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Epidemiology of the colonization and acquisition of methicillin-resistant staphylococci and vancomycin-resistant enterococci in dogs hospitalized in a clinic veterinary hospital in Spain

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ABSTRACT

Antibiotic resistance is one of the biggest threats to human and animal health. Methicillin-resistant *Staphylococcus* spp. (MRS) and vancomycin-resistant *Enterococcus* spp. (VRE) are of increasing importance in hospital and/or nosocomial infections and represent a potential risk of transmission to humans from infected or colonized companion animals. Studies on the risk factors associated with colonization by multiresistant bacteria in animals are scarce. The present study aimed to estimate the prevalence and incidence of MRS and VRE in canine patients hospitalized in a veterinary hospital and to identify the risk factors for its acquisition and persistence.

Nasal and perianal swabs were obtained from 72 dogs. Antimicrobial susceptibility assays and molecular detection of *mec*A and *van* genes were performed.

A prevalence of 13.9% and incidence of 26.5% was observed in dogs colonized by MRS at hospital admission and release, respectively, higher values than those described in most veterinary studies. Thirty-five *Staphylococcus* isolates had *mec*A gene and showed higher resistance levels to most of the antimicrobials evaluated. Previous and concomitant use of antibiotics and corticosteroids has been associated with an increase in MRS colonization. The use of antibiotics in other animals living with the canine patients has also been identified as an associated factor, suggesting cross transmission. The presence of *van*-resistant genes from *Enterococcus* spp. was not detected.

Pets should be considered possible vehicles of transmission and reservoirs for MRS bacteria and veterinary hospitals should be considered high-risk environments for the occurrence and spread of nosocomial infections and resistant bacteria.

1. Introduction

Antimicrobial resistance (AR) is an increasingly serious threat to global public health that requires coordinated actions to control and minimize its emergence and spread [1]. The World Health Organization (WHO) has issued warning regarding the high level of resistance to antibiotics worldwide, which affects both high- and low-income countries.

The term "multi-resistance" is used when a bacterial strain is resistant to multiple antimicrobial agents, classes or subclasses of antimicrobial agents (frequently when it is resistant to three or more antimicrobial classes) [2].

Infections caused by multidrug-resistant bacteria have a great impact on morbidity and mortality of humans and animals and pose a challenge to clinicians due to the low number of treatment options [3].

For many decades, AR has threatened human health and has been considered a medical concern, resulting in substantial increases of dangerous infections. AR has been associated with lessened treatment

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efficacy, longer hospital stays, increased treatment and post disease costs [4].

Data on antimicrobial drug resistance in companion animals is scarce. However, a progressive increase in antibiotic resistance rates has been observed in bacteria isolated from companion animals [5]. Therefore, animal health care is crucial for AR control as animals can act as reservoirs of resistant organisms and lead to an increased potential for its transmission between humans and their pets. Moreover, veterinary science acknowledges that a unique and critical aspect of AR in pets is their close physical contact with humans [6].

Some works have reported the threat that companion animals colonized with bacteria resistant to all registered veterinary drugs represent to human health [7].

Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus pseudintermedius* (MRSP), as well as other methicillin-resistant *Staphylococcus* (MRS) are acquiring an increasing importance in hospital and healthcare associated infections and represent a potential risk of transmission to humans from infected or colonized companion animals [7].

Coagulase-positive staphylococci, as *Staphylococcus aureus*, *Staphylococcus intermedius* group, particularly *Staphylococcus pseudintermedius*, and *Staphylococcus schleiferi* subsp. *coagulans*, are the most clinically relevant in veterinary medicine. MRS strains have been isolated in dogs [8–11] and more recently, methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) infections were reported in this animal species [12–14]. The rapid spread of MRSP in veterinary small animal practice is of greater concern. It has become a major therapeutic challenge in veterinary practice worldwide as *S. pseudintermedius* is the primary staphylococcal species colonizing healthy dogs and cats [15,16], and also a human health concern due to its potential zoonotic risk [7]. MRSP may cause skin and ear infections, surgical site infections, gingivitis, hepatitis, urinary and respiratory infections, arthritis, peritonitis and septicemia in dogs [17]. Nosocomial outbreaks have also been described [18]. In Europe and North America, MRSP shows resistance to all oral and most parenteral antimicrobials approved for veterinary use [18].

Methicillin resistance is usually conferred by the presence of the *mec*A gene, which encodes for the production of an altered penicillin binding protein (PBP) (PBP2a or PBP2') located on a chromosomal cassette (SCCmec) that has low affinity for all beta-lactam antimicrobials (penicillins, cephalosporins, carbapenems) [19]. Although MRSP is more relevant in animals and rarely colonizes or infects humans, transfer of SCCmec elements and/or other AR genes to other staphylococcal species such as *S. aureus*, a major human pathogen, is possible [7]. Moreover, there is an increasing number of reports on MRSP infection in humans,some associated with dog contact, others not [20–24]. Rates of carriage of MRSP are higher in owners of infected animals and in veterinary surgeons than in the general human population [25,18].

On the other hand, Enterococci, mainly *Enterococcus faecium* and *Enterococcus faecalis,* commensal bacteria of the intestinal microbiota in humans and animals, are among the most important opportunistic pathogens responsible for human nosocomial and animal infections [26]. VRE have emerged as a worldwide health problem. Although ten different genotypes of glycopeptide resistance have been described in enterococci, *van*A and, to a lesser extent, *van*B are the acquired vancomycin resistance genes most widely spread and most worrying in the clinical setting [27]. Several studies suggest that animals carrying VRE in their gastrointestinal tract could be the source of VRE infections to humans [28], capable of transferring resistance genes to other human intestinal bacteria [29]. However, there are only a limited number of studies that discuss the occurrence and impact of VRE in companion animals [30–34].

The epidemiology and risk factors for acquiring multidrug-resistant organisms in humans have been extensively studied and include age, malnutrition, immunological status, hospital stay, associated pathologies, transfusions, inappropriate prescription, inappropriate dispensation and incorrect consumption of antimicrobials, as well as their indiscriminate use in the food industry. However, in veterinary medicine studies are scarce. Therefore it is important to identify factors that increase patients' risk of acquiring a multidrug-resistant infection.

The present study aimed to estimate the prevalence and incidence of MRS and VRE in canine patients hospitalized in a veterinary hospital, and to study the risk factors for the acquisition and persistence of these antimicrobial-resistant bacteria.

2. Materials and methods

2.1. Study design and setting

A prospective longitudinal (incidence) and descriptive cross-sectional study (prevalence) was carried out on small animals during their hospitalization at the Veterinary Hospital of Alfonso X El Sabio University between January and October 2015. Written consent was obtained from the dogs' owners.

2.2. Participants

For this survey, a total of 72 dogs that required hospitalization for a minimum of 24 hours were included in the study. The patients were enrolled in the study consecutively, through a non-probabilistic sample, as they were admitted during the study period. All of them met the established inclusion criteria, including patients who remained hospitalized a minimum of 24 hours, patients who did not die during the first 24 hours of hospitalization and non-aggressive patients.

2.3. Variables

To identify associated factors for MRSP and VRE prevalence and acquisition, the following data were collected from the study population: breed, sex, age, reproductive status, habitat, date of hospital admission, date of hospital release, number of people or pets living in the same household as the dogs, contact with people working in clinics, the existence of previous or present concomitant diseases and antibiotic and anti-inflammatory treatments received before (three-month period prior to entering the study) and during hospitalization.

2.4. Bacterial isolation and identification

Nasal, perianal and rectal samples were collected from each dog with Amies agar gel with charcoal transport swabs at hospital admission and discharge. All samples were transferred in an icebox to the laboratory, where the specimens were kept refrigerated at 4 °C and processed within 12 -hs of sampling.

Nasal and perianal swabs were plated on Brilliance MRSA 2 selective chromogenic agar (Oxoid, Basingstoke, UK) for MRS isolation and rectal swabs were seeded on Brilliance VRE chromogenic agar (Oxoid, Basingstoke, UK) for VRE isolation. All inoculated media were incubated at 37 °C for 24-48 hours. A colony-forming unit (CFU) of each presumptive positive MRS and VRE was selected and sub-cultured on blood agar at 37 °C for 24-48 hours. Species identification was performed by using MALDI-TOF Mass Spectrometry (Bruker Daltonics, Germany). All identified bacterial isolates were stored at −80 °C in a preservative medium containing milk as a cryoprotective agent.

2.5. Detection of mecA and van genes

Isolates that grew in selective chromogenic agar and were identified as *Staphylococcus* spp. were grown in enrichment broth Luria-Bertani (LB) medium and subjected to DNA extraction. The automatic extraction system EasyMag® (Biomerieux, France) based on the use of magnetic particles, was used.

Table 1
Primers used for the determination of *van*A and *van*B resistance genes from *Enterococcus* spp.

Primers[a]	Sequence (5´- 3´)	Gene	Position[b]	PCR product size (pb)	GenBank Accession Nr.
EA1(+)	GGGAAAACGACAATTGC	vanA	176-192	732	M97297
EA2(-)	GTACAATGCCGGCCGTTA		907-891		
EB3(+)	ACGGAATGGGAAGCCGA	vanB	169-185	647	U00456/ AF550667
EB4(-)	TGCACCCGATTTCGTTC		815-799		

[a] +: primer sense; -: primer antisense; [b]Nucleotide numbering begins at the start of the gene codon.

Presence of *mec*A gene was studied by PCR using specific primers designed for the amplification of a 309 bp fragment (positions 318 to 627) of the *mecA* gene: mecA-F: 5'GTAGAAATGACTGAACGTCCGA TAA3' and mecA-R:5'CCAATTCCACATTGTTTTCGGTCTAA 3'. The PCR reaction was performed as described previously [35]. As positive and negative *Staphylococcus aureus* controls, the methicillin-resistant strain (MRSA): ATCC43300 and methicillin-susceptible strain (MSSA): ATCC25923 were used. As positive and negative controls of *Staphylococcus pseudintermedius*, the methicillin-resistant (MRSP) C2597 and methicillin-susceptible (MSSP) C2719 strains were used.

Enterococci growing in Brilliance VRE chromogenic agar and exhibiting a MIC to vancomycin > 4 mg/L or to teicoplanin > 2 mg/L in the antimicrobial susceptibility assay were further screened for *van*-resistance genes. For this purpose, an alkaline lysis method was used for DNA extraction [36] and a multiplex PCR was performed as previously described by Depardieu et al. [37] (Table 1).

2.6. Antimicrobial susceptibility assays

Antimicrobial susceptibility was tested in *Staphylococcus* spp. strains grown in Brilliance MRSA 2 selective chromogenic agar. A microdilution test was performed using Microtiter plates (Micronaut S Kleintiere, Merlin Diagnostika GmbH, Bornheim-Hersel) in the *mec*A positive *Staphylococcus* spp. to determine the resistance patterns to the following antimicrobials: enrofloxacin, marbofloxacin, orbifloxacin, difloxacin, ibafloxacin, pradofloxacin, gentamicin, kanamycin, tobramycin, sulfamethoxazole-trimetroprim, doxycycline, tetracycline, lincomycin, clindamycin, spiramycin, erythromycin, fusidic acid, chloramphenicol, nitrofurantoin, rifampicin, penicillin G, ampicillin, amoxicillin, amoxicillin-clavulanic acid, cephalexin, cefquinome, cefoperazone and cefovecin.

All strains growing in Brilliance VRE chromogenic medium and identified as *Enterococcus* spp. were further screened for vancomycin resistance using gradient diffusion strips, *Etest*® (Biomérieux, France). Additionally, high level streptomycin resistance, levofloxacin and quinupristin-dalfopristin susceptibility was tested by the disk diffusion method and results were interpreted according EUCAST criteria (http://www.eucast.org/clinical_breakpoints/).

2.7. Clonal relatedness

Pulsed-field gel electrophoresis (PFGE) was performed to analyze genetic relatedness of *Staphylococcus* spp. isolates using a CHEF DR-III apparatus (Bio-Rad Laboratories; Hercules, CA, USA), DNA was isolated and restricted with *Apa*I (Promega) and *Sma*I (MBI Fermentas) and PFGE was performed as described by Vela et al. [38]. Gels were stained with ethidium bromide (0.5 μg/ml) for 15 min and photographed under UV light. Lambda ladder PFGE marker (Boehringer Mannheim) was used for molecular weight size determination. Macro restriction fragments were compared and interpreted both visually and with the BioNumerics 4.61 software (Applied Math, St-Martens-Latem, Belgium). Strains with similar pulsed-field pattern were not included in the incidence analysis.

2.8. Statistical analysis

Continuous variables are presented as means and standard deviations, or medians and interquartile ranges. Categorical variables are expressed as frequencies and percentages.

To analyze the influence of different variables in the prevalence of MRS colonization in dogs at hospital admission, a multivariate logistic regression model was created. Variables with $P < 0.100$ were considered clinically relevant and included in the multivariate logistic regression analysis. The final model was built with a stepwise forward selection and backward elimination technique. The significance levels for forward selection were $P < 0.050$ and for backward elimination were $P < 0.100$.

To analyze the influence of different variables on the incidence of MRS during hospitalization, a COX regression model was created. Variables with $P < 0.100$ were considered clinically relevant and included in the multivariate COX regression analysis.

The final model was built with a stepwise forward selection and backward elimination technique. The significance levels for forward selection were $P < 0.050$ and for backward elimination were $P < 0.100$.

All tests were two-sided, and differences were considered statistically significant at $P < 0.05$. Bonferroni adjustments were used to correct for multiple comparisons. Statistical analyses were performed using Stata software version 13.0 (Stata Corp) and the Statistical Package for the Social Sciences (SPSS 15.0, IBM, NY, USA).

3. Results

3.1. Descriptive population analysis

Seventy-two dogs (45.8% males and 54.2% females) were included in this study. The median weight was 14.0 Kg (IQR: 7.0-29.7), and the median age was 6.4 years (IQR: 4, 4-9.3). The most representative breeds were the Yorkshire Terrier and the Belgian Shepherd. Among evaluated patients, 44.4% lived indoors with access to the outdoors, 41.7% lived exclusively inside the household and 13.9% outside (garden or farm). The most common reason for hospital admission was the presence of neurological disease (26.4%), followed by postoperative complications (22.2%) and gastrointestinal disease (13,9%). Most (73.6%) of the animals did not present concomitant diseases, while digestive disease was the most frequent (6.9%) among those who did.

Regarding the treatments received before hospitalization, 12.5% and 19.4% of the dogs had received previous treatment with corticosteroids and antimicrobials, respectively. The average number of people with whom the animal lived was 3 (IQR: 2-4) and 34.7% of the patients lived with other dogs. Additionally, 30.6% patients were gonadectomized and 40.3% of the animals had been previously hospitalized in the last three months.

The patients included in this study were hospitalized a median of 3 days (IQR: 2-4) with a minimum of 24 hours and a maximum of 33 days. During hospitalization, 16.7% and 79.2% of the dogs received treatment with corticosteroids and antibiotic therapy, respectively (50.0% amoxicillin-clavulanate, 29.2% cephalosporins, 25% quinolones, 12.5% metronidazole and 1.4% doxycycline).

Table 2
Frequency distribution of AR of MRS (with the presence of the *mec*A gene). β-lactams have not been included in the table.

Antimicrobial drug	Resistant isolates	
	n/N	%
Enrofloxacin	30/35	85,7
Marbofloxacin	28/35	80,0
Orbifloxacin	30/35	85,7
Difloxacin	30/35	85,7
Ibafloxacin	30/35	85,7
Pradofloxacin	18/35	51,4
Gentamicin	15/35	42,9
Kanamycin	12/35	34,3
Tobramycin	1/7	14,3
Sulfamethoxazole-Trimetroprim	28/35	80,0
Doxycycline	13/35	37,1
Tetracycline	13/35	37,1
Lincomycin	29/35	82,9
Clindamycin	29/35	82,9
Spiramycin	31/35	88,6
Erythromycin	32/35	91,4
Fusidic acid	14/35	40,0
Chloramphenicol	2/35	5,7
Nitrofurantoin	0/35	0,0
Rifampicin	8/35	22,9

Abbreviations: n number of isolates resistant to the specific antibiotic; N total number of isolates with the *mec*A gene.

3.2. *Staphylococcus spp.*

From the samples taken from the nose and perianal area, 45 *Staphylococcus* isolates were recovered from the selective chromogenic medium, 35 of which were resistant to methicillin, as confirmed by PCR. Ten of the 35 strains were isolated at the time of hospital admission and 25 at hospital release. The PFGE results showed that six out of the 10 isolates at admission (6/10) showed > 80% similarity with six isolates of the 25 (6/25) obtained at hospital release, suggesting that 19 out of the 25 strains obtained at the time of hospital release had been acquired at the hospital.

MRS were detected in the nasal, perianal and both locations in 26.9%, 44.4% and 25.9% of the animals, respectively. 88.6% (31/35) of MRS isolates were identified as *Staphylococcus pseudintermedius*, and 11.4% (4/35) as *Staphylococcus haemolitycus*.

The strains of *Staphylococcus* with *mec*A gene (n = 35) had a higher resistance levels to most of the antimicrobials evaluated, obtaining over 80% of strains resistant to half of the studied antimicrobials (Table 2), highlighting the high resistance to quinolones, sulfamides and macrolides. On the other hand, most of the *Staphylococcus* strains not carrying the *mec*A gene were susceptible to all tested antimicrobials.

Overall, the study revealed a MRS colonization prevalence of 13.9% (10/72, 95% CI = 6.9-24.1) at hospital admission.

The multivariable logistic model explaining MRS colonization at hospital admission revealed that corticosteroid administration prior to hospitalization (OR = 14.3, 95% CI = 1.4-147.3, p = 0.025), antimicrobial administration prior to hospitalization (OR = 9.7, 95% CI = 1.4-65.1, p = 0.020), antimicrobial treatment in other animals that coexist with the patient (OR = 30.8, 95% CI = 3.1-300.4, p = 0.003) and female sex (OR = 19.1, 95% CI = 1.5-242.2, p = 0.023) were associated with the colonization of MRS in the dogs. The area under the curve was 0.894 (95% CI 0.80-0.96; p < 0.001). The Hosmer and Lemeshow test was 0.803. The OR results are presented in Table 3.

Although the total number of patients colonized by MRS after hospitalization was 34.7% (25/72), six strains were the same as those isolated before hospitalization, as shown by PFGE results, and 19 strains were new, indicating that hospital-acquired colonization was 26.4% (19/72; 95% CI = 16.7-38.1). The incidence rate of MRS colonization was 6.3 per 100 dogs and day of hospitalization (95% CI = 3.9-10.0), with 297 days of risk exposure.

Table 3
Multivariable model of colonization by MRS at hospital admission.

		OR	95% CI Lower	95% CI Upper	P
Corticoids prior hospitalization					0,025
	No	1	-	-	
	Yes	14,3	1,4	147,3	
Antimicrobials prior hospitalization					0,020
	No	1	-	-	
	Yes	9,7	1,4	65,1	
Antimicrobials other animals					0,003
	No	1	-	-	
	Yes	30,8	3,1	300,4	
Sex					0,023
	Male	1	-	-	
	Female	19,1	1,5	242,2	

During hospitalization, the factors associated with the acquisition of MRS patients observed in the multivariable COX model were the presence of a concomitant disease different from admission to hospitalization (HR = 2.8, 95% CI = 1-7.7, p = 0.026), age over 6.4 years (HR = 2.8, 95% CI = 1-1.8, p = 0.047) and the fact of being colonized by MRS at admission (HR = 3.1, 95% CI = 1.1-8.4, p = 0.027) (Table 4). C de Harrel test was 0.791.

3.3. *Enterococcus spp.*

Of the *Enterococcus* isolates obtained, 53.0% (26/49) were identified as *E. faecium*, 38.8% (19/49) as *E. faecalis* and 8.2% (4/49) as *E. canintestini*. None of them showed a CMI for vancomycin > 4 mg/L or teicoplanin > 2 mg/L, except for one isolate of *E. faecalis* which presented a Minimum Inhibitory Concentration (MIC) for vancomycin > 4. However, the presence of either *van*A or *van*B genes was not detected in this isolate. Therefore, no VRE were isolated from any dog enrolled in the study.

4. Discussion

In this study, samples were taken from the nasal and perianal areas. Both areas of mucocutaneous union are preferred targets for *Staphylococcus* spp. accumulation in healthy dogs [39] and are designated as the best areas for sampling [40–42].

In the present survey, we found a prevalence of 13.9% and incidence of 26.5% of dogs colonized by MRS at admission and release in a reference Veterinary Hospital.

This study reports a higher prevalence of MRS in dogs compared to other studies performed in veterinary hospitals in different countries, which found values between 2 and 6% [43–47]. However, higher prevalence for MRS (specifically methicillin-resistant coagulase-positive staphylococcus, *S. pseudintermedius* and *S. schleiferi* subsp. *coagulans*)

Table 4
Multivariable COX model considering age, concomitant disease and MRS at the time of hospital release.

		HR	95% CI Lower	95% CI Upper	P Breslow
Age					0,047
	≤ 6,4	1	-	-	
	> 6,4	2,8	1	8,1	
Concomitant disease		8			0,026
	No	1	-	-	
	Yes	2,8	1,0	7,7	
MRS at hospital admission					0,027
	No	1	-	-	
	Yes	3,1	1,1	8,4	

have been reported in dogs in Japan, with prevalence values around 30-65% [14,48]. Although prevalence of multidrug-resistant bacteria is increasing in recent years, variability in prevalence rates could be explained by geographical differences, differences in the methods used to detect colonization and antimicrobial use and policy.

Studies in veterinary medicine on the incidence of MRS in dogs and cats are very rare and, to the authors' knowledge, they are absent in Spain. Studies comparing *Staphylococcus* spp. prevalence and incidence in hospitalized animal patients at admission and hospital release are also scarce [49,50]. Therefore, additional studies are needed so that veterinarians from hospitals and small animal clinics are better able to define AR in small animals and be aware of the problem derived from the colonization by MRS in dogs and the risk of intra-hospital dissemination of those strains in order to implement sanitary measures and antibiotic prescription practices.

Among the MRS strains isolated in our study, we found a predominance of *S. pseudintermedius* over *S. haemolitycus*. Additionally, non-resistant isolates of *S. pseudintermedius*, *S. delphini* and *S. aureus* were found. *Staphylococcus aureus* colonization is less common in companion animals, maybe due to the greater affinity of *S. pseudintermedius* for the dogs' corneocytes [51]. *S. haemolitycus* is a coagulase-negative *Staphylococcus* acting as an opportunistic pathogen; however, this species should be taken into consideration as plasmid carriers.

Studies on the risk factors associated with MRS colonization in animals are scarce. In this study, the administration of corticosteroids and antimicrobials prior to hospitalization, treatments with antimicrobials in other animals that live with the patient and female sex were possible associated factors for MRS colonization. On the other hand, during hospitalization, presenting a concomitant disease, age over 6.4 years and being previously colonized by MRS seem to influence the acquisition of these bacterial strains. To the author´s knowledge, no relationship was found between the age and the presence of concomitant diseases in the acquisition and persistence of MRS in veterinary studies. However, in human medicine these factors were associated with an increased risk of infection by MRS [52]. More studies are needed in order to determine the implication of age for MRS colonization.

Antimicrobial use in small animals has been recognized as a risk factor for colonization or infection with resistant bacteria in the present and previous studies [53–55]. Specifically, antimicrobial therapy, hospitalization and surgical interventions could be a risk factor for acquiring MRSP in dogs [39,43,46,53]. These risk factors are similar to those described for MRSA infections in humans, suggesting that the etiology of staphylococcal infections is likely to be similar in dogs. In this study, prolonged hospitalization was related to MRS acquisition in dogs, described also as a factor associated with AR in humans. Other associated factors described in humans are gastrointestinal surgery or transplantation, exposure to invasive devices, underlying diseases, severity of illness and advanced age [56,57]. Owners of MRSA-positive households, healthcare workers or veterinarians, exposure to medical hospitals, extensive wounds, prolonged hospitalization and immunosuppression also constitute possible risk factors for MRSA colonization [58,59]. Further investigation would be needed to better define the risk factors and clinical relevance of colonization in animals, as well as the potential for interspecies transmission and clinical infection.

Antimicrobial treatment in other animals from the patient's household was described in this study as a risk factor for MRS colonization in dogs and could suggest cross-contamination between animal species. Other studies describe the presence of other pets as a significant factor for MRS colonization [60].

Treatment with systemic glucocorticoids has been evaluated in some studies, most of them showing similar results [60,61]. Moreover, in this study, female sex was considered an associated factor for the acquisition of MRS infection. However, some studies in dogs and cats with MRSP [61] show, with non-significant results, a greater predisposition to acquiring these bacterial strains among males.

Dog colonization with MRS harboring the *mec*A gene at hospital admission could be associated with an increase in the acquisition of resistance to methicillin by other *Staphylococcus* clones due to the transmission of the plasmid where the *mec*A gene is inserted [62].

Furthermore, we have detected high resistance profiles associated with the *mec*A gene carriers to quinolones, sulfonamides and macrolides. Most MRS strains showed high percentages of resistance to the antimicrobials most frequently used in small animal veterinary medicine. As observed in other studies [22], many of the *mec*A positive *Staphylococcus* isolates from this study present resistance to other antibiotics besides than β-lactams.

Colonization by different species of *Enterococcus* in dogs was reported in this study, but VRE was not isolated from any dog enrolled in the study, neither upon hospital admission nor during hospitalization.

The prevalence of dogs colonized by *E. faecium* in the study was higher than that of dogs colonized by *E. faecalis* and *E. canintestini*, which differs from other studies [63], maybe due to geographical differences.

VRE have a global and growing impact on human medicine, but only few studies have focused on the resistance of this bacterial species in veterinary medicine. Several studies from different countries report a VRE prevalence in domestic animals ranging between 0% and 76% [31,33,34,63–65], including VRE strains harboring *van*A gene in dogs in Spain [66]. In our study, although several *Enterococcus* spp. strains were identified in dogs, none of the isolated strains had vancomycin resistance genes. This may be due to differences in sampling site, antibiotic use guidelines in each institution or the Spanish antibiotic policy, among others.

Rectal or fecal samples are considered the best choice for the isolation of *Enterococcus* spp. in animals [33,34,67]. However, some authors observed higher prevalence rates when samples were obtained from other locations [63]. As rectal area holds only one third of *Enterococcus* spp. strains that inhabit the patient, it is recommended that other body regions be considered for sampling.

Several bacteria are shared between companion animals and humans. Molecular studies are needed in order to understand the cross transmission from animal to human or *vice-versa*. The use of antimicrobials in companion animals may imply selection and spread of antimicrobial drug resistance to humans. In recent years, the number of pets has increased, and given the close contact that exists between them and their owners, a route of transmission of multiresistant bacteria between both is favored, contributing to the dissemination of resistant strains and constituting a potential risk to public health [68]. Although sample size is a limiting factor, results from this survey contribute to the concern that pets can serve as vehicles of transmission and reservoirs for MRS multiresistant bacteria, as suggested by other authors [7].

Moreover, veterinary hospitals and clinics, which make an intensive use of antimicrobials and have a high density of patients, constitute high-risk environments for the occurrence and spread of nosocomial infections and resistant bacteria [22,69,70]. These places play a role in the dissemination of multidrug resistant bacteria between the animal patients and veterinary practitioners as well as to the environment and society [18]. In the veterinary hospital environment, MRS has been described with greater frequency in laboratories, intensive care units and surgery rooms, compared to consulting rooms. MRS also present in the soil, door handles, hospitalization rooms, in surgically-treated dogs and in veterinarians' clothes [71]. Thus, these environments should be especially considered for the implementation of control measures to reduce AR.

There is a need to deepen the knowledge of the epidemiology and the role of these multiresistant bacteria in small-animal veterinary medicine. Hence, studies of the epidemiology of multidrug-resistant bacteria in human and veterinary medicine, the interaction between humans and animals, antimicrobial use, and infection control are needed. The correct identification of resistance problems, early sampling in risk groups and, most importantly, the responsible and prudent

use of antimicrobials at the veterinary practice level should be urgently implemented to mitigate the risk of MRS infection in dogs. Risk assessment methodology should be used to evaluate new antimicrobial treatment options for bacterial infections in companion animals [7]. Additionally, implementation of the guidelines to prevent contamination in animal clinics and minimize the risk of transferring multidrug-resistant bacteria to other patients is crucial to controlling emerging pathogens, like MRS, in small-animal veterinary medicine [72].

5. Conclusions

The use of antimicrobials in companion animals may imply selection and spread of antimicrobial drug resistance to humans, therefore representing a potential risk to public health. MRS and VRE are of increasing importance in hospital and/or nosocomial infections. In this study, a prevalence of 13.9% and incidence of 26.5% of dogs colonized by MRS was observed at hospital admission and release, and several risk factors were associated with MRS colonization. Thus, pets should be considered as possible reservoirs and vehicles of transmission of MRS multiresistant bacteria to humans, and special attention should be given to veterinary hospitals and clinics for the implementation of control measures to reduce antibiotic resistance.

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CRediT authorship contribution statement

Gustavo Ortiz-Díez: Conceptualization, Data curation, Formal analysis, Investigation, Writing - original draft. **Raúl López:** Methodology, Investigation, Validation, Visualization. **Ana María Sánchez-Díaz:** Investigation, Methodology. **María-Carmen Turrientes:** Investigation, Methodology. **María-Rosario Baquero:** Conceptualization, Funding acquisition, Project administration, Resources, Writing - review & editing. **Ruth Luque:** Investigation, Methodology. **Alba Maroto:** Investigation, Methodology. **Cristina Fernández:** Data curation, Formal analysis. **Tania Aylló:** Conceptualization, Investigation, Project administration, Supervision, Writing - original draft, Writing - review & editing.

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