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## Introduction

*Staphylococcus intermedius* (*S. intermedius*) and *pseudintermedius* (*S. pseudintermedius*) are two commensal bacteria that lives in many different animal species and can also be opportunistic pathogen responsible for skin infections, such as pyoderma, in dogs and cats. Both species rarely causes infections in humans and, when it happens, it is usually wound infections after animal contacts. Methicillin-resistant *S. intermedius* (MRSI) and *S. pseudintermedius* (MRSP) have recently emerged as two significant nosocomial pathogens in companion animals. Their rising incidence makes them an alarming inflicting by limited therapeutic options in animals and humans. Moreover, in addition to have been isolated from cats and dogs, MRSP have also been isolated from humans highlighting the public health issue for veterinarians and pet owners.

The aim of this study is to investigate MRSI and MRSP presence in a collection of *S. intermedius* and *S. pseudintermedius* isolated from dogs and cats in Japan and to compare their genotypic and phenotypic characteristics.

## Materials and methods

Fifty-one *S. intermedius* (5 isolated from cats and 46 isolated from dogs) and 149 *S. pseudintermedius* (17 isolated from cats and 132 isolated from dogs) isolated in Japan were tested for methicillin resistance.

Positive isolates were characterised for:

- virulence genes: *siet*, *lukS-PV* and *lukF-PV* genes coding respectively for *S. intermedius* exfoliative toxin and Panton-Valentine leukocidin (LukF-PV and LukS-PV);
- biofilm formation in Tryptic Soy Broth (TSB) + 0,25% glucose;
- antibiotics resistances to 15 commonly used antibiotics.

Besides, the strains were typed using SCCmec-typing, Pulsed Field Gel Electrophoresis (PFGE) and MultiLocus Sequence Typing (MLST).

## Results

Out of the 200 *S. intermedius* and *S. pseudintermedius* isolates collected from cats and dogs dermatitis cases, 27 (13.5%) were characterised as MRSI (n=5) or MRSP (n=22). Most positive strains were isolated from dogs (n=25).

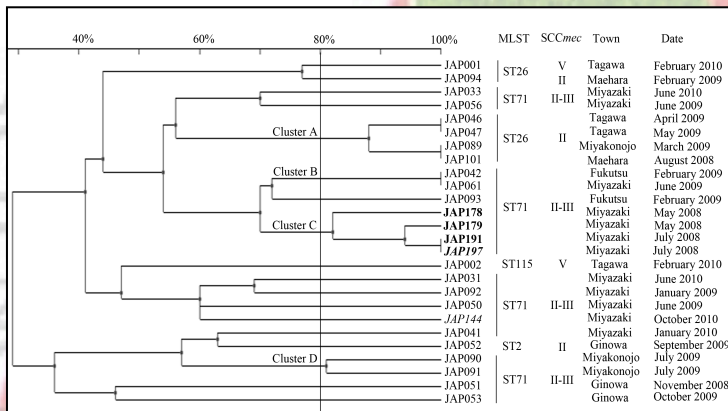


Figure 1: PFGE, MLST and SCCmec-typing results for MRSI and MRSP strains. Strains highlighted in bold correspond to MRSI strains and strains indicated in italic correspond to strains isolated from cats. Dendrogram was constructed using UPGMA (Dice coefficient, 2% position tolerance).

After *SmaI* digestion of total DNA, **22 different pulsotypes** were obtained for the 26 isolates. DNA from one MRSI isolate could not be digested with *SmaI*. By using a cut-off at 80% similarity, only few isolates could be grouped together, forming 4 distinct clusters (A, B, C and D) (Figure 1). MLST results revealed that all 27 isolates belonged to five groups: ST2, ST26, ST29, ST71 and ST115 (Figure 1). **Most MRSP and MRSI belonged to ST71 group** (18 isolates) and six MRSP isolates belonged to ST26. The other three isolates belonged to ST2 and ST115 types. SCCmec-typing identified **3 types of SCCmec cassette**, namely the II, II-III and V types (Figure 1). A good correlation was observed between the MLST results and SCCmec-typing.

All but one isolate from a cat (JAP144) tested positive for the *siet* gene. Conversely, none of the isolates harboured the *lukS-PV* and *lukF-PV* genes. Biofilm production results estimated that all but two isolates produced strong biofilm in TSB<sub>glc</sub>. The remaining two isolates (JAP001 and JAP178) were moderate and weak biofilm producers, respectively.

All isolates were resistant to at least five of the fifteen tested antibiotics (Figure 2): gentamicin, kanamycin, spectinomycin, erythromycin and trimethoprim-sulfamethoxazole. Moreover all but one isolate were also resistant to penicillin, clindamycin and ciprofloxacin. By contrast, more than 40% of the isolates were sensitive to cefoperazone, cefalexin and ceftiofur. One isolate was resistant to all antibiotics.

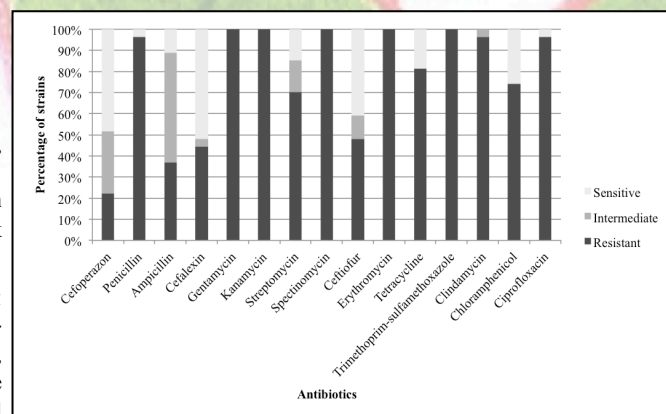


Figure 2: Antibiotic susceptibility profiles to fifteen antibiotics.

## Discussion

This study provides an insight into MRSI and MRSP by demonstrating that: (i) ST71 represents one of the main MLST lineage in Japan; (ii) ST26 MRSP could represent an emerging MLST lineage in Japan; (iii) most MRSP and MRSI of our study are strong biofilm producers and possess the exfoliative toxin gene (*siet*); and (iv) all strains are resistant to a large number of antibiotics.

In conclusion, results obtained showed that MRSI and MRSP are present in the studied animals and do not seem to form a homogeneous group. Phenotypic features as strong biofilm formation and high antibiotics resistance probably help bacteria to infect and persist in animals and veterinary hospital. Moreover, such strains could represent a risk for pets owners and veterinarians.

## Acknowledgments

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