a 2 o más antimicrobianos no relacionados fue hallada en el 30,6% de los ECN. *S. haemolyticus* exhibió una frecuencia de resistencia a 2 o más antimicrobianos no relacionados más elevada que *S. chromogenes*.

Introduction

Bovine mastitis is one of the most costly and complex diseases of the dairy industry[3,9]. The complexity is reflected in the numerous causative pathogens, the variety and magnitude of the physiological responses to these pathogens and the variation in efficacy of control measures for different causative organisms[4,7,17,18,23,32]. Coagulase-negative staphylococci (CNS) have been traditionally considered minor pathogens. However, their importance has increased because they have become the most frequently isolated group of species from bovine milk in many areas around the world[16,29,33]. CNS usually cause subclinical mastitis (SCM), resulting in an increase in the somatic cell count (SCC) and a decrease in milk quality with the resulting economic losses that this implies[30]. The understanding and control of CNS mastitis are complicated by the heterogeneity of this group of bacteria. So far, based on Jean Euzéby's List of Procaryotic names with Standing in Nomenclature (http://www.bacterio.net/s/staphylococcus.html), the genus *Staphylococcus* contains 47 species and 24 subspecies. Even though, the prevalence of CNS species varies among the studies; six species have been reported to be commonly involved in intramammary infections (IMI)[20,30]. Recently, it has been found that CNS speciation based on the biochemical profile is not accurate enough for bovine CNS identification[26]. Hence, molecular methods have become an important diagnostic tool to improve CNS species differentiation[5,15,39]. CNS tend to be more resistant to antibiotics than *Staphylococcus aureus* and easily develop multiresistance[2,27,32]. In addition, limited information is available regarding differences in antimicrobial susceptibility among CNS species identified by genotyping[27,38]. As yet, nothing has been reported about the prevalence and susceptibility antibiotics of CNS species of dairy cows in Argentina. Recently, a research conducted in our laboratory[8] showed that CNS represent the most prevalent bacterial group of minor pathogens isolated from the subclinical infection of dairy cows in Córdoba province, Argentina. The aim of the present study was to determine the prevalence and antibiotic resistance profiles of CNS species isolated from bovine subclinical mastitis in 51 dairy herds in the central region of Argentina.