Influence of hospital size on antimicrobial resistance and advantages of restricting antimicrobial use based on cumulative antibiograms in dogs with *Staphylococcus pseudintermedius* infections in Japan

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Background – Antimicrobial resistance in *Staphylococcus pseudintermedius* (SP) and the prevalence of meticillin-resistant SP (MRSP) is increasing in dogs worldwide.

Objectives – To evaluate the influence of hospital size on antimicrobial resistance of SP and whether restricted use of antimicrobials based on antibiograms could reduce the identification of antimicrobial resistance in SP from infected dogs.

Methods and materials – In Study 1, a total of 2,294 SP isolates from dogs with pyoderma (*n* = 1,858, 52 hospitals) or otitis externa (*OE*; *n* = 436, 44 hospitals) taken between 2017 and 2019 were analysed. Clinics were categorised into small, medium and large based on numbers of practicing veterinary surgeons. In Study 2, a cumulative antibiogram was constructed for 12 antimicrobials from one large veterinary clinic from 2017 to 2018. Referring to this antibiogram, the clinic introduced strict antimicrobial selection criteria to treat dogs with pyoderma and OE, starting in 2018.

Results – MRSP was identified in 981 dogs (42.8%). In large clinics, the isolation rate of MRSP was 51.1% (404 of 791), which was significantly higher (*P* < 0.01) than in small clinics with less than two veterinary practitioners (34.0%, 154 of 453). In the antibiogram study, the susceptibility rates of oxacillin (MPIPC, 61.5%), cefpodoxime (CPDX, 55.8%) and minocycline (MINO, 55.8%) were significantly higher in 2019 (*n* = 52) than in 2017 to 2018 (*n* = 54; MPI/PC, 37.0%; CPDX, 33.3%; MINO, 20.4%; *P* < 0.05).

Conclusions and clinical relevance – Hospital size could affect the isolation rate of MRSP in dogs. Restricted use of antimicrobials for over a year based on cumulative antibiograms could reduce the resistance rate of multiple antimicrobials in SP isolated from dogs with pyoderma and OE.

Introduction

Canine pyoderma and otitis externa (*OE*) are among the most common diseases encountered in veterinary practice.1–6 *Staphylococcus pseudintermedius* (SP) is a commensal and common bacterial pathogen in dogs with pyoderma and OE.7–9 In recent years, SP has gained considerable attention because of the emergence of antimicrobial resistance of SP and meticillin-resistant SP (MRSP) in dogs worldwide.10–12 MRSP expresses the penicillin-binding protein 2a, encoded by the *mecA* gene, and shows low affinity to all β-lactam antimicrobials, including cephalosporins and carbapenems.13 In Japan, the isolation of MRSP in dogs was not reported until 2000.14,15 Since then, MRSP has been reported in dogs with pyoderma or OE in several regions in Japan; however, the isolation rates of MRSP have varied greatly depending on the research areas and institutions.14–19

Because MRSP isolates often are resistant not only to β-lactams, but also to several other classes of antimicrobial drugs, the treatment of MRSP infection in dogs has been a challenge in veterinary medicine.20 The recommendations for MRSP infections in small animals by the Clinical Consensus Guidelines of the World Association for Veterinary Dermatology state that restriction policies for certain antimicrobial drugs might help to mitigate the progressive development and dissemination of multidrug-resistant staphylococci.10 Recent studies in Japan revealed that the restricted use of antimicrobials for over a period of approximately two years, especially the use of third-generation cephalosporins and fluoroquinolones, was effective in reducing antimicrobial resistance rates in the *Staphylococcus intermedius* group, including MR...
strains, and *Escherichia coli* isolated from diseased dogs in an animal hospital.\textsuperscript{21}

A cumulative antibiogram is a periodic summary of the test results of the antimicrobial susceptibility of specific micro-organisms to batteries of antimicrobial drugs during a specific period of time (e.g. 12 months).\textsuperscript{22} Cumulative antibiograms are used to select appropriate empirical antimicrobial treatments and monitor the trends of antimicrobial resistance. In humans, the use of hospital cumulative antibiograms to guide the choice of empirical antimicrobial therapy has been identified as a key strategy to prevent and control the spread of antimicrobial-resistant micro-organisms in hospitals.\textsuperscript{23,24}

The influence of hospital size on meticillin resistance or the antimicrobial susceptibility pattern is unclear in SP isolated from dogs with pyoderma and OE. Moreover, only a few studies have evaluated the usefulness of antibiograms to establish the criteria for antimicrobial restriction in small animal practices.\textsuperscript{25} One objective of this study was to investigate the antimicrobial resistance pattern of SP in infected dogs from animal hospitals in Japan categorized into three different sizes based on numbers of practicing veterinary surgeons. The other objective was to evaluate whether the restricted use of antimicrobials based on cumulative antibiograms could reduce the frequency of resistance of several types of antimicrobials in clinical SP isolates from dogs with pyoderma and OE.

Materials and methods

Ethics

This study was conducted in compliance with applicable animal welfare regulations relating to the care and use of animals for scientific purposes.\textsuperscript{26} The study was conducted in accordance with good clinical practice guidelines,\textsuperscript{27} and informed consent was obtained from the owner of each participating dog.

Study design

This study analysed the antimicrobial resistance patterns of SP isolates from lesions of dogs with pyoderma or OE in different animal hospitals (Study 1), and evaluated the usefulness of restricting antimicrobial use based on an antibiogram for SP infections in dogs (Study 2).

Study 1: Analysis of antimicrobial resistance patterns of SP isolates from different animal hospitals

Bacterial samples and animal hospitals

Samples of SP were obtained from 2,294 dogs with pyoderma or OE that were treated in our affiliated veterinary clinics between 1 January 2017 and 31 December 2019. These samples were collected initially for bacterial culture and susceptibility testing by a commercial veterinarian. The samples were stored in Luna–Bertani broth (Sigma-Aldrich Corp.; St Louis, MO, USA) with 10% glycerol at \( -80^\circ\text{C}\) until further use. Pyoderma or OE had been confirmed by the attending veterinary surgeons based on clinical signs, cytological findings and bacterial culture. There were 1,858 SP isolates from pyoderma from 52 veterinary clinics and 436 from OE from 44 veterinary clinics in 17 cities (Hokkaido, Miyagi, Fukushima, Tokyo, Kanagawa, Chiba, Saitama, Ibaraki, Shizuoka, Aichi, Gifu, Nara, Kyoto, Osaka, Hyogo, Okayama and Hiroshima). The veterinary clinics were categorized into three sizes: large clinics with \(>10,000\) medium clinics with three to nine, and small clinics with two or fewer practising veterinary surgeons.

Species identification

Each swab (Seed Swab TechnoAmenity Inc.; Kyoto, Japan) was inoculated onto 5% sheep blood agar (Eiken Chemical Co., Ltd; Tokyo, Japan) and manganese salt agar (Eiken Chemical Co., Ltd) and incubated aerobically at \(37^\circ\text{C}\) for 18–24 h. Identification of SP was performed with thermonuclease genes using a primer pair reported previously.\textsuperscript{28} Crude DNA for PCR was extracted with achromopeptidase (Wako Chemical Co. Ltd.; Osaka, Japan), as described previously.\textsuperscript{29}

Antimicrobial susceptibility testing and identification of MRSP

Antimicrobial susceptibility analyses were carried out on SP isolates by the disk diffusion susceptibility test using the KB disk (Eiken Chemical Co., Ltd.) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.\textsuperscript{29-31} The following commonly used drug classes in Japan were tested: oxacillin (MPIPC, 1 \(\mu\text{g/disk}\)), clavulanic acid-amoxicillin (AMPC/CVA, 20 \(\mu\text{g/disk}\)), cefalexin (ICEX; 30 \(\mu\text{g/disk}\)), cefoperazone (CPDX; 10 \(\mu\text{g/disk}\)), enrofloxacin (ERFX; 5 \(\mu\text{g/disk}\)), gentamicin (GM; 10 \(\mu\text{g/disk}\)), trimethoprim-sulfamethoxazole (ST; 23.75 \(\mu\text{g} \times 1.25 \mu\text{g/disk}\)), clindamycin (CLDM; 2 \(\mu\text{g/disk}\)), doxycycline (DOXY; 30 \(\mu\text{g/disk}\)), minocycline (MINO; 30 \(\mu\text{g/disk}\)), chloramphenicol (CP; 30 \(\mu\text{g/disk}\)) and fosfomycin (FOM; 50 \(\mu\text{g/disk}\)). In the disk diffusion testing, the interpretative criteria for susceptible (SI), intermediate (I) or resistant (R) were taken from: the CLSI VET\textsuperscript{32} for MPIPC, CPDX and DOXY; the CLSI M100-S3\textsuperscript{33} for AMP/CVA, ERFX, GM, ST, CLDM, MINO, CP and FOM according to the CLSI guidelines.\textsuperscript{29} The methods for disk diffusion testing were as described above. The CLSI guidelines recommend compiling the antibiogram at least annually, including only the first isolate per case in the period analysed, as well as only organisms for which \(\geq30\) isolates were tested in the period analysed. The susceptibility rate of each antimicrobial was calculated based on the number of susceptible isolates, not including intermediate isolates.

Based on the results of an antibiogram from 2017 to 2018, veterinary clinics introduced strict antimicrobial prescribing criteria starting on 1 November 2018 for the treatment of dogs with pyoderma and OE. Furthermore, the clinics preferred topical antimicrobial treatments, such as 0.5–2% chlorhexidine lotion or shampoo, and antimicrobial ear drops or cleaner, over systemic antimicrobial treatment. Following the restricted use of antimicrobials for over a year, a cumulative antibiogram was reconstructed using the susceptibility test data between 1 January 2019 and 31 December 2019.

Statistical analysis

Antimicrobial susceptibility patterns from different clinic sizes were analysed using a logistic regression equation. The susceptibility patterns before and after antimicrobial restriction were compared using the Chi-square test.

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Table 1. Antimicrobial susceptibility test results of Staphylococcus pseudintermedius isolated from dogs with pyoderma according to veterinary clinic size

| Number of SP isolates (%) | Small (n = 356) | Medium (n = 886) |
|---------------------------|-----------------|-----------------|
|                           | R   | I   | S   | R   | I   | S   |
| MPIPC                     | 124 (34.8%)    | 0 (0.0%)       | 232 (65.2%)   | 358 (40.4%) | 0 (0.0%) | 528 (59.6%)   |
| AMPC/CVA                  | 44 (12.4%)     | 0 (0.0%)       | 312 (87.6%)   | 126 (14.2%) | 0 (0.0%) | 760 (85.8%)   |
| CEX                       | 79 (22.2%)     | 19 (5.5%)     | 258 (72.5%)   | 217 (24.5%) | 39 (4.4%) | 630 (71.1%)   |
| CPDX                      | 111 (32.9%)    | 22 (6.5%)     | 204 (60.5%)   | 296 (34.3%) | 83 (9.6%) | 487 (56.1%)   |
| ERFX                      | 204 (57.3%)    | 21 (5.9%)     | 132 (37.8%)   | 521 (60.8%) | 43 (4.9%) | 322 (36.3%)   |
| GM                        | 120 (35.6%)    | 30 (8.9%)     | 187 (55.5%)   | 314 (36.2%) | 72 (8.3%) | 482 (55.5%)   |
| ST                        | 140 (40.7%)    | 19 (5.5%)     | 155 (43.8%)   | 307 (35.3%) | 46 (5.3%) | 353 (40.0%)   |
| CLDM                      | 182 (52.9%)    | 32 (9.3%)     | 118 (37.8%)   | 430 (50.0%) | 63 (7.0%) | 493 (54.7%)   |
| MINO                      | 85 (23.9%)     | 29 (8.2%)     | 231 (67.9%)   | 296 (33.8%) | 70 (8.2%) | 436 (49.0%)   |
| CP                        | 98 (27.1%)     | 38 (11.3%)    | 201 (59.6%)   | 238 (27.4%) | 131 (15.1%) | 499 (55.5%)   |
| FOM                       | 17 (5.0%)      | 28 (8.7%)     | 294 (86.3%)   | 83 (9.6%) | 62 (7.0%) | 753 (84.4%)   |

| Number of SP isolates (%) | Large (n = 616) | Total (n = 1,888) |
|---------------------------|-----------------|-----------------|
|                           | R   | I   | S   | R   | I   | S   |
| MPIPC                     | 307 (49.8%)    | 0 (0.0%)       | 309 (50.2%)   | 789 (42.5%) | 0 (0.0%) | 1069 (57.5%)   |
| AMPC/CVA                  | 137 (22.2%)    | 0 (0.0%)       | 479 (77.8%)   | 307 (16.5%) | 0 (0.0%) | 1551 (83.5%)   |
| CEX                       | 209 (33.9%)    | 37 (6.0%)     | 172 (61.1%)   | 505 (27.2%) | 95 (5.1%) | 1258 (67.7%)   |
| CPDX                      | 272 (45.8%)    | 54 (9.1%)     | 268 (55.1%)   | 681 (37.9%) | 159 (8.8%) | 929 (53.3%)   |
| ERFX                      | 445 (72.2%)    | 24 (3.9%)     | 147 (23.9%)   | 1,170 (63.0%) | 88 (4.7%) | 600 (32.3%)   |
| GM                        | 245 (41.2%)    | 85 (14.3%)    | 264 (44.4%)   | 679 (37.7%) | 187 (10.4%) | 933 (51.9%)   |
| ST                        | 271 (49.0%)    | 29 (5.3%)     | 243 (45.8%)   | 718 (45.1%) | 89 (5.6%) | 786 (43.9%)   |
| CLDM                      | 364 (67.2%)    | 49 (9.0%)     | 126 (22.8%)   | 949 (56.9%) | 144 (9.9%) | 499 (31.3%)   |
| DOXY                      | 414 (68.8%)    | 24 (4.0%)     | 155 (27.2%)   | 1,166 (64.8%) | 100 (5.6%) | 532 (29.6%)   |
| MINO                      | 85 (13.9%)     | 292 (47.7%)   | 235 (38.4%)   | 213 (11.6%) | 767 (41.8%) | 853 (46.5%)   |
| CP                        | 204 (34.4%)    | 116 (19.6%)   | 273 (46.0%)   | 540 (30.0%) | 285 (15.9%) | 797 (45.1%)   |
| FOM                       | 51 (8.6%)      | 51 (8.6%)     | 491 (82.8%)   | 151 (8.4%) | 129 (7.2%) | 1,518 (84.4%) |

Statistical analyses. A P-value < 0.05 was considered statistically significant.

Results

Study 1

Tables 1 and 2 show the antimicrobial susceptibility testing results of SP isolated from dogs with pyoderma or OE in veterinary clinics of different sizes. The isolation rate of MRSP with the MPIPC disk diffusion test in large clinics was 51.1% (404 of 791), which was significantly higher than that in the small (34.0%, 154 of 453) and medium clinics (40.3%, 423 of 1,050; Table 3). Each susceptibility rate of AMPC/CVA, CEX, CPDX, ERFX, GM, ST, CLDM, MINO and CP in pyoderma, and that of CEX, CPDX, ERFX, ST, CLDM, CP and FOM in OE, was significantly lower in small clinics than in large clinics (P < 0.05; Table 3). Each susceptibility rate of MPIPC, AMPC/CVA, CEX, CPDX, ERFX, GM, ST, CLDM, MINO and CP in pyoderma, and that of MPIPC, AMPC/CVA, CEX, CPDX and ERFX in OE, was significantly higher in medium clinics than in large clinics (P < 0.05; Table 3). No antimicrobials used in both small and medium clinics had significantly lower susceptibility than those used in large clinics. There were no significant differences in the susceptibility rates of all tested antimicrobials in pyoderma between small and medium clinics. The susceptibility rates of CPDX and CLDM in OE were significantly higher in small clinics than in medium and large clinics (P < 0.05; Table 3).

Study 2

From 2017 to 2018, 54 SP isolates were collected from dogs with pyoderma (n = 30) and OE (n = 24). The resulting cumulative antibiogram from 2017 to 2018 showed the following susceptibility rates (in ascending order): DOXY (14.8%), CLDM (16.7%), ERFX (18.5%), MINO (20.4%), ST (31.5%), CPDX (33.3%), MPIPC (37.0%), GM (40.7%), CEX (50.0%), CP (50.0%), FOM (57.4%) and AMPC/CVA (66.7%). Based on these results, the large veterinary clinic introduced strict antimicrobial prescribing criteria to treat dogs with pyoderma and OE, which included the following: (i) systemic treatments with fluoroquinolones and β-lactam antimicrobials, including first- and third-generation cephalosporins should be used only when life-threatening infection is expected; (ii) CP and FOM could be used for empirical treatment; and (iii) ST, CLDM, DOXY and MINO should be used according to the results of susceptibility tests. Following the restricted use of antimicrobials, a cumulative antibiogram was reconstructed using the susceptibility test data between 1 January and 31 December 2019. A total of 52 SP strains were isolated from dogs with pyoderma (n = 30) and OE (n = 22). Although the frequency of susceptibility was higher for all antimicrobials in 2019 compared to 2017 to 2018, with the exception of ST,
these differences were significant only for MPIPC (61.5%, \( P = 0.02 \)), CPDX (55.8%, \( P = 0.03 \)) and MINO (55.8%, \( P = 0.001 \)), as shown in Table 4.

**Discussion**

This study investigated the influence of hospital size (number of practising veterinary surgeons) on the antimicrobial resistance of SP, and determined whether the restricted use of antimicrobials with antibiograms could reduce the antimicrobial resistance of SP in infected dogs. Study 1 included a total of 2,294 SP isolates from 17 cities in Japan. Previous Japanese studies analysed 31 to 282 strains of SP in dogs.14–19 To the best of the authors’ knowledge, the present study used the largest number of SP strains isolated from dogs with pyoderma and OE in Japan. Previous reports revealed that the antimicrobial susceptibility patterns of MRSP differed between North America and Europe, which indicates differences in the susceptibility patterns between different MRSP clones across different countries.15 Although the present study revealed that the isolation rate of MRSP was 42.8% in total, the isolation rates of MRSP varied across different hospitals,14–19 as shown in Table 4.

Two studies performed in referral clinics showed higher isolation rates of MRSP in dogs with pyoderma (2007–2009: 66.5%; 2010: 57%).16–17 than those in a study performed in 11 animal hospitals (2009: 11.4%).15 Our study showed that the susceptibility rates of several classes of antimicrobials in SP isolated from dogs with pyoderma or OE were significantly lower in large veterinary clinics than in small and medium clinics. Furthermore, the isolation rate of MRSP was significantly higher in large clinics than in small and medium clinics. These findings indicate that the number of practising veterinary surgeons in a clinic could influence the antimicrobial resistance of SP in dogs.

A significant correlation between antimicrobial resistance and consumption of antimicrobials for *S. aureus* has been reported.32 Although the present study did not confirm the antimicrobial consumption in each clinic, or the medical history in each case, it was presumed that large veterinary clinics or referral clinics would use larger amounts of antimicrobials and have a larger number of recurrent cases than smaller clinics.

It has been reported that patterns of antimicrobial use could influence the antimicrobial resistance of *S. aureus* in humans.33,34 For appropriate antimicrobial use, two guidelines were created independently in North America and the European Union in 2013 to 2014 for the antimicrobial treatment of canine skin diseases.35–37 However, these guidelines are not relevant to particular countries. There are no guidelines on the antimicrobial treatment of...
from dogs affected with pyoderma or otitis externa

Table 3. Comparisons between small, medium and large clinics for antimicrobial susceptibility rates in Staphylococcus pseudintermedius isolated from dogs affected with pyoderma or otitis externa

|                      | Pyoderma |                        |                        |                        |
|----------------------|----------|------------------------|------------------------|------------------------|
|                      | Small (versus Large) | Medium (versus Small) | Medium (versus Large) |                      |
|                       | OR  | 95% CI     | P-value | OR  | 95% CI     | P-value | OR  | 95% CI     | P-value |
| MPIPC                | 1.86 | 1.42–2.44  | <0.001* | 0.79 | 0.61–1.02  | 0.07    | 1.47 | 1.19–1.8    | <0.001* |
| AMPC/CVA             | 2.03 | 1.41–2.96  | <0.001* | 0.85 | 0.58–1.22  | 0.39    | 1.73 | 1.32–2.26   | <0.001* |
| CEK                  | 1.75 | 1.32–2.33  | <0.001* | 0.93 | 0.71–1.23  | 0.63    | 1.64 | 1.32–2.03   | <0.001* |
| CPDX                 | 1.87 | 1.42–2.45  | <0.001* | 0.83 | 0.64–1.08  | 0.16    | 1.55 | 1.26–1.92   | <0.001* |
| ERFX                 | 1.86 | 1.4–2.47   | <0.001* | 0.98 | 0.76–1.27  | 0.88    | 1.82 | 1.45–2.3    | <0.001* |
| GM                   | 1.56 | 1.19–2.04  | <0.001* | 1    | 0.78–1.29  | 0.99    | 1.56 | 1.27–1.93   | <0.001* |
| ST                   | 1.44 | 1.1–1.89   | 0.01*   | 0.88 | 0.68–1.14  | 0.35    | 1.27 | 1.01–1.59   | 0.04*   |
| CLDM                 | 1.94 | 1.45–2.61  | <0.001* | 0.85 | 0.65–1.11  | 0.23    | 1.77 | 1.28–2.12   | <0.001* |
| DOXY                 | 1.28 | 0.95–1.72  | 0.10    | 1.01 | 0.77–1.33  | 0.95    | 1.29 | 1.02–1.63   | 0.03*   |
| FOM                  | 1.73 | 1.32–2.27  | <0.001* | 0.91 | 0.71–1.18  | 0.49    | 1.59 | 1.29–1.96   | <0.001* |
|                      | 1.42 | 0.97–2.1   | 0.07    | 0.79 | 0.54–1.14  | 0.22    | 1.13 | 0.85–1.49   | 0.40    |

Table 4. Results of antibiograms from Staphylococcus pseudintermedius isolated from dogs affected with pyoderma or otitis externa before (2017–2018) and after (2019) the restriction of antimicrobial use

|                      | 2017–2018 | 2019 | Number of SP isolates (%) |
|----------------------|-----------|------|----------------------------|
|                      | n  | %   | n   | %   | P-value |
| Total                | 54 | 100.0 | 52 | 100.0 |        |
| MPIPC                | 20 | 37.0 | 32 | 61.5 | 0.02*  |
| AMPC/CVA             | 36 | 68.7 | 41 | 78.8 | 0.23   |
| CEK                  | 27 | 50.0 | 35 | 67.3 | 0.11   |
| CPDX                 | 18 | 33.3 | 29 | 55.8 | 0.03*  |
| ERFX                 | 10 | 18.5 | 15 | 28.8 | 0.31   |
| GM                   | 22 | 40.7 | 30 | 57.7 | 0.10   |
| ST                   | 17 | 31.5 | 14 | 26.9 | 0.76   |
| CLDM                 | 9  | 16.7 | 15 | 28.8 | 0.21   |
| DOXY                 | 8  | 14.8 | 15 | 28.8 | 0.13   |
| MINO                 | 11 | 20.4 | 29 | 55.8 | 0.001* |
| CP                   | 27 | 50.0 | 30 | 57.7 | 0.34   |
| FOM                  | 31 | 57.4 | 32 | 61.5 | 0.63   |

SP: Staphylococcus pseudintermedius; MPIPC, oxacillin; AMPC/CVA, clavulanic acid-amoxicillin; CEX, cefalexin; CPDX, cefpodoxime; ERFX, enrofloxacin; GM, gentamicin; ST, trimethoprim-sulfamethoxazole; CLDM, clindamycin; DOXY, doxycycline; MINO, minocycline; CP, chloramphenicol; FOM, fosfomycin

pyoderma and OE in dogs in Japan. The Guideline Committee of the Japanese Society of Veterinary Dermatology stated that it was difficult to propose a guideline for Japanese practitioners in 2017, because evidence for the practice was quite limited. The lack of guidelines leads to an inconsistent selection of antimicrobials by veterinary practitioners, which may contribute to an increase in resistant strains, especially in large veterinary clinics.

In Study 2, the restricted, antibiogram-based use of antimicrobials significantly improved the susceptibility rate of MPIPC (37.0–61.5%) and CPDX (33.3–55.8%). We restricted the systemic use of fluoroquinolones and β-lactam antimicrobials, including first- and third-generation cephalosporins, as well as AMPC/CVA, which showed the highest susceptibility rate (66.7%) before restriction in this study. The susceptibility to MPIPC was low (37.0%) from 2017 to 2018, indicating a high prevalence of MRSP. For S. aureus, it has been reported that the use of fluoroquinolones and β-lactam antimicrobials is a risk factor for meticillin resistance. A previous study indicated the importance of fluoroquinolones in promoting the survival and spread of multidrug-resistant MRSP. Furthermore, the restriction of antimicrobials –
mainly, third-generation cephalosporins and fluoroquinolones – reduced the isolation rate of the MR *S. intermedius* group from 41.5% to 9.3%.21

These findings suggest that the restriction of fluoroquinolones and β-lactam antimicrobials for over a year could be useful in reducing the meticillin resistance rate of SP in dogs. The susceptibility rate of ERFX increased slightly from 18.5% to 28.8% and was not significantly changed in the present study. In a previous study, from 2016, the use of fluoroquinolones in the treatment of *S. intermedius* infections in dogs and cats was restricted; subsequently, the resistance rate of ERFX was significantly decreased in 2017 (39.0%) and 2018 (22.2%) compared to that in 2015 (59.4%).21 In the present study, the resistance rate of ERFX in SP isolates before the restriction was 81.5% (2017 to 2018), which was higher than that reported previously. Although further studies are needed to confirm the change in fluoroquinolone resistance after antimicrobial use restriction, the high resistance rate of ERFX and short duration of antimicrobial restriction could influence the recovery of fluoroquinolone resistance in SP.

Cumulative antibiograms help establish the criteria for empirical systemic treatment with antimicrobials in each hospital. However, in the treatment of canine OE, topical antimicrobial therapy is commonly used in small animal practices. The large hospital enrolled in this study usually has chronic severe or referral cases of canine OE that require systemic antimicrobial treatment. Therefore, our study analysed and established antibiograms for both pyoderma and OE. The antimicrobials that show a high susceptibility rate (>80%) in antibiograms are commonly recommended for empirical use. However, there were no antimicrobials with a susceptibility rate >80% in the present study. Therefore, we recommend the use of topical antiseptic therapy, especially chlorhexidine lotion or shampoo products for pyoderma, and antimicrobial ear drops or cleaner for OE, before systemic antimicrobial treatment, as much as possible. Previous reports showed that a twice-weekly chlorhexidine shampoo combined with daily chlorhexidine spray was as effective as oral AMPC/CVA for treatment in dogs with pyoderma, including MRSP infection.40 A Japanese study revealed that the minimal inhibitory concentration (MIC) for chlorhexidine remained low, and that there were no significant differences in the MIC of chlorhexidine between mecA-positive and mecA-negative SP isolated from dogs with pyoderma.17 After the restriction of systemic antimicrobial treatment and the recommendation of topical treatment, MINO revealed a significant elevation in susceptibility rate (from 20.4% to 55.8%), while other classes of antimicrobials did not show a significant decrease in susceptibility rates in the present study. Although this study did not investigate the detailed use of each antimicrobial, topical treatment with antiseptics may be used as an alternative to antimicrobial use in veterinary clinics to prevent the resistance of SP, if systemic antimicrobials with susceptible rates >80% cannot be used based on the results of antibiograms in dogs.

Conclusions

In summary, antimicrobial resistance, including meticillin resistance in SP, may be influenced by the number of veterinary practitioners in the clinic. The restricted use of antimicrobials for over a year, based on antibiograms, reduced the rate of antimicrobial resistance of SP strains, including MRSP isolated from dogs with pyoderma and OE. Although the number of dogs is gradually decreasing in Japan, the estimated sale of antimicrobials has been increasing in recent years.26 It is important to select and restrict antimicrobials and to create antibiograms regularly at each veterinary clinic to prevent future antimicrobial resistance in dogs.

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**RESUMÉ**

**Contexte** – La résistance antimicrobienne de Staphylococcus pseudintermedius (SP) et la prévalence des SP résistants à la méticililine (MRSP) est en augmentation chez les chiens à travers le monde.

**Objectifs** – Évaluer l’influence de la taille d’hôpital sur la résistance antimicrobienne de SP et si l’utilisation restreinte des antibiogrammes basée sur les antibiogrammes pourrait réduire l’identification des résistances antimicrobiennes des SP des chiens infectés.

**Matériels et méthodes** – Dans l’étude 1, un total de 2 294 Souches de SP de chiens avec pyoderme (n = 1,858, 52 hôpitaux) ou otite externe (OE; n = 436, 44 hôpitaux) pris entre 2017 et 2019 a été analysé.

Les cliniques étaient catégorisées en petite, moyenne et large, selon le nombre de vétérinaires praticiens. Dans l’étude 2, un antibiogramme cumulatif a été construit pour 12 antimicrobiens d’une grande clinique vétérinaire de 2017 à 2018. Se référant à cet antibiogramme, la clinique a introduit des critères de sélection des antimicrobiens pour traiter les chiens avec pyoderme et OE, à partir de 2018.

**Résultats** – MRSP a été identifié chez 981 chiens (42,8%). Dans les grandes cliniques, le taux d’isolement des MRSP était de 51.1% (404 sur 791), qui était significativement plus élevé (P < 0,01) que dans les petites cliniques avec moins de deux vétérinaires (34,0%, 154 de 453). Dans l’étude antibiogramme, les taux de sensibilité de l’oxacilline (MPIPC, 61,5%), cefsopodoxime (CPDX, 55,8%) et minocycline (MINO, 55,8%)
La resistencia a los antimicrobianos en *Staphylococcus pseudintermedius* (SP) y la prevalencia de SP resistente a la meticilina (MRSP) está aumentando en perros en todo el mundo. Los estudios han mostrado que el uso restringido de antimicrobianos podría reducir la identificación de resistencia antimicrobiana en SP de perros infectados.

### Resultados
En el Estudio 1 se analizaron un total de 2294 aislamientos de SP de perros con pioderma (n = 1858, 52 hospitales) u otitis externa (OE; n = 436, 44 hospitales) obtenidos entre 2017 y 2019. Las clínicas se clasificaron en pequeñas, medianas y grandes según el número de veterinarios en ejercicio. En el Estudio 2, se construyó un antibiograma acumulativo para 12 antimicrobianos de una gran clínica veterinaria de 2017 a 2018. En referencia a este antibiograma, la clínica introdujo criterios estrictos de selección de antimicrobianos para tratar perros con pioderma y OE, a partir de 2018.

### Conclusión y relevancia clínica
El tamaño del hospital podría afectar la tasa de aislamiento de MRSP en perros. El uso restringido de antimicrobianos durante más de un año basado en antibiogramas acumulativos podría reducir la tasa de resistencia de múltiples antimicrobianos en SP aislado de perros con pioderma y OE.

### Zusammenfassung
**Ziel** – Eine Evaluierung des Einflusses der Spitalsgröße auf die antimikrobielle Resistenz gegenüber SP und eine Feststellung, ob ein restriktiver Einsatz der Antibiotika basierend auf einem Antibiogramm die Identifizierung antimikrobieller Resistenzen auf SP bei infizierten Hunden reduzieren könnte.

**Methoden und Materialien** – In Studie 1 wurden insgesamt 2.294 SP Isolate von Hunden mit einer Pyodermie (n = 1.858; 52 Kliniken) oder Otitis externa (OE; n = 436; 44 Kliniken), die zwischen 2017 und 2019 genommen wurden, analysiert. Die Kliniken wurden anhand der praktizierenden Tierärzte in klein, medium und groß eingeteilt. In Studie 2 wurde ein kumulatives Antibiogramm für 12 Antibiotika aus einer großen Veterinärmedizinischen Klinik aus den Jahren 2017 bis 2018 konstruiert. Bezugnehmend auf dieses Antibiogramm führte die Klinik ab 2018 strikte antimikrobielle Selektionskriterien zur Behandlung von Hunden mit Pyodermie und OE ein.

**Ergebnisse** – Ein MRSP wurde bei 981 Hunden (42,8%) identifiziert. In großen Kliniken betrug die Isolationsrate für MRSP 51,1% (404 von 791), was signifikant höher war (P < 0,01) als in kleinen Kliniken mit weniger als zwei praktizierenden TierärztInnen (34,0%, 154 von 453). In der Antibiogramm Studie lagen die Empfindlichkeitswerte von Oxacillin (MPIPC, 61,5%), Cefpodoxim (CPDX, 55,8%) und Minocyclin (MINO; 55,8%) 2017 bis 2018 (n = 54; MPIPC, 37,0%; CPDX, 33,3%; MINO; 20,4%; P < 0,05).

**Schlussfolgerungen und klinische Bedeutung** – Die Klinikgröße könnte die Isolationsrate von MRSP bei Hunden beeinflussen. Ein restriktiver Einsatz von Antibiotika für über ein Jahr basierend auf einem kumulativen Antibiogramm könnte die Resistenzrate von multien Antibiotika auf SP, welcher von Hunden mit Pyodermie und OE isoliert wird, reduzieren.
炎（OE：n=436、44病院）の犬から分離された合計2,294個のSPを解析した。診療所は、開業している獣医外科医の数に基づいて、小、中、大規模に分類した。研究2では、2017年から2018年にかけて、1つの大規模な動物病院から12種類の抗生物質について累積的なアンチバイオグラムを構築した。このアンチバイオグラムを参考に、同クリニックは2018年から薬剤師やOEの犬の治療に厳格な抗菌薬選択基準を導入した。

結果 – 981頭（42.8%）の犬でMRSPが確認された。大規模クリニックにおけるMRSPの分離率は51.1%（791頭中404頭）であり、獣医師が2名以下の小規模クリニック（34.0%、453頭中154頭）よりも有意に高かった（P<0.01）。アンチバイオグラム調査では、オキサシリン（MPIPC、61.5%）、セフボドキシム（CPDX、55.8%）、ミノサイクリン（MINO、55.8%）の感受性率は、2017～2018年（n=54、MPIPC、37.0%、CPDX、33.3%、MINO、20.4%、P<0.05）に比べ、2019年（n=52）は有意に高かった。

結論と臨床的関連性 – 病院の規模は、犬のMRSPの分離率に影響を与える可能性がある。累積アンチバイオグラムに基づいて1年以上抗菌薬の使用を制限することで、犬の膿皮症やOEから分離されたSPの複数の抗生物質に対する耐性率を低下させることができた。

Resumo

Contexto – A resistência a antimicrobianos em Staphylococcus pseudintermedius (SP) e a prevalência de SP resistente à meticilina (MRSP) vem aumentando em cães em todo o mundo.

Objetivos – Avaliar a influência do tamanho do hospital na resistência antimicrobiana de SP e se o uso restrito de antimicrobianos com base em antibiogramas poderia reduzir a identificação de resistência a antimicrobianos em SP de cães infectados.

Métodos e materiais – No Estudo 1, um total de 2.294 isolados de SP de cães com pododermite (n=1.858, 52 hospitais) ou otite externa (OE; n=436, 44 hospitais) coletados entre 2017 e 2019 foram analisados. As clínicas foram categorizadas em pequenas, médias e grandes com base no número de cirurgias veterinárias em atividade. No Estudo 2, um antibiograma cumulativo foi elaborado para 12 antimicrobianos de uma grande clínica veterinária de 2017 a 2018. Referindo-se a este antibiograma, a clínica introduziu critérios de seleção de antimicrobianos estritos para tratar cães com pododermite e OE, a partir de 2018.

Resultados – A MRSP foi identificada em 981 cáes (42.8%). Em grandes clínicas, a taxa de isolamento de MRSP foi de 51,1% (404 de 791), que foi significativamente maior (P<0,01) do que em pequenas clínicas com menos de dois médicos veterinários (34,0%, 154 de 453). No estudo de antibiograma, as taxas de suscetibilidade de oxaciclina (MPIPC, 61,5%), cefpodoxima (CPDX, 55,8%) e minociclina (MINO, 55,8%) foram significativamente maiores em 2019 (n=52) do que em 2017-2018 (n=54; MPIPC, 37,0%; CPDX, 33,3%; MINO, 20,4%; P<0,05).

Conclusões e relevância clínica – O tempo do hospital pode afetar a taxa de isolamento de MRSP em cães. O uso restrito de antimicrobianos por mais de um ano com base em antibiogramas cumulativos pode reduzir a taxa de resistência de vários antimicrobianos em SP isolados de cães com pododermite e OE.