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Prevalence and anti-microbial resistance of *Staphylococcus* spp. isolated from the environment and veterinary personnel in a Spanish veterinary teaching hospital

Ana-María Ríos[1], Silvia Penelo[2]*, María-Rosario Barquero[3], Tania Ayllón[3,4]*, Gustavo Ortiz-Díez[5]

[1] Veterinary Service, Puchol Veterinary Hospital, Madrid, Spain; [2] Emergency, Hospitalization and ICU Service, Complutense Veterinary Clinical Hospital, Complutense University of Madrid, Madrid, Spain; [3] Faculty of Health Sciences, Alfonso X el Sabio University, Madrid, Spain; [4] Department of Genetics, Physiology and Microbiology, School of Biological Sciences, Complutense University of Madrid, Madrid, Spain; [5] Department of Medicine and Surgery, Faculty of Veterinary Medicine, Complutense University of Madrid, Madrid, Spain.

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Abstract

Methicillin-resistant *Staphylococcus* (MRS) bacteria, including methicillin-resistant *S. aureus* and methicillin-resistant *S. pseudintermedius* (MRSP), pose a significant threat in veterinary medicine, given their potential for zoonotic transmission and their implications for companion animals and humans' health. This study aimed to assess the prevalence of MRS and anti-microbial resistance patterns at a university clinical hospital in Madrid, Spain. Samples were collected from both the environment and hospital staff at Veterinary Clinical Hospital of Alfonso X el Sabio University. Anti-microbial susceptibility assays, molecular detection of *mec*A gene and genetic relationships among the identified bacterial strains were performed. The study revealed an MRS prevalence of 1.50% in environmental samples, with MRSP accounting for 0.75% of the cases. Genetically related MRSP strains were found in different hospital areas. Among hospital staff, there was a MRS prevalence of 14.03%, including *S. pseudintermedius* and *S. epidermidis* strains. Antibiogram tests revealed multi-drug resistance among MRSP strains. Additionally, methicillin-resistant coagulase-negative staphylococci were isolated, suggesting potential cross-species transmission. This study underscores the presence of MRS in a veterinary clinical hospital, highlighting the significance of infection control through the implementation of protective measures, stringent hygiene practices among personnel and in the environment and responsible use of antibiotics. Further research is necessary to assess MRS incidence in animal patients and explore geographical variations, enhancing our understanding of MRS in veterinary medicine and addressing its challenges.

Introduction

Methicillin-resistant *Staphylococcus* (MRS) bacteria, mainly methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant *S. pseudintermedius* (MRSP), are the major causes of morbidity and mortality in companion animals. These bacteria, that constitute a zoonotic risk, can potentially affect the human-animal bond and their inter-actions, posing health concerns for both animals and humans.[1]

The genus *Staphylococcus* consists of Gram-positive, catalase-positive and anaerobic facultative cocci. The most pathogenic species possess coagulase, an enzyme coagulating plasma by converting fibrinogen to fibrin. Coagulase-negative staphylococci (CoNS) are minor pathogens usually causing opportunistic infections in immuno-compromised patients.[2] The coagulase-positive species with the greatest veterinary clinical significance are *S. pseudintermedius*, *S. aureus*, *S. hyicus* and *S. schleiferi* subsp. *coagulans.* The *S. aureus* is the most common pathogen in humans; while, *S. pseudintermedius* and *S. schleiferi* are the most important pathogens in dogs.[3] Recent molecular studies consolidate the emergence of the term "*Staphylococcus intermedius* group", which includes three major subgroups: *S. intermedius*, *S. delphini* (more common in horses) and *S. pseudintermedius.*[4]

***Correspondences:**

Silvia Penelo. DVM, PhD
Emergency, Hospitalization and ICU Service, Complutense Veterinary Clinical Hospital, Complutense University of Madrid, Madrid, Spain
E-mail: spenelo@ucm.es
Tania Ayllón. DVM, PhD
Faculty of Health Sciences, Alfonso X el Sabio University, Madrid, Spain | Department of Genetics, Physiology and Microbiology, School of Biological Sciences, Complutense University of Madrid, Madrid, Spain
E-mail: tayllsan@uax.es

Studies have shown that veterinary hospitals play a significant role in the transmission of multi-drug-resistant (MDR) organisms, not only because of the administration of anti-bacterial drugs, but also due to the close contact between humans and animals.[4,5] The scientific community emphasizes the importance of several factors in the management of MRSA and MRSP infections, such as the understanding of the epidemiology of these organisms in humans and animals (both food and companion animals), appropriate use of antibiotics in all species, effective management of production species and adherence to aseptic standards in surgical and medical procedures. In addition, the implementation of rigorous hygiene and health education measures for pet owners and those in contact with MRSA- or MRSP-infected pets is essential.[6] Given the close relationship between humans and animals, it is essential to coordinate efforts in addressing the challenges posed by MRS, recognizing the environment as an important factor in the transmission and management of these organisms.[5]

The aim of this work was to determine the prevalence of MRS in the staff and environment of a university clinical hospital and to study the patterns of anti-microbial resistance; thereby, contributing to a better understanding of the dynamics of MRSA and MRSP in veterinary settings.

Materials and Methods

Study design. A cross-sectional descriptive survey (prevalence) was conducted at Veterinary Clinical Hospital of Alfonso X el Sabio University, Madrid, Spain. Two different types of samples were collected, one comprising environmental samples and the other consisting of samples collected from the staff. The collection was carried out on a single day, June 20, 2011. It should be noted that no hospitalized animals with MRSA and/or MRSP infection had been identified in the six months prior to sampling.

Environmental microbiological samples. The required sample size calculation for environmental sampling was based on a minimum of 120 samples, assuming an infinite population of sites, and using an estimated prevalence of 10.00%[7] with a 95.00% confidence interval to detect prevalence with a margin of ± 5.00%. The hospital was divided into 21 areas, including four small animal consulting rooms, two small animal operating rooms, one small dog admission, one large dog admission, one infectious disease admission, one cat admission, one anesthesia induction area, one small animal anesthesia recovery, one laboratory, one diagnostic image room, two equine intensive care unit stalls, two equine examination rooms, two equine operating room and one equine intensive care unit room (Fig. 1). These locations were selected due to their similar equipment and facilities, to provide a subsequent comparison. Samples were collected from door handles, worktops, examination and diagnostic tables, clinical and surgical materials, work desks, cages and hospitalization boxes. Specific and relevant locations in each area were also included. A total of 123 environmental samples were collected using sterile cotton swabs moistened with sterile distilled water. Stuart-Amies transport medium (Oxoid, Basingstoke, UK) was used for each swab. The sampling process consisted of rotating and moving the swab horizontally from the inside to the outside of a 10.00 cm^2 area for 10 sec at each site.

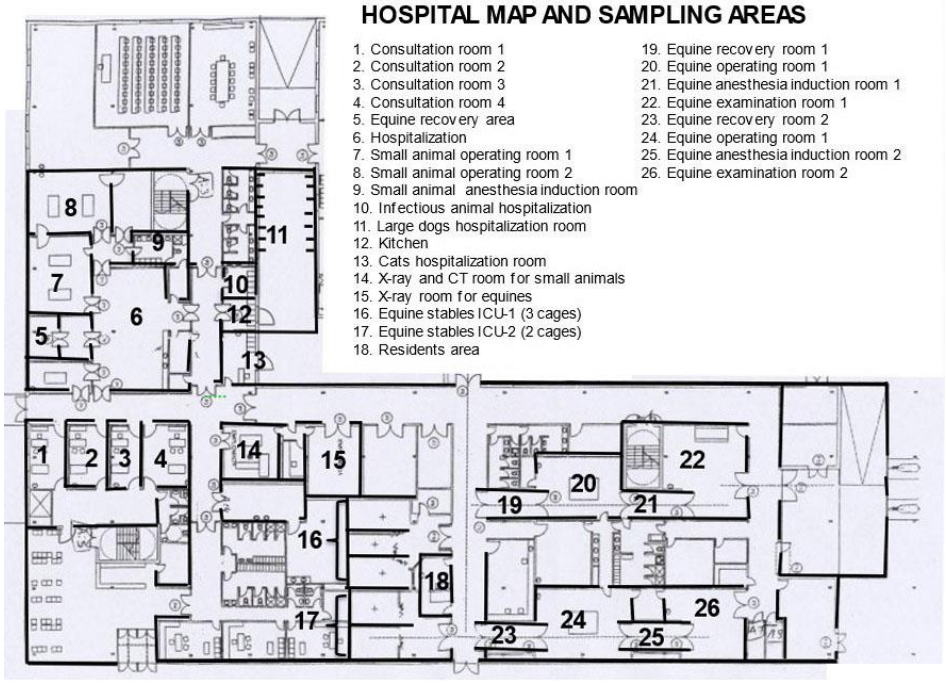

Fig. 1. Distribution of Veterinary Clinical Hospital of Alfonso X el Sabio University (Madrid, Spain) areas.

Samples were collected from clinical facilities and furnishings after patient departure and following routine cleaning procedures as follows: Floors were swept and mopped with a water dilution of Dimethyl benzyl ammonium (Sutter Professional, Borghetto DI Borbera, Italy) at a concentration of 0.40%; surfaces such as examination tables and cages were cleaned twice daily with the same product. Operating rooms were disinfected once a week with a protease-based enzymatic compound (Ultra Concentrate Enzymatic Cleaner, Prolystica™, Steris Solutions Limited, Leicester, UK) at the recommended dilution (20.00 mL of Prolystica™ *per* L of water).

Human microbiological samples. The sample size calculation for human samples was based on a population of 60 individuals with an estimated prevalence of 10.00%[7] and a 95.00% confidence interval to detect prevalence with a margin of ± 5.00%. Therefore, the sample size required at least 38 subjects. In this study, samples were obtained from 42 individuals out of a total population of 57, considering different work shifts, as not all staff was present at the hospital at the time of sampling. All hospital staff was invited to voluntarily participate in the study through a letter explaining the purpose of the study and providing general information about MRSA and MRSP. Signed informed consent was obtained from all participants and personal information was kept anonymous. Information was also collected on professional profile (veterinarians, assistants and administrative staff) and the work area (medical, surgical and other), as well as possible contact with health personnel or institutions. For human sampling, a sterile cotton swab with Stuart-Amies transport medium (Oxoid) was rotated in each nostril for 5 sec. Results obtained on the presence or absence of MRSA and/or MRPS were sent to the participants by ordinary mail using the postal addresses associated with the assigned codes. The letters included information about MRSA and/or MRSP colonization and the possibility of discussing this information with their family doctor. All samples from individuals who had received antibiotic treatment in the 3 months prior to collection were rejected.

Bacterial identification and *mec*A gene detection. Gram staining and catalase tests were performed on all strains obtained from the culture. The results showed Gram-positive, catalase-positive cocci, being then seeded on Chapman agar, and the bacteria growing on this medium were considered to belong to the *Micrococcaceae* family. These bacteria were subjected to the cefoxitin diffusion test to determine resistance. A 30.00 µg cefoxitin antibiotic disc was used and specific criteria were applied to classify the strains as either susceptible or resistant. Strains found to be resistant to cefoxitin were identified by MALDI-TOF laser-assisted spectrometry (Microflex LT; Bruker Daltonik, Bremen, Germany), cultured in Luria-Bertani enrichment broth and incubated at 37.00 ˚C. Genomic DNA was extracted using the EasyMag® automated extraction system (Biomerieux, Craponne, France), which is based on the use of magnetic particles. An aliquot of 200 µL of culture broth was used and polymerase chain reaction (PCR) was performed to detect the *mec*A gene. The PCR reaction was carried out using specific primers capable of amplifying a 309 bp fragment (positions 318 to 627) of the *mec*A gene. Based on the previously described methodology,[8] the following primers were used: *mec*A-F: 5'-GTAGAAATGACTGAACGT CCGATAA and *mec*A-R: 5'-CCAATTCCACATTGTTTCGGT CTAA. The PCR reaction was performed in a final volume of 50.00 µL containing reaction buffer (1.00 X), $MgCl_2$ (1.50 mM), dNTPs mixture (0.20 mM), 1.00 µM of each primer and 0.50 U of Taq Gold™ DNA polymerase (Thermo Fisher scientific, Madrid, Spain). Each reaction included 5.00 µL of previously extracted DNA. The DNA was amplified in a thermocycler (Eppendorf, Hamburg, Germany) and the amplicons electrophoresed on a 2.00% agarose gel containing 0.50 µg mL^{-1} ethidium bromide. The following strains were used as positive and negative controls for *S. aureus:* MRSA ATCC43300 and penicillin-sensitive *S. aureus* ATCC25923. Positive and negative controls for *S. pseudintermedius* included SPRM C2597 and SPSM C2719 (strains kindly provided by Dr. Carmen Torres, Department of Molecular Biology, University of La Rioja, Logroño, Spain).

Antibiogram by micro-dilution test. All strains with cefoxitin resistance were subjected to an antibiogram of 31 antibiotics by micro-dilution in an external laboratory (Laboklin, Bad Kissingen, Germany). Resistance patterns were determined according to the recommendations of Clinical and Laboratory Standards Institute guidelines,[9] and clinical breakpoints established by the European Committee of Anti-microbial Susceptibility Testing.[10] The antibiotics included were oxacilin, penicillin G, ampicilin, amoxicillin, amoxicillin/clavulanic acid, cefoxitin, cefovecin, cefoperazona, cefquinoma, enrofloxacin, marbofloxacin, ciprofloxacin, difloxacin, ibofloxacin, pradofloxacin, gentamicin, kanamycin, neomycin, spectinomycin, tobramycin, streptomycin, doxycycline, tetracycline, erythromycin spiramycin, clindamycin, lincomycin, chloramphenicol, rifampicin, sulfa-methoxazole-trimetroprim, fusidic acid and polymyxin-B. All *S. pseudintermedius* strains containing the *mec*A gene being not susceptible to at least three or more categories of the following antibiotics were considered MDR *Staphylococcus*: aminoglycosides (gentamicin), ansamycins (rifampicin), fluoroquinolones (enrofloxacin), folate synthesis inhibitors (trimethoprim and sulfamethoxazole), fudicans (fusidic acid), lincosamides (clindamycin), macrolides (erythromycin), phenicols (chloramphenicol), phosphonic acids (fosphomycin), tetracyclines (doxycycline/tetracycline) and phenicols (chloramphenicol).[11]

Pulsed field gel electrophoresis (PFGE) technique. Pulsed-field gel electrophoresis with SmaI digestion (PFGE-SmaI) was used to characterize the isolated MRSP strains in this study. Electrophoresis was performed on a CHEF DR-III instrument (Bio-Rad, Birmingham, UK) for 24 hr at 6.00 V per cm^2 with intervals of 5 to 30 sec. Lambda Ladder PFGE Marker (New England Biolabs, Beverly, USA) was used as a molecular weight marker. Macro-restriction fragments were compared and interpreted visually.

Statistical analysis. Categorical variables are expressed as frequencies and percentages. The prevalence of *Staphylococcus* spp. with the *mec*A gene in environmental and human microbial samples was calculated as a ratio of positive samples to the total, multiplied by 100, with confidence intervals provided (95.00%). Statistical analysis was conducted using Stata Statistical Software (version 13.1; StataCorp, College Station, USA).

Results

In this study, 408 microorganisms were isolated from a total of 123 environmental samples. Of these 408 strains, 12 were fungal contaminants, 263 were Gram-positive bacteria and 133 were Gram-negative bacteria. Among the Gram-positive bacteria, 107 samples (87.00%) belonged to the *Micrococcaceae* family, including 11 coagulase-positive and 96 coagulase-negative strains. Among the coagulase-positive samples,three were resistant to cefotixin and three *S. pseudintermedius* were isolated. Among the coagulase-negative samples, three isolates were resistant to cefotixin, two were *Staphylococcus beta-hemolyticus* and one was identified as *S. epidermidis* (Fig. 2).

No MRSA or *S. schleiferi* subsp. *coagulans* was isolated from the hospital settings. Hence, the prevalence of MRS among the total bacterial population was 1.50%, while it reached 5.60% for the *Micrococcaceae* family. Regarding MRSP, it constituted 0.75% of the overall bacterial count and 2.80% within the *Micrococcaceae* family.

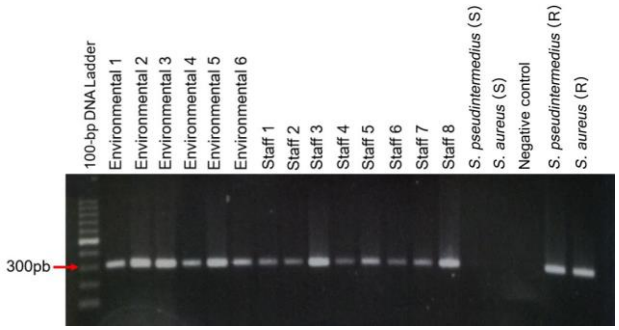

Fig. 2. Results of PCR for cefotixin-resistant staphylococci. S = sensitive and R = resistant.

The MRSPs identified in the study were found in different locations including a small dog hospitalization cage, a small dog hospitalization oxygen cage and a small animal anesthesia recovery cage. In addition, two strains of *S. beta-hemolyticus* were found on the computed tomography table and in a small animal anesthetic recovery cage. The *S. epidermidis* was also isolated from the internal door handle of a small animal practice.

The PFGE results revealed a genetic relationship between the MRSPs found in the oxygen cage and anesthetic recovery cage of small dogs, as they showed a similar pattern of resistance (Fig. 3). A map of the hospital was constructed and genetically related MRSPs were highlighted in blue.

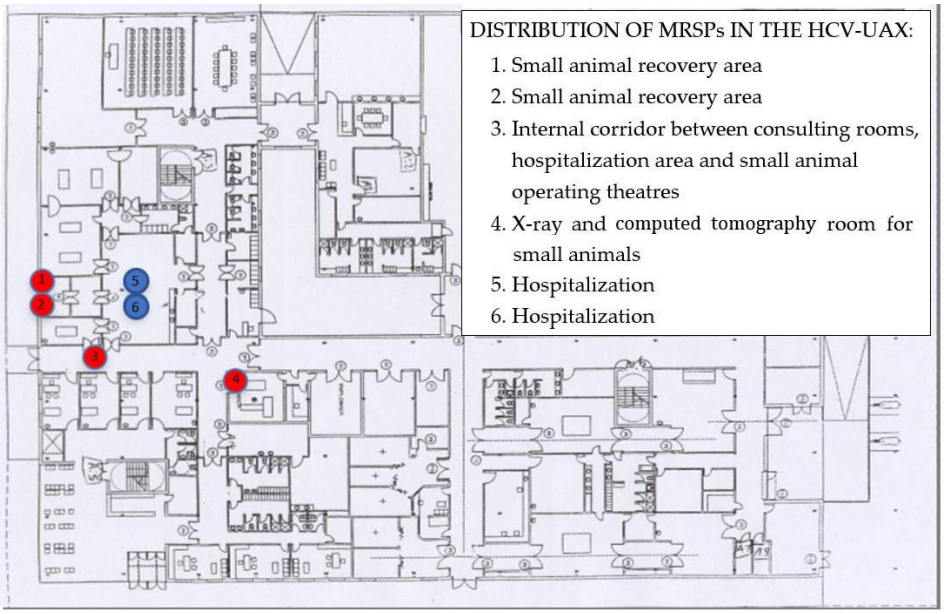

Fig. 3. Distribution of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) in the veterinary clinical hospital (blue and red circles). The blue circles indicate genetically related location of MRSP.

Regarding the samples taken from the hospital personnel, 57 microorganisms were isolated from 42 individuals (54 Gram-positive and three Gram-negative bacteria). All Gram-positive bacteria were members of the *Micrococcaceae* family, of which 17 were coagulase-positive and 37 were coagulase-negative. The prevalence of MRS was 14.03% among the total number of isolated bacteria and 14.80% within the *Micrococcaceae* family. Of the 54 bacteria from the *Micrococcaceae* family, eight were resistant to cefoxitin, identified as one *S. pseudintermedius* and seven *S. epidermidis* (Fig. 2).

Concerning the antibiogram results (Table 1), all *S. pseudintermedius* isolates were resistant to the most of fluoroquinolones tested (enrofloxacin, difloxacin, ibafloxacin and marbofloxacin), but sensitive to pradofloxacin. They were also resistant to gentamicin, streptomycin, spectinomycin, tetracyclines (tetracycline and doxycycline), macrolides (erythromycin and spiramycin), lincosamides (lincomycin and clindamycin), phenicols (chloramphenicol), potentiated sulfamides (trimethoprim-sulfamethoxazole) and polymyxin-B. Resistance to fusidic acid was observed in one of the four isolates (25.00%); while, none was resistant to rifampicin. All *S. pseudintermedius* isolates were considered MDR.

Among coagulase-negative *Staphylococcus* strains detected, four out of 10 isolates (40.00%) were resistant to quinolones; while, five (50.00%) were resistant to aminoglycosides, four (40.00%) to doxycycline, eight (80.00%) to erythromycin and four (40.00%) to clindamycin and lincomycin. None was resistant to chloramphenicol and only one out of 10 was resistant to trimethoprim-sulfamethoxazole and rifampicin.

Within the *S. epidermidis* strains, only two isolates, one from environmental samples and the other from a staff member, did not show MDR characteristics.

Discussion

The prevalence of MRS obtained from the environment was 1.50%. Coagulase-negative MRSP and MRS were isolated with a prevalence of 0.75% each. Among the *S. pseudintermedius* strains isolated, two genetically closely related strains were found in two different locations (one in the anesthesia recovery boxes and the other in an oxygen therapy cage; both in the small animal area). On the other hand, the prevalence of MRS isolated from staff was 14.03%, with one *S. pseudintermedius* and seven *S. epidermidis* strains identified. The presence of methicillin-resistant coagulase-negative staphylococci (MRCoNS) in both the environment and among personnel is of paramount significance in terms of resistance gene transmission. The prevalence of isolated MRS in the environment is consistent with other surveys, ranging from 1.40% to 12.00%.[1,12] The main source of environmental contamination with MRS seems to stem from canine patients.[13] It is worth noting that even in the absence of recognized outbreaks, MRS was present in the environment, albeit at lower percentages.[14] This could possibly be explained by the absence of recognized outbreaks in other studies, leading to lower prevalence rates in these cases. The MRSP was the most common organism detected in other studies. However, in this survey the prevalence of *S. pseudintermedius* was similar to that of MRCoNS, and two *S. hemolyticus* and one *S. epidermidis* were isolated.

Table 1. Resistance (%) of *Staphylococcus pseudintermedius* and methicillin-resistant coagulase-negative staphylococci (MRCoNS).

Antibiotics	*Staphylococcus pseudintermedius* (n = 4)	MRCoNS (n = 10)
Enrofloxacin	4 (100)	4 (40.00)
Marbofloxacin	4 (100)	4 (40.00)
Difloxacin	4 (100)	5 (50.00)
Ibafloxacin	4 (100)	4 (40.00)
Pradofloxacin	0 (0.00)	2 (20.00)
Gentamicin	4 (100)	5 (50.00)
Kanamycin	1 (25.00)	1 (10.00)
Neomycin/Framycetin	1 (25.00)	1 (10.00)
Tobramycin	1 (25.00)	4 (40.00)
Streptomycin	4 (100)	2 (20.00)
Spectinomycin	4 (100)	10 (100)
Doxycycline	4 (100)	4 (40.00)
Tetracycline	4 (100)	5 (50.00)
Erythromycin	4 (100)	8 (80.00)
Spiramycin	4 (100)	2 (20.00)
Clindamycin	4 (100)	4 (40.00)
Lincomycin	4 (100)	4 (40.00)
Chloramphenicol	4 (100)	0 (0.00)
Trimethropim-sulfamethoxazole	4 (100)	1 (10.00)
Fusidic acid	1 (25.00)	4 (40.00)
Polymyxin-B	4 (100)	4 (100)
Rifampicin	0 (0.00)	1 (10.00)

Although their role as pathogens is not well-defined, *S. epidermidis*, *S. warnery*, *S. hominis*, *S. sciuri*, *S. hemolyticus* and *S. xylosus* have been isolated from canine and feline samples;[15] either alone or in association with coagulase-positive staphylococci. The MRCoNS have been described in veterinary hospitals.[16] Although further studies are needed to assess the significance of these organisms in veterinary clinics and hospitals, recent publications have raised concerns about emerging MRCoNS, especially after the coronavirus disease 2019 pandemic.[17]

The MRCoNS exhibit high clonal diversity and represent a reservoir of anti-microbial resistance genes. These bacteria have a higher prevalence of the *mec*A gene, considered a potential reservoir of the staphylococcal cassette chromosome, and pose a potential for cross-species transmission.[18] In prior surveys, concurrent isolation of *S. epidermidis* and *S. beta-hemolyticus* was observed; whereas, an alternative study documented the specific isolation of *S. beta-hemolyticus*. Moreover, previous research identified *S. hominis.*[19] In previous studies, *S. aureus* has been isolated alongside *S. pseudintermedius* in environmental samples.[20,21]

No MRS was isolated in the equine area of the hospital. In this study, *S. delphini*, the predominant species from the *S. intermedius* group affecting horses and not displaying resistance patterns, was found in horses.[22] In addition, the veterinary staff, facilities and most of the clinical material of the hospital are separated by species, which may have contributed to the absence of MRS in the equine area.[22]

Although no predominant clone was found in this study, two strains of bacteria that were highly genetically related in the PFGE test were isolated from different small animal hospital sites. In terms of transmission, it has been hypothesized that the same MRS strain can be transferred between different hospital sites through materials such as blankets, feeding bowls, uniforms and equipment, or through colonization by hospital staff themselves. Staphylococci are spread by direct skin-to-skin contact, sneezing, coughing, dust particles and saliva, and can survive for more than 90 days on hospital fabrics (blankets, sheets, *etc*) and plastic materials used in human hospitals.[23] It can also survive for months in the environment and can be isolated from various hospital surfaces, highlighting the importance of routine disinfection and cleaning to effectively eliminate MRS.[24]

Failure to wash hands after handling pets has been associated with colonization by MRS.[25] Contamination by MRS has also been found in clinical uniforms and mobile phones.[25,26] In terms of resistance patterns, most of the MRS strains were MDR, with only two *S. epidermidis* strains being non-MDR. It is common for MRS to exhibit co-resistance to various combinations of antibiotics, including aminoglycosides, fluoroquinolones, macrolides, lincosamides, tetracyclines, potentiated sulfonamides and rifampicin. The percentage of resistance in this survey was lower in CoNS compared to coagulase-positive strains, as described in other studies.[17,27] The prevalence of MRS and MRSP observed in hospital staff was lower than that reported in other surveys.[28] Nasal carriage of MRSP is now documented in veterinarians and veterinary clinic staff, with a prevalence ranging from 3.00 to 5.30%.[28] The *S. pseudintermedius* does not routinely colonize humans, but humans can become transient carriers if they are in very close contact with an infected patient, resulting in dog-owner transmission, being recently described in several surveys representing an emerging pathogen.[29,30]

Moreover, MRSP colonization was higher in owners of atopic dogs and dogs with pyoderma, as well as in veterinary staff members who are in frequent contact with dogs.[31] The prevalence of MRS in veterinary personnel appears to be influenced by geographical factors. A study conducted in the United States reported prevalence rates ranging from 6.50% to 17.00%, being significantly higher than those in healthy non-veterinarians (≤ 2.00%).[32] In recent years, pet ownership (especially of dogs and cats) has increased significantly.[33] Therefore, bacterial species could be transmitted from dogs to humans (especially dog owners and small animal veterinarians) due to their close contact. The emergence of MRSP represents a loss of anti-microbial efficacy and further complicates the management of MRSP infections in both veterinary and human medicine. Furthermore, global epidemiological reports indicate that most MRSP strains infecting humans belong to dominant clones in Asia and Europe, further confirming their global epidemiological success.[29,30] Guidelines promoting the appropriate use of antibiotics have been published in various countries, particularly in those where animal-assisted therapy is available, such as the German Society for Hospital Hygiene.[34] In cases where dogs need to be screened, such as during outbreak investigations, it is advisable to screen dog's handlers, as cross-transmission cannot be excluded. To date, there is a limited experience with the effectiveness of MRSA/MRSP decolonization therapies in dogs.[35]

Regarding the MRCoNS, seven strains of *S. epidermidis* were isolated, accounting for 12.28% of the total bacteria isolated from the personnel. The MRCoNS are common colonizers of homeowners and pets. In humans, CoNS generally have a benign relationship with the host as saprophytic commensals.[2] They act as opportunistic pathogens and cause infections, particularly in situations associated with hospitalization, prostheses and immuno-suppression (intensive care, premature infants and/or cancer patients).[36] The *S. epidermidis* is the most common CoNS species colonizing human skin and the most common cause of infection and contamination of permanent medical devices.[37] In contrast to other studies, no sample of MRSA was isolated from personnel participating in the survey.[19,29]

"Methicillin-resistance" means resistance to all penicillins and cephalosporins, apart from some new-generation cephalosporins such as ceftobiprole and ceftaroline, regardless of *in vitro* results.[38] Resistance to penicillins and *β*-lactam antibiotics is encoded by the *mec*A gene, containing the penicillin binding protein 2a (PBP2a). Interestingly, among 57 *S. pseudintermedius* strains isolated in another study, only two were resistant to methicillin, but half of them expressed PBP2a, suggesting a genetic capacity for resistance under more optimal conditions.[38] In recently published reviews, low MRSP isolates, defined by an oxacillin minimum inhibitory concentration (MIC) below 4.00 mg L^{-1}, exhibited susceptibility to cephalexin but not to amoxicillin-clavulanic acid, regardless of their strain genotype. This variability in MIC to oxacillin is due to mutations in several PBPs and corresponds to susceptibility to cephalexin.[38] In contrast, all MRSP isolates in our survey were resistant to all penicillins, potentiated penicillins and cephalosporins, except for one environmental strain that was susceptible to cefoperazone (3rd generation cephalosporins) and the staff strain that was susceptible to cefquinome (4th generation cephalosporin). All environmental and human MRSP isolates were resistant to cefovecin.

Regarding CoNS, a recent survey at a veterinary hospital isolated primarily MRCoNS strains.[39] In contrast, our survey revealed that eight out of 10 CoNS isolates displayed *in vitro* susceptibility to cefoperazone; while, nine out of 10 were susceptible to cefquinome and cefovecin. The MRS showed a high degree of resistance to fluoroquinolones, consistent with various publications.[40] Moreover, some studies report a lower *in vitro* resistance rate for pradofloxacin, a 3rd generation quinolone;[41] while, others report resistance rates similar to other fluoroquinolones.[42] Among the aminoglycosides, older drugs such as gentamicin, spectinomycin and streptomycin have shown high resistance rates. In contrast, newer drugs such as amikacin, neomycin/framycetin and tobramycin have demonstrated lower resistance rates, although moderate to high resistance patterns have been reported.[43] Rifampicin has recently gained attention due to its potent activity against MRS.[44] Nonetheless, the presence of resistant strains has also been documented.[45] In this study, *in vitro* resistance to rifampicin was observed at a very low rate. The percentages of resistance to tetracyclines observed in this survey are consistent with those reported in other publications.[46] Generally, tetracyclines are more active *in vitro* than *in vivo* against different *Staphylococcus* species.[47] Resistance percentages observed for fusidic acid were slightly higher than those reported by other authors,[48] although more studies are needed to correlate *in vitro* studies with clinical efficacy.[49] In contrast to the generally low to intermediate resistance percentages observed in MRS for chloramphenicol,[11] this survey revealed a disparity, as 100% of MRSP demonstrated resistance to chloramphenicol; whereas, only 10.00% of MRCoNS exhibited resistance.

Overall, MRS strains showed high resistance rates against potentiated sulfonamides (trimethoprim-sulfamethoxazole), lincosamides (lincomycin and clindamycin), macrolides (erythromycin and spiramycin) and polymyxin-B. This aligns with the consensus in most literature, which does not regard these drugs as suitable therapeutic alternatives for MRS.[50]

In this study, the prevalence and resistance patterns of *Staphylococcus* spp. in samples from the hospital environment and its personnel were determined. Resistant strains of MRS and MRSP have been identified, within the same strain at different locations, indicating potential transmission through hospital staff. However, clones from hospital personnel were not isolated, and no analysis was conducted on animals, leaving the origin of these clones unknown. Inadequate measures against bacterial transmission may result in the transport of MRS across locations. Although some of these bacteria, such as CoNS, might lack pathogenicity, they could serve as carriers for resistance genes that can be transferred to pathogenic strains like coagulase-positive *Staphylococcus*, exacerbating the risk of infections.

Furthermore, concerns arise regarding the potential transmission of infections to both humans and animals through contact with hospital surfaces. The underdeveloped state of hospital preventive medicine, attributed to the lack of specialized training in contrast to human medicine, underscores an opportunity for extended research and advancement. This emphasizes the critical importance of comprehensive training programs and rigorous enforcement of protective measures and hygiene practices among personnel. Additionally, it highlights the necessity for thorough cleaning and disinfection protocols for all surfaces to effectively prevent the transmission of infections within hospital settings. Further research is needed to investigate these aspects, particularly in different geographical regions, to improve our understanding of MRS in veterinary medicine and help address the challenges it presents.

This study provides valuable insights into the prevalence and resistance patterns of MRS in both the environment and among veterinary personnel within a veterinary clinical hospital in Spain. Resistant strains were detected, with a prevalence of 1.50% for MRS in the overall bacterial population; while, MRSP accounted for 0.75% of the total. Antibiotic resistance was notable in MRSP and CoNS, emphasizing MDR in MRSP strains. The present study stresses the importance of infection control measures and further research due to the varying prevalence of MRS and the potential for cross-species transmission.

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Conflicts of interest

The authors declare no conflict of interest.

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