The SCCmec Types and Antimicrobial Resistance among Methicillin-Resistant Staphylococcus Species Isolated from Dogs with Superficial Pyoderma

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Abstract: This study characterizes clinical methicillin-resistant staphylococcal (MRS) isolates obtained from superficial pyoderma infections in dogs. Our interest was to determine the staphylococcal cassette chromosome mec (SCCmec) type and the antimicrobial susceptibility among MRS isolates from clinical cases. Skin swabs were collected and cultured. Staphylococcus species were identified and characterized with biochemical tests and MALDI-TOF-MS and antimicrobial susceptibility testing by disk diffusion. mecA detection and staphylococcal cassette chromosome mec (SCCmec) typing were achieved by PCR. Of the 65 clinical samples, 56 (86.2%) staphylococcal infections were identified. Twelve (21%) of 56 isolates were MRS infections. All MRS isolates were multidrug resistant. The ccrC and class-C2 mec, which were SCCmec type V, were the most prevalent (66.7%) among the 12 MRS isolates. The predominant SCCmec type V was found in Staphylococcus aureus, Staphylococcus intermedius group, Staphylococcus arlettae, and Staphylococcus xylosus. Treatment failure is a concern with the emergence of highly resistant MRS in dogs associated with superficial pyoderma. The detection of type V SCCmec MRS has previously been reported among veterinarians and dog owners but not in Northern Thailand. These infections serve as a reminder to improve infection prevention and control measures including reducing environmental contamination and potential zoonotic exposures to MRS. In addition, educational awareness of these risks in small animal hospitals needs to be increased among veterinary hospital staff, clients, and patients.

Keywords: pyoderma; type V SCCmec; skin swab; MRS; staphylococcal infection; zoonotic disease

1. Introduction

Infection with methicillin-resistant staphylococci (MRS) is an ongoing and emerging health concern among both humans and animals. Methicillin-resistant Staphylococcus aureus (MRSA) not only causes hospital-, community-, and livestock-associated infections in humans, but it can also lead to infections in pet animals [1]. Methicillin-resistant Staphylococcus pseudintermedius, which belongs to the Staphylococcus intermedius group (MRSIG), can...
cause serious wound infections in domestic companion animals such as dogs and cats. It may also be capable of spreading between animals and humans, including veterinarians, companion animal owners, and veterinary nurses [2,3]. Methicillin-resistant coagulase-negative staphylococci can persist in the patient environment and serve as a source of human infection in hospital settings [4]. MRS is recognized as a public health threat and is a significant opportunistic pathogen in both human and veterinary medicine [5].

The evolution of MRS emerged from the development of the penicillin-binding protein 2a (PBP2a), which is encoded by the \textit{mecA} gene [1]. This protein has a significantly lower affinity for beta-lactams and thus cell wall synthesis can continue even in the presence of normally inhibitory concentrations of beta-lactam antibiotics. Thus, the detection of \textit{mecA} by polymerase chain reaction (PCR) is used to characterize MRS [2,5]. The \textit{mecA} gene is the mobile genetic element located in the chromosome of \textit{Staphylococcus} known as the staphylococcal cassette chromosome \textit{mec}, or SCC\textit{mec} [6]. There are four important components of the SCC\textit{mec} element: (1) the group comprised of the \textit{mec} genes; the \textit{mecA} gene and the regulatory genes, (2) the group comprised of the \textit{ccr} genes responsible for the mobility of the SCC\textit{mec} element, (3) the direct repeated nucleotide at the end of both sides of SCC\textit{mec} making the movable structure of the SCC\textit{mec} element, and (4) the 3' ending part of the \textit{orfX} gene [7]. Currently, SCC\textit{mec} is classified into 13 types, type I to type XIII, according to the differences of the \textit{mec} gene and the \textit{ccr} gene [8]. A multiplex PCR is used to determine the structure of the \textit{mec} complex, while the presence of different \textit{ccr} genes is a popular method for SCC\textit{mec} typing [9]. Staphylococcal infections in companion animals, particularly in dogs and cats, include superficial pyoderma, otitis externa, and superficial bacterial folliculitis. Occasionally, MRS is also detected among companion animal owners. These are mostly multidrug-resistant MRS infections [10,11]. According to prior reports, the identification of various MRS isolates, such as MRSA, MRSIG, and MRCNS, are rarely reported in Thailand. The objectives of this study were to characterize the type of staphylococcal infections associated with superficial pyoderma among dogs at the Small Animal Teaching Hospital, Chiang Mai University, Thailand.

2. Materials and Methods

2.1. Data and Sample Collection

Cases of superficial pyoderma in dogs were identified by clinical staff at our teaching hospital from 2015 to 2017. Sixty-five dogs presenting with superficial pyoderma at the Small Animal Teaching Hospital, Faculty of Veterinary Medicine, Chiang Mai University, Thailand were swabbed and submitted to the veterinary diagnostic laboratory for microbiological analysis.

2.2. Bacterial Isolation and Identification

The skin swabs were kept in Stuart transport medium (BD BBLTM, US) and submitted to the microbiology laboratory within 4–6 h for bacterial identification and antimicrobial susceptibility testing. Standard bacterial isolation procedures were used as follows. Staphylococcal isolates were identified in cultures using 5% of sheep blood agar (Merck, Darmstadt, Germany) along with a biochemical testing process that employed Gram stain, catalase, tube coagulase, Voges–Proskauer (VP) test, and the clumping factor [12]. All staphylococcal species were confirmed by MALDI-TOF-MS analysis.

2.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed by the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) recommendations [13]. Sixteen antimicrobial agents, including penicillin, ampicillin, cefoxitin, amoxicillin/clavulanate, cefazolin, cefpodoxime, amikacin, gentamicin, doxycycline, ciprofloxacin, chloramphenicol, clindamycin, erythromycin, linezolid, rifampin, and trimethoprim/sulfamethoxazole, were used in this study. The minimum inhibitory concentration (MIC) of oxacillin was determined for all \textit{Staphylococcus} isolates by Vitek 2 system. All MRS isolates were iden-
tified using the CLSI oxacillin MIC breakpoints: MIC ≥ 4 µg/mL for *S. aureus* and MIC ≥ 0.5 µg/mL for other *Staphylococcus* spp. [13]. *Staphylococcus aureus* ATCC 25923 was used as a quality control strain.

2.4. PCR Detection of mecA Gene

DNA was extracted using a commercial test kit for genomic DNA obtained from tissue samples (NucleoSpin R Tissue). The mecA genotype of staphylococci was characterized. The 50-µL PCR reaction mixtures contained 100 ng chromosomal DNA, oligonucleotide primers (10 pmols), 2.5 mM each deoxynucleotide triphosphates, Taq buffer, and 5 U Taq polymerase (I-TagTM plus iNtRON Biotechnology, Korea) at a final volume of 50 µL. A PCR thermal cycler was used for amplification with an initial denaturation step (94 °C, 5 min) comprised of 40 cycles of denaturation (95 °C, 45 s), an annealing step (59 °C, 45 s), and an extension step (72 °C, 45 s); and a final extension step at 72 °C for 5 min. Regarding the primer pairs used for PCR experiments, the forward primer was 5′-TGGCTATCGTGTCACAATCG-3′, the reverse primer was 5′-CTGGAACTTTGT TGAGCAGAG-3′, and the product size was 309 base pairs [14].

2.5. SCCmec Typing

The SCCmec typing of all mecA gene-positive staphylococcal isolates was performed using the multiplex PCR, M-PCR1, and MPCR2, as previously described by [15]. The M50-µL reaction mixtures of M-PCR1 contained 100 ng chromosomal DNA, oligonucleotide primers (0.1 µM), 200 µM each deoxynucleotide triphosphates, Taq buffer, and 2.5 U DreamTaq DNA polymerase (Thermo Fisher Scientific UAB, Vilnius, Lithuania) at a final volume of 50 µL. The concentration of MgCl$_2$ was 3.2 mM. A PCR thermal cycler was used for amplification with an initial denaturation step (94 °C, 2 min) comprised of 30 cycles of denaturation (94 °C, 2 min), an annealing step (57 °C, 2 min), and an extension step (72 °C, 2 min); and a final extension step at 72 °C for 2 min. The 50-µL reaction mixture of M-PCR2 contained the same components as the M-PCR1 except that the concentration of MgCl$_2$ was 2 mM and the annealing temperature was raised to 60 °C for 2 min. The primer pairs used for PCR experiments are listed in Tables S1 and S2. The M-PCR 1 for ccr type assignment contained two primers used to detect mecA and eight primers used for the identification of five ccr genes: four primers including a common forward primer (common to ccrB1–3) and three reverse primers specific for ccrA1, ccrA2, and ccrA3 used to identify ccr1–3 based on the differences present in the ccrA genes; two primers used to identify ccr4; and two primers used to identify ccr5. Furthermore, the M-PCR2 for mec class assignment contained four primers that were used to identify the gene lineages of mecA–mecI (class A mec), mecA-IS1272 (class B mec) and mecAIS431 (class C mec). The positive control strains for SCCmec typing were 4 MRSA strains, including epidemic MRSA (EMRSA)-8 (SCCmec type I), N315 (SCCmec type II), EMRSA-4 (SCCmec type III), and EMRSA-10 (SCCmec type IV).

2.6. Statistical Analysis

Percentages of antimicrobial susceptibility were calculated for staphylococci and MRS isolates. The antimicrobial susceptibility was categorized as 3 groups, including susceptible, intermediate, or resistant, according to the breakpoint of the inhibition zone or MIC as recommended by CLSI M100-S26 guidelines [13]. Data were visualized and analyzed using R version 4.0.3 [16].

3. Results

3.1. Staphylococcal Infections in Dogs with Superficial Pyoderma

Staphylococci were isolated from 56 (86.2%) of 65 clinical samples. Two isolates (3.1%) were identified as *Staphylococcus aureus* and 50 isolates (76.9%) as the *S. intermedius* group. In addition, one isolate each of coagulase-negative staphylococci, *S. lentus*, *S. xylosus*, *S. lugdunensis*, and *S. arlettae*, were found. For nine culture-negative samples, non-*Staphylococcus*
bacteria were detected including beta-hemolytic Streptococcus Group C, Escherichia coli, Aerococcus viridans, Proteus mirabilis, Pseudomonas aeruginosa, Rothia nasimurium, Klebsiella pneumoniae, Moraxella sp., Corynebacterium auriscanis, and Enterococcus faecalis. From available information, the mean age of the patients was 6.2 years of age (range from 0.5 to 14 years). Twenty-eight dogs were intact males, 20 were female, and one was a spayed female. Eight (16%) dogs presented with papules, 19 (34%) presented with pustules, 35 (63%) presented with crusts, and 20 (36%) presented with epidermal collarettes. Eleven patients had recurring pyoderma and 37 had other underlying medical conditions such as atopic dermatitis, food allergies, demodicosis, hyperadrenocorticism, or hypothyroidism. Fifteen (31%) of 49 dogs with reviewable records were previously treated with antibiotics (cephalexin \( n = 8 \), amoxicillin–clavulanate \( n = 4 \), doxycycline \( n = 3 \)).

3.2. Antimicrobial Susceptibility Testing Results

Most staphylococci were found to be resistant to penicillin and ampicillin. All staphylococci were susceptible to linezolid and rifampin (Table 1). Twelve staphylococcal isolates were resistant to oxacillin and cefoxitin and were confirmed as MRS isolates. One isolate was S. aureus, eight isolates were in the S. intermedius group, and three isolates were identified as coagulase-negative staphylococci: S. lentus, S. xylosus, and S. arlettae. All MRS isolates were resistant to penicillin, cephalosporin, and fluoroquinolone. Notably, the mecA gene was found to be present in all methicillin-resistant staphylococci (Figure 1). Epidemiologic data were available for 10 dogs. The mean age of these 10 dogs was 6.3 years of age (ranging from 1 to 13 years). Six of the dogs were intact males and four were female dogs. Two (20%) dogs presented with papules, four (40%) presented with pustules, eight (80%) presented with crusts, and four (40%) presented with epidermal collarettes. Five of ten dogs had recurring pyoderma and five had underlying medical conditions. Furthermore, eight of the ten subjects had previously been prescribed antibiotics.

| Antimicrobial Susceptibility Test Result of 56 Staphylococcal Isolates from Superficial Pyoderma Dogs |
|------------------------------------------------------------------------------------------------|
| Antimicrobial Agent | Antimicrobial Susceptibility n (%) |
|---------------------|-----------------------------------|
|                     | Susceptible | Intermediate | Resistant |
| Penicillin          | 19 (33.93%) | 0 (0%)       | 37 (66.07%) |
| Ampicillin          | 21 (37.50%) | 0 (0%)       | 35 (62.50%) |
| Cefoxitin           | 44 (78.57%) | 0 (0%)       | 12 (21.43%) |
| Amoxicillin/clavulanate | 43 (76.79%) | 0 (0%)       | 13 (23.21%) |
| Cefazolin           | 43 (76.79%) | 0 (0%)       | 13 (23.21%) |
| Cefpodoxime         | 43 (76.79%) | 0 (0%)       | 13 (23.21%) |
| Amikacin            | 38 (67.86%) | 15 (26.79%)  | 3 (5.36%)  |
| Gentamicin          | 39 (69.64%) | 14 (25%)     | 3 (5.36%)  |
| Doxycycline         | 52 (92.86%) | 4 (7.14%)    | 0 (0%)     |
| Ciprofloxacin       | 44 (78.57%) | 0 (0%)       | 12 (21.43%) |
| Chloramphenicol     | 46 (82.14%) | 2 (3.57%)    | 8 (14.29%) |
| Clindamycin         | 38 (67.86%) | 6 (10.71%)   | 12 (21.43%) |
| Erythromycin        | 37 (66.07%) | 5 (8.93%)    | 14 (25%)   |
| Linezolid           | 56 (100%)   | 0 (0%)       | 0 (0%)     |
| Rifampin            | 56 (100%)   | 0 (0%)       | 0 (0%)     |
| Trimethoprim/Sulfamethoxazole | 38 (67.86%) | 7 (12.50%)   | 17 (30.36%) |

3.3. SCCmec Types of Methicillin-Resistant Staphylococcal Isolates

There were 12 isolates of MRS that were SCCmec typed using the multiplex PCR. M-PCR1 successfully amplified DNA fragments corresponding in size to each ccr gene (Figure 2) and M-PCR2 was applied to assign the mec class (Figure 3). Characterization results from all 12 MRS isolates revealed that ccrC and class-C2 mec were found from one isolate (8.3%) of S. aureus; thus, it could be classified as SCCmec type V. These components were also found in four isolates (33.3%) of the S. intermedius group. The ccrA1B1, ccrC,
and class-C2 mec were found in four isolates (33.3%) of the *S. intermedius* group and they were categorized as SCCmec non-typeable strains. The coagulase-negative staphylococci, including *S. lentus, S. xylosus, and S. arlettae*, were found to carry *ccrC* and class-C2 *mec*. These strains were classified as SCCmec type V (Table 2).

![Figure 1](image1.png)

**Figure 1.** Results of *mecA* gene (309 bp) detection by the conventional PCR among 12 MRS strains. Lane 1, 100 bp plus marker; lane 2 (CMU1) and lane 3 (CMU2), samples with undetected *mecA* gene; lane 4 (CMU3), lane 5 (CMU68), lane 6 (CMU27), lane 7 (CMU29), lane 8 (CMU52), lane 9 (CMU62), lane 10 (CMU88), and lane 11 (CMU71), samples with detected *mecA* genes; lane 12 (CMU64) as a positive control for which *mecA* was detected using DNA sequence method; lane 13, negative control (distilled water).

![Figure 2](image2.png)

**Figure 2.** M-PCR1 for identification of *ccr* genes for assignment of the type of *ccr* gene complex. Lane 1, 100 bp plus marker; lane 2, positive control types I *ccrA1B1* (695 bp); lane 3, positive control type II *ccrA2B2* (937 bp); lane 4, positive control type III *ccrA3B3* (1791 bp) and *ccrC* (518 bp); lane 5, positive control type IV *ccrA2B2* (937 bp) and *ccrC* (518 bp); lane 6, positive control type IX *ccrA1B1* (695 bp); lane 7 (CMU64), lane 8 (CMU3), lane 9 (CMU27), lane 11 (CMU29), lane 12 (CMU33), and lane 13 (CMU42), samples with *ccrC* (518 bp); lane 10 (CMU52), lane 14 (CMU62), lane 15 (CMU68), and lane 16 (CMU88), samples with *ccrA1B1* (695 bp) and *ccrC* (518 bp); lane 17, negative control (distilled water). The 286 bp amplification products that appear in each lane represent *mecA*. 
Figure 3. M-PCR2 for identification of three gene alleles for assignment of the mec gene complex. Lane 1, 100 bp plus marker; lane 2, positive control type I mec class B (2827 bp); lane 3, positive control type II mec class A (1963 bp); lane 4, positive control type III mec class A (1963 bp); lane 5, positive control type IV mec class B (2827 bp); lane 6, positive control type IX mec class C2 (804 bp); lane 7 (CMU64), lane 8 (CMU3), lane 9 (CMU27), lane 10 (CMU52), lane 11 (CMU29), lane 12 (CMU30), lane 13 (CMU42), lane 14 (CMU62), lane 15 (CMU68), and lane 16 (CMU88), sample mec class C2 (804 bp); lane 17, negative control (distilled water).

Table 2. Results of methicillin-resistant staphylococci by oxacillin MIC, cefoxitin disk diffusion test, mecA gene detection, and SCCmec typing.

| NO. | Isolate ID | Bacteria                        | Oxacillin MIC (µg/mL) | Cefoxitin (30 µg) Disk Diffusion Test | mecA Gene | ccr Gene Complex | mec Gene Complex | SCCmec Typing |
|-----|------------|---------------------------------|-----------------------|---------------------------------------|-----------|------------------|------------------|---------------|
| 1   | CMU 3      | *S. pseudintemidius*/S. intermedius | >4, Resistant         | NA                                    | Positive  | C                | C2               | V             |
| 2   | CMU 27     | *S. pseudintemidius*/S. intermedius | >4, Resistant         | NA                                    | Positive  | C                | C2               | V             |
| 3   | CMU 29     | *S. pseudintemidius*/S. intermedius | >4, Resistant         | NA                                    | Positive  | C                | C2               | V             |
| 4   | CMU 33     | *Staphylococcus lentus*          | 0.5, Resistant        | Resistant                             | Positive  | C                | C2               | V             |
| 5   | CMU 42     | *Staphylococcus arlettae*        | 1, Resistant          | Resistant                             | Positive  | C                | C2               | Non-typeable   |
| 6   | CMU 52     | *S. pseudintemidius*/S. intermedius | >4, Resistant         | NA                                    | Positive  | A1B1, C          | C2               | V             |
| 7   | CMU 61     | *Staphylococcus xylosus*         | 0.5, Resistant        | Resistant                             | Positive  | C                | C2               | V             |
| 8   | CMU 62     | *S. pseudintemidius*/S. intermedius | 1, Resistant          | NA                                    | Positive  | A1B1, C          | C2               | Non-typeable   |
| 9   | CMU 64     | *Staphylococcus aureus*          | >4, Resistant         | Resistant                             | Positive  | C                | C2               | V             |
| 10  | CMU 68     | *S. pseudintemidius*/S. intermedius | 1, Resistant          | NA                                    | Positive  | A1B1, C          | C2               | Non-typeable   |
| 11  | CMU 71     | *S. pseudintemidius*/S. intermedius | >4, Resistant         | NA                                    | Positive  | C                | C2               | V             |
| 12  | CMU 88     | *S. pseudintemidius*/S. intermedius | >4, Resistant         | NA                                    | Positive  | A1B1, C          | C                | Non-typeable   |

NA, not applicable.

4. Discussion

This study represents, to our knowledge, the first molecular characterization of MRS isolates obtained from canines with superficial pyoderma in Northern Thailand. The presence of mecA gene encoding PBP2a in all MRS isolates was confirmed along with the antimicrobial susceptibility profiles highlighting potential treatment failures with commonly used antimicrobials [17]. One MRSA isolate was recovered from a dog with superficial pyoderma. This emphasizes the need for clinical awareness of antimicrobial-resistant organisms and potential treatment failures and the need for optimum infection...
control measures in clinical settings to prevent healthcare-associated infections. Direct contact with an infected animal can be a risk for human pet owners, especially those with underlying health conditions [18]. Dogs are at risk for carrying MRS and MRSA and can be colonized with resistant strains without showing clinical signs [10,19,20]. Transmission can occur through close contact [21,22]. These findings highlight the need for veterinarian awareness of potential multidrug-resistant infections, and the importance of providing client information about the management and care of patients and the appropriate hand hygiene practices to reduce the transmission risk of multidrug-resistant infections between animals and humans.

Staphylococcal infections in domestic companion animals, especially in dogs and cats, can commonly cause superficial pyoderma, otitis externa, superficial bacterial folliculitis, and bacterial rhinitis. The main cause of these infections are often *S. pseudintermedius* [10,23,24]. The *S. intermedius* group (SIG) accounted for the majority of the isolates recovered from clinical pyoderma cases in this study. These SIG isolates from dogs with pyoderma could not be conclusively identified in the absence of molecular testing and they were indicated as *S. pseudintermedius/S. intermedius*. Subsequent analysis of two strains by MALDI-TOF-MS systems (VITEK MS, bioMérieux, Marcy l’Etoile France) gave the same results. Although *S. pseudintermedius* has been recognized as a major pathogen among SIG isolates, the multiplex PCR method for species identification of coagulase-positive staphylococci by targeting the nuc gene locus [25] should be performed. Currently, the concept of SIG is becoming more and more broad. Recently, two species, *S. cornubiensis* and *S. ursi*, have been added to the SIG [26,27]; however, they were not present in the dogs tested here. There are some reports which document MRS infection in companion animals, their owners, veterinarians, and other animal caretakers [22,28–31]. MRS can readily spread and can contaminate the immediate environment, including surfaces in veterinary clinics [32].

Previous studies have documented the increasing frequency of the recovery of MRS in canine pyoderma [33,34]. These multidrug-resistant infections identified in companion animals are difficult to treat [2,35]. For our study, MRS isolates were resistant to fluoroquinolones. The International Society for Companion Animal Infectious Diseases (ISCAID) has devised some important diagnostic and treatment approaches [36]. It is, therefore, necessary and important for Thailand and other South East Asian countries with limited culture and epidemiologic data to recognize the emergence of resistant staphylococcal infections and to follow appropriate antimicrobial stewardship principles. Awareness of the emergence of MRS is important to veterinary practitioners and future veterinarians. Due to the limited effectiveness of antimicrobials to treat MRS, good hygienic and infection control practices are needed to prevent potential environmental contamination and spread to other animals and humans.

Our study also documented that staphylococcal isolates contained SCCmec types V. This SCCmec type has previously been found in dogs living in the central part of Thailand [10]. However, these MRS strains were isolated by swabbing the nares and perineum of healthy dogs and not from the dogs with pyoderma. In addition, SCCmec type V MRS samples were isolated from veterinarians and the owners of dogs in the study area [10]. Recently, studies of SCCmec typing in cats and dogs from other geographic areas indicated the presence of type V and type VII SCCmec MRS in Asia. The type V SCCmec was the most commonly detected type in MRS in Europe and America [11,37–40].

5. Conclusions

MRS, including *S. aureus*, *S. intermedius* group, and coagulase-negative *Staphylococcus* with multidrug resistance phenotypes, was isolated from dogs with superficial pyoderma. These MRS infection findings pose certain diagnostic and treatment challenges for South East Asian veterinary practitioners and highlight the need for improved antimicrobial stewardship and hygienic practices. This includes diagnostic recognition and new treatment approaches. It also reminds veterinarians about the potential for zoonotic transmission
of multidrug-resistant organisms. Veterinarians should consider appropriate treatment options, including effective topical treatments for mild cases to reserve the use of important parenteral treatments if needed. Newly released recommendations can serve as established approaches for the treatment of superficial pyoderma. The emergence of type V SCC<em>mec</em> MRS has broad implications for companion animals, pet owners, veterinarians, and animal caretakers. The emergence of these MRS strains can contaminate the hospital environment and are a public health concern.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/vetsci8050085/s1, Table S1: M-PCR1 for amplification of <em>ccr</em> gene complex type with <em>mecA</em>, Table S2: M-PCR2 for amplification of <em>mec</em> gene complex class.

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**Informed Consent Statement:** Informed Consent Statement was obtained from the owners of Sixty-five dogs presenting with superficial pyoderma.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author. The data are not publicly available due to institutional privacy policy.

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**References**

1. Pomba, C.; Rantala, M.; Greko, C.; Baptiste, K.E.; Catry, B.; van Duijkeren, E.; Mateus, A.; Moreno, M.A.; Pyörälä, S.; Ružauskas, M.; et al. Public health risk of antimicrobial resistance transfer from companion animals. *J. Antimicrob. Chemother.* 2017, 72, 957–968. [CrossRef] [PubMed]

2. Couto, N.; Monchique, C.; Belas, A.; Marques, C.; Gama, L.T.; Pomba, C. Trends and molecular mechanisms of antimicrobial resistance in clinical staphylococci isolated from companion animals over a 16 year period. *J. Antimicrob. Chemother.* 2016, 71, 1479–1487. [CrossRef] [PubMed]

3. Wu, M.T.; Burnham, C.A.; Westblade, L.F.; Dien Bard, J.; Lawhon, S.D.; Wallace, M.A.; Stanley, T.; Burd, E.; Hindler, J.; Humphries, R.M. Evaluation of oxacillin and cefoxitin disk and MIC Breakpoints for prediction of methicillin resistance in human and veterinary isolates of *Staphylococcus intermedius* group. *J. Clin. Microbiol.* 2016, 54, 535–542. [CrossRef] [PubMed]
4. Wilson, A.P.; Smyth, D.; Moore, G.; Singleton, J.; Jackson, R.; Gant, V.; Jeanes, A.; Shaw, S.; James, E.; Cooper, B.; et al. The impact of enhanced cleaning within the intensive care unit on contamination of the near-patient environment with hospital pathogens: A randomized crossover study in critical care units in two hospitals. Crit. Care Med. 2011, 39, 651–658. [CrossRef] [PubMed]

5. Igbinosa, E.O.; Beshiru, A.; Akporehe, L.U.; Ogofure, A.G. Detection of methicillin-resistant staphylococci isolated from food producing animals: A public health implication. Vet. Sci. 2016, 3, 14. [CrossRef] [PubMed]

6. IWG-SCC. Classification of staphylococcal cassette chromosome mec (SCCmec): Guidelines for reporting novel SCCmec elements. Antimicrob. Agents Chemother. 2009, 53, 4961–4967. [CrossRef] [PubMed]

7. Turlej, A.; Hryniewicz, W.; Empel, J. Staphylococcal cassette chromosome mec (SCCmec) classification and typing methods: An overview. Pol. J. Microbiol. 2011, 60, 95–103. [CrossRef]

8. Baig, S.; Johannesen, T.B.; Overballe-Petersen, S.; Larsen, J.; Larsen, A.R.; Stegger, M. Novel SCCmec type XIII (9A) identified in an ST152 methicillin-resistant Staphylococcus aureus. Infect. Genet. Evol. 2018, 61, 74–76. [CrossRef]

9. Deurenberg, R.H.; Stobberingh, E.E. The evolution of Staphylococcus aureus. Infect. Genet. Evol. 2008, 8, 747–763. [CrossRef]

10. Chanchaithong, P.; Perreten, V.; Schwendener, S.; Tribuddharat, C.; Chongthaleong, A.; Niyomtham, W.; Prapasarakul, N. Strain typing and antimicrobial susceptibility of methicillin-resistant coagulase-positive staphylococcal species in dogs and people associated with dogs in Thailand. J. Appl. Microbiol. 2014, 117, 572–586. [CrossRef]

11. Van Duijkeren, E.; Catry, B.; Greko, C.; Moreno, M.A.; Pomba, M.C.; Pyörälä, S.; Sanders, P.; Threlfall, E.J.; Torren-Edo, J.; et al. Review on methicillin-resistant Staphylococcus pseudintermedius. J. Antimicrob. Chemother. 2011, 66, 2705–2714. [CrossRef] [PubMed]

12. Baron, E.J.; Peterson, L.R.; Finegold, S.M. Baily & Scotts Diagnostic Microbiology, 9th ed.; Mosby-Yearbook, Inc.: Missouri, MO, USA, 1994; pp. 321–330, ISBN 0-8016-6987-1.

13. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. CLSI Supplement M100, 30th ed.; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2020.

14. Alipour, F.; Ahmadi, M.; Javadi, S. Evaluation of different methods to detect methicillin resistance in Staphylococcus aureus (MRSA). J. Infect. Public Health 2014, 7, 186–191. [CrossRef] [PubMed]

15. Kono, Y.; Ito, T.; Ma, X.X.; Watanabe, S.; Kreiswirth, B.N.; Etienne, J.; Hiramatsu, K. Combination of multiplex PCRs for staphylococcal cassette chromosome mec type assignment: Rapid identification system for mec, ccr, and major differences in junkyard regions. Antimicrob. Agents Chemother. 2007, 51, 264–274. [CrossRef]

16. RCoreTeam. R: A Language and Environment for Statistical Computing. Available online: https://www.R-project.org/ (accessed on 11 February 2021).

17. Loeffler, A.; Lloyd, D.H. What has changed in canine pyoderma? A narrative review. Vet. J. 2018, 235, 73–82. [CrossRef] [PubMed]

18. Köck, R.; Ballhausen, B.; Bischoff, M.; Cuny, C.; Eckmann, T.; Fetsch, A.; Harmsen, D.; Goerge, T.; Oberheitmann, B.; Schwarz, S.; et al. The impact of zoonotic MRSA colonization and infection in Germany. Berl. Munch. Tierarztl. Wochenschr. 2014, 127, 384–398. [PubMed]

19. Catry, B.; Van Duijkeren, E.; Pomba, M.C.; Greko, C.; Moreno, M.A.; Pyörälä, S.; Ruzauskas, M.; Sanders, P.; Threlfall, E.J.; Ungemach, F.; et al. Reflection paper on MRSA in food-producing and companion animals: Epidemiology and control options for human and animal health. Epidemiol. Infect. 2010, 138, 626–644. [CrossRef] [PubMed]

20. Bramble, M.; Morris, D.; Tolomeo, P.; Lautenbach, E. Potential role of pet animals in household transmission of methicillin-resistant Staphylococcus aureus: A narrative review. Vector Borne Zoonotic Dis. 2011, 11, 617–620. [CrossRef]

21. Kaspar, U.; von Lützau, A.; Schlattmann, P.; Roesler, U.; Köck, R.; Becker, K. Zoonotic multidrug-resistant microorganisms among small companion animals in Germany. PLoS ONE 2018, 13, e0208364. [CrossRef]

22. Ishihara, K.; Saito, M.; Shimokubo, K.; Muramatsu, Y.; Maetani, S.; Tamura, Y. Methicillin-resistant Staphylococcus aureus carriage among veterinary staff and dogs in private veterinary clinics in Hokkaido, Japan. Microbiol. Immunol. 2014, 58, 149–154. [CrossRef]

23. Menandro, M.L.; Dotto, G.; Mondin, A.; Martini, M.; Ceglie, L.; Pasotto, D. Prevalence and characterization of methicillin-resistant Staphylococcus pseudintermedius from symptomatic companion animals in Northern Italy: Clonal diversity and novel sequence types. Comp. Immunol. Microbiol. Infect. Dis. 2019, 66, 101331. [CrossRef]

24. González-Domínguez, M.S.; Carvajal, H.D.; Calle-Echeverri, D.A.; Chinchilla-Cárdenas, D. Molecular Detection and Characterization of the mecA and nuc genes from staphylococcus species (S. aureus, S. pseudintermedius, and S. schleiferi) isolated from dogs suffering superficial pyoderma and their antimicrobial resistance profiles. Front. Vet. Sci. 2020, 7, 376. [CrossRef]

25. Sasaki, T.; Tasukahata, S.; Tanaka, Y.; Sakusabe, A.; Ohtsuka, M.; Hirota, S.; Kawakami, T.; Fukata, T.; Hiramatsu, K. Multiplex-PCR method for species identification of coagulase-positive staphylococci. J. Clin. Microbiol. 2010, 48, 765. [CrossRef]

26. Perreten, V.; Kania, S.A.; Bemis, D. Staphylococcus ursi sp. nov., a new member of the ‘Staphylococcus intermedius group’ isolated from healthy black bears. Int. J. Syst. Evol. Microbiol. 2008, 58, 3404–3408. [CrossRef] [PubMed]

27. Murray, A.K.; Lee, J.; Bendall, R.; Zhang, L.; Sunde, M.; Schau Slettemeås, J.; Gaze, W.; Page, A.J.; Vos, M. Staphylococcus cornubiensis sp. nov., a member of the Staphylococcus intermedius Group (SIG). Int. J. Syst. Evol. Microbiol. 2018, 68, 3404–3408. [CrossRef] [PubMed]

28. Aires-de-Sousa, M. Methicillin-resistant Staphylococcus aureus among animals: Current overview. Clin. Microbiol. Infect. 2017, 23, 373–380. [CrossRef] [PubMed]
29. Loncaric, I.; Lepuschitz, S.; Ruppitsch, W.; Trstan, A.; Andreadis, T.; Bouchlis, N.; Marbach, H.; Schauer, B.; Szostak, M.P.; Feßler, A.T.; et al. Increased genetic diversity of methicillin-resistant Staphylococcus aureus (MRSA) isolated from companion animals. *Vet. Microbiol.* 2019, 235, 118–126. [CrossRef]
30. Worthing, K.A.; Brown, J.; Gerber, L.; Trott, D.J.; Abraham, S.; Norris, J.M. Methicillin-resistant staphylococci amongst veterinary personnel, personnel-owned pets, patients and the hospital environment of two small animal veterinary hospitals. *Vet. Microbiol.* 2018, 232, 79–85. [CrossRef]
31. Van Balem, J.C.; Landers, T.; Nutt, E.; Dent, A.; Hoet, A.E. Molecular epidemiological analysis to assess the influence of pet-ownership in the biodiversity of Staphylococcus aureus and MRSA in dog- and non-dog-owning healthy households. *Epidemiol. Infect.* 2017, 145, 1135–1147. [CrossRef]
32. Bender, J.B.; Schiffman, E.; Hiber, L.; Gerads, L.; Olsen, K. Recovery of staphylococci from computer keyboards in a veterinary medical centre and the effect of routine cleaning. *Vet. Rec.* 2012, 170, 414. [CrossRef]
33. Joffe, D.; Goulding, F.; Langelier, K.; Magyar, G.; McCurdy, L.; Milstein, M.; Nielsen, K.; Villemaire, S. Prevalence of methicillin-resistant staphylococci in canine pyoderma cases in primary care veterinary practices in Canada: A preliminary study. *Can. Vet. J.* 2015, 56, 1084–1086.
34. Nakaminami, H.; Okamura, Y.; Tanaka, S.; Wajima, T.; Murayama, N.; Noguchi, N. Prevalence of antimicrobial-resistant staphylococci in nares and affected sites of pet dogs with superficial pyoderma. *J. Vet. Med. Sci.* 2021, 83, 214–219. [CrossRef]
35. Ference, E.H.; Danielian, A.; Kim, H.W.; Yoo, F.; Kuan, E.C.; Suh, J.D. Zoonotic Staphylococcus pseudintermedius sinonasal infections: Risk factors and resistance patterns. *Int. Forum Allergy Rhinol.* 2019, 9, 724–729. [CrossRef]
36. Hillier, A.; Lloyd, D.H.; Weese, J.S.; Blondeau, J.M.; Boothe, D.; Breitschwerdt, E.; Guardabassi, L.; Papich, M.G.; Rankin, S.; Turnidge, J.D.; et al. Guidelines for the diagnosis and antimicrobial therapy of canine superficial bacterial folliculitis (antimicrobial guidelines working group of the international society for companion animal infectious diseases). *Vet. Dermatol.* 2014, 25, 163-e143. [CrossRef] [PubMed]
37. Feng, Y.; Tian, W.; Lin, D.; Luo, Q.; Zhou, Y.; Yang, T.; Deng, Y.; Liu, Y.H.; Liu, J.H. Prevalence and characterization of methicillin-resistant Staphylococcus pseudintermedius in pets and horses. *J. Antimicrob. Chemother.* 2013, 68, 1256–1266. [CrossRef] [PubMed]
38. Park, Y.K.; Paik, Y.H.; Yoon, J.W.; Fox, L.K.; Hwang, S.Y.; Park, Y.H. Dissimilarity of ccrAB gene sequences between methicillin-resistant Staphylococcus epidermidis and methicillin-resistant Staphylococcus aureus among bovine isolates in Korea. *J. Vet. Sci.* 2013, 14, 299–305. [CrossRef]
39. Youn, J.-H.; Koo, H.C.; Ahn, K.J.; Lim, S.-K.; Park, Y.H. Determination of staphyloccocal exotoxins, SCCmec types, and genetic relatedness of Staphylococcus intermedius group isolates from veterinary staff, companion animals, and hospital environments in Korea. *J. Vet. Sci.* 2011, 12, 221–226. [CrossRef] [PubMed]