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Identification and susceptibility testing of staphylococcal isolates from subclinical mastitis in ewes in Greece

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Background: Subclinical mastitis is the most financially important disease of dairy animals, primarily caused by *Staphylococcus* spp. The organisms may also be transmitted to humans, e.g., through dairy product consumption or direct contact with animals. Objective of this study was species identification of staphylococcal isolates from milk samples from ewes with subclinical mastitis collected from farms throughout Greece, as well as investigation of antibiotic resistance patterns of these isolates.

Material/methods: Milk samples were collected aseptically from dairy ewes 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 ceftiofur, erythromycin, clindamycin and tetracycline was performed by PCR. Clonality of isolates to each species was tested by pulse field gel electrophoresis (PFGE).

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, ceftiofur, erythromycin, clindamycin, tetracycline and fusidic 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 ceftiofur 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, while *S. epidermidis* isolates were distributed equally to three pulsotypes.

Conclusions: Of the predominant species identified, *S. aureus*, *S. epidermidis*, *S. simulans* and *S. chromogenes* are pathogenic for the mammary gland. Antibiotic resistance profiles are of assistance for designing antibiotic treatment schemes in staphylococcal infections, in human and veterinary medicine. It is noteworthy that as fusidic acid is rarely administered to sheep, strains resistant to that are likely of human origin.