P2089

Identification and susceptibility testing of staphylococcal isolates from sub-clinical mastitis in ewes in Greece

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Introduction and purpose

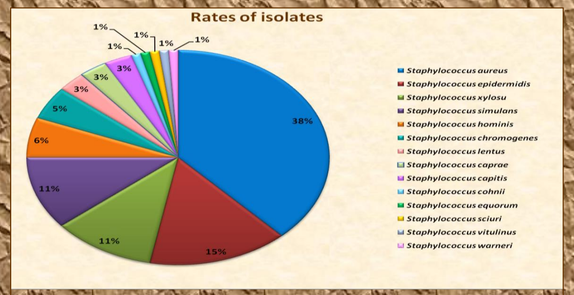
Mastitis is one of the common health problems in dairy animals, reducing the quantity and quality of milk. Subclinical form of the disease due to a higher incidence, decrease in milk yield and changes in the physicochemical properties of milk is more important than acute mastitis. Because of the importance of subclinical mastitis, it is necessary to control the disease and to this end, and so, identification of causative agents of subclinical mastitis is important.

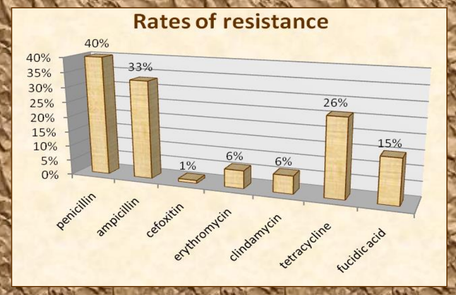
Among the various pathogens, *Staphylococcus* spp. are the most common microorganisms that cause subclinical mastitis in sheep. Specifically, coagulase-negative staphylococci (CNS) have a major role in the development of subclinical mastitis in sheep than other members of this family. Subclinical mastitis caused by CNS leads to decrease of milk production, increase in the number of somatic cell counts (SCC) in milk and production of the thermostable enterotoxins. *Staphylococcus epidermidis*, *S. simulans, S. chromogenes, S. xylosus,* and *S. hemolyticus* are the most common CNS, causing the subclinical mastitis in sheep.

The aim of the present study was species identification of staphylococci isolated from milk samples from ewes with subclinical mastitis collected from farms throughout Greece, as well as investigation of antibiotic resistance patterns of these isolates.

Methods

Milk samples were collected aseptically from dairy ewes with sub-clinical mastitis (defined as lack of clinically evident systemic or mammary abnormalities, but with simultaneous bacterial isolation and increased somatic cell counts) for bacteriological examination. In total, 74 isolates were randomly selected for identification to staphylococcal species level and testing of antimicrobial susceptibility (VITEK® 2; BioMerieux, France). Detection of genes that correlated with resistance to cefoxitin (*mecA, mecC*), erythromycin /clindamycin (*erm, msrA*, *lnu*) and tetracycline (*tetK, L, M, O* etc) was performed by PCR. Clonality of isolates to each species was tested by pulse field gel electrophoresis (PFGE). Representative isolates from each pulsotype were further characterized by Multi Locus sequence typing (MLST).





Results

Of 74 isolates, 28 were identified as *Staphylococcus aureus* (38%) and 46 as coagulase-negative staphylococci (62%). These were specifically identified as *Staphylococcus epidermidis* (n=11, 15% of all strains), *Staphylococcus xylosus* (n=8, 11%), *Staphylococcus simulans* (n=8, 11%), *Staphylococcus chromogenes* (n=4, 5%), *Staphylococcus hominis* (n=4, 6%), *Staphylococcus lentus* (n=2, 3%), *Staphylococcus caprae* (n=2, 3%), *Staphylococcus capitis* (n=2, 3%), *Staphylococcus cohnii* (n=1, 1%), *Staphylococcus equorum* (n=1, 1%), *Staphylococcus sciuri* (n=1, 1%), *Staphylococcus vitulinus* (n=1, 1%) and *Staphylococcus warneri* (n=1, 1%). Rates of resistance to penicillin, ampicillin, cefoxitin, erythromycin, clindamycin, tetracycline and fucidic acid were 40%, 33%, 1%, 6%, 6%, 26% and 15%, respectively, and were particularly prominent for the coagulase-negative isolates (59%, 46%, 2%, 4%, 4%, 37%, 24%). Resistance to cefoxitin was detected in only one *S. capitis* strain, associated with presence of *mecA* gene. The *ermC* gene was found in all erythromycin/clindamycin-resistant isolates (*S. aureus*, *S. xylosus*, *S. equorum*, *S. hominis*), whilst the tetracycline resistant isolates (*S. aureus, S. epidermidis, S. chromogenes, S. hominis, S. lentus, S. xylosus*) carried either the *tetK* or the *tetL* gene. Most *S. aureus* isolates (19 of 28) belonged to one pulsotype, which corresponded to ST 133, while *S. epidermidis* isolates were distributed equally to three pulsotypes (ST152, ST140, ST100).

Conclusions

In conclusion, the results of this study showed that various *Staphylococcus* species, play an important role in the development of subclinical mastitis in sheep in Greek provinces. Molecular characterization of staphylococcal isolates revealed that common zoonotic clones (ST133, ST140) cause subclinical mastitis and are disseminated in sheep. Additionally, the high resistance rate to tetracycline, an antibiotic widely used in veterinary, emphasizes the need for new therapeutic strategies for treatment of staphylococcal intra-mammary infections. Further studies on the role of environmental and management factors in occurrence of staphylococcal mastitis, as well as identification of virulence factors in the identified dominant species involved in mastitis, can be helpful in prevention and control of this disease

References

1. Schalm OW, Carroll EJ, Jain NC, Jain NC. Bovine mastitis. Philadelphia, USA: Lea and Febiger; 1971. Number and types of somatic cells in normal and mastitic milk; pp. 94–127
2. Contreras A, Sierra D, Sanchez A, et al. Mastitis in small ruminants. Small Rumin Res. 2007;68:145–153
3. Gebrewahid TT, Abera BH, Menghistu HT. Prevalence and etiology of subclinical mastitis in small ruminants of tigray regional state, North Ethiopia. Vet World. 2012;5:103–109
4. Albenzio M, Taibi L, Muscio A, et al. Prevalence and etiology of subclinical mastitis in intensively managed flocks and related changes in the yield and quality of ewe milk. Small Rumin Res. 2002;43:219–226
5. Frey Y, Rodriguez JP, Thomann A, et al. Genetic characterization of antimicrobial resistance in coagulase-negative staphylococci from bovine mastitis milk. J Dairy Sci. 2013;96:2247–2257
6. Mohanty NN, Das P, Pany SS, et al. Isolation and antibiogram of *Staphylococcus*, *Streptococcus* and *E. coli* isolates from clinical and subclinical cases of bovine mastitis. Vet World. 2013;6:739–743