Antimicrobial Susceptibility of *Staphylococcus intermedius* Isolated from Healthy and Diseased Dogs

Akira SHIMIZU1), Yoshihisa WAKITA1), Sayuri NAGASE2), Mika OKABE3), Takuya KOJI1), Toshikatsu HAYASHI2), Naoko NAGASE1), Asako SASAKI1), Junichi KAWANO1), Kenji YAMASHITA1) and Michihiro TAKAGI1)

1)Department of Microbiology and Immunology, Faculty of Agriculture, Kobe University, 1–1 Rokkodai-cho, Nada-ku, Kobe-shi, Hyogo 657–0013 and 2)Animal Health Research Laboratories, Agro Company, Takeda Chemical Industries, Ltd., 4428–2 Osada, Fukuchiyama-shi, Kyoto 620–0843, Japan

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**ABSTRACT.** A total of 90 strains of *Staphylococcus intermedius* isolated from dogs were examined for antimicrobial susceptibility. There were no significant differences in the distribution patterns of MICs between strains from 1982 to 1985 and those from 1999, and between strains from healthy dogs and those from diseased dogs. All of the strains were susceptible to ABPC, DMPPC, CEX, TDM, ERFX, BFLX, and FF at concentrations of 0.05 to 6.25 µg/ml. The MICs of OTC, KM, EM, AIV-TS, and LCM were distributed in a broad range of 0.1 to >100 µg/ml, indicating the existence of resistant as well as susceptible populations of *S. intermedius*. Thirty-three strains (36.7%) were resistant to one or more antimicrobial agents such as OTC (n=32), KM (n=9), EM (n=7), AIV-TS (n=7), and LCM (n=7).

**KEY WORDS:** canine, drug resistance, *Staphylococcus intermedius*.

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**NOTE** Bacteriology

Coagulase-positive *Staphylococcus intermedius* was first described as a new species by Hajek [8] in 1976. *S. intermedius* is one of the causative agents of bacterial skin infections, such as otitis externa [3, 11, 16], pyoderma [7, 9, 11, 12], and abscesses [3, 16], in dogs. The organism is present on the skin surface and coat of healthy dogs [2]. It also has been frequently isolated from the external auditory meatus, mouth, nose, and anus of healthy dogs [1]. No vaccines are available to control these diseases. Thus, at present, chemotherapy is the most practical way to treat staphylococcal infections. There have been many studies on the *in vitro* effect of antimicrobial agents against strains of *S. intermedius* isolated from dogs [3, 4, 9, 11–15,17, 18] and humans [21] in countries outside of Japan. However, no study of the antimicrobial susceptibility patterns of these isolates from dogs in Japan has been reported.

Determination of the susceptibility of *S. intermedius* to antimicrobial agents is of great importance in the selective use of chemotherapeutics, the evaluation of new antimicrobial agents, and the development of drug resistance through continuous use of antimicrobial agents against field isolates. The present study was designed to investigate the antimicrobial susceptibility of strains of *S. intermedius* isolated from healthy and diseased dogs during two different time periods, from 1982 to 1985 and in 1999.

A total of 90 *S. intermedius* strains were used. Twenty-four strains isolated in 1982 to 1985 were isolated from 24 dogs suffering from otitis externa (n=8), pyoderma (n=3), and mastitis (n=1), and from the external auditory meatus (n=6) and nares (n=6) of clinically healthy dogs in 2 Prefectures (Tokyo and Hiroshima). Sixty-six strains isolated in 1999 were isolated from dogs suffering from otitis externa (n=1), pyoderma (n=5), impetigo (n=4), eczema (n=8), dermatitis (n=6), folliculitis (n=9), skin tumor (n=1), alveolar blennorrhrea (n=1), and from the external auditory meatus (n=11), mouth (n=3), nares (n=8), and skin (n=9) of healthy dogs. Strains in 1999 were isolated from 66 dogs which had visited eleven veterinary clinics for various diagnostic procedures and vaccinations in 6 Prefectures (Hokkaido, Saitama, Aichi, Osaka, Hyogo, and Fukuoka). Strain ATCC 29663 was also used for reference. These strains were stored in 10% skim milk suspensions at −80°C until they were used. Identification of *S. intermedius* was performed using the API-Staph system (bio Merieux S.A., France), and its presence was confirmed by some additional characteristics such as colony pigment, staphylocoagulase, clumping factor, heat-stable DNase, hemolysins, hyaluronidase, novobiocin resistance (1.6 µg/ml), and acid production anaerobically from mannitol [5, 8].

The antimicrobial agents tested were as follows: ampicillin (ABPC), methicillin (DMPPC), cephalaxin (CEX), oxytetracycline (OTC), kanamycin (KM), erythromycin (EM), acetylsaloverlytylosin (AIV-TS), terdekamycin (TDM), lincomycin (LCM), enrofloxacin (ERFX), benoxacin (BFLX), and florfenicol (FF). Minimum inhibitory concentrations (MICs) were determined by means of a standardized agar dilution method as described by the Japanese Society of Chemotherapy [19]. Isolates were grown in Mueller-Hinton broth (Difco) for 18 hr at 37°C. The inocula were adjusted to give approximately 10⁶ colony-forming units/ml in saline and spotted onto Mueller-Hinton agar plates (Difco) containing serial two-fold dilutions of the antimicrobial agents with a microplanter (Sakuma Co., Ltd., Tokyo, Japan). The plates were incubated for 20 hr at 37°C. MICs were defined as the lowest concentrations at which no visible growth was observed. β-lactamase production was determined using cefinase discs (BBL).

The ranges of MICs of 12 antimicrobial agents for 90 strains are shown in Table 1. The ranges of MICs for recent strains collected in 1999 were almost the same as those for
earlier strains collected from 1982 to 1985. Also, there were no significant differences in MICs between strains isolated from healthy dogs and those of diseased dogs.

All of the 90 strains were highly susceptible to ABPC, ERFX, and BFLX (MIC, 0.05 to 1.56, 0.1 to 0.78, and 0.39 to 1.56 \( \mu g/ml \), respectively). DMPPC, CEX, TDM, and FF showed the distribution of MICs ranging from 0.78 to 6.25 \( \mu g/ml \) for all the strains tested. These results indicated that the above-mentioned antimicrobial agents were still active in vitro against field isolates regardless the period from which they were recovered (1982 to 1985 or 1999). However, careful usage of the drugs is required for the effective chemotherapy against the disease caused by \textit{S. intermedius}.

While the MICs of OTC, KM, EM, AIV-TS, and LCM were distributed in a broad range of 0.1 to >100 \( \mu g/ml \), indicating that resistant as well as susceptible populations of \textit{S. intermedius} strains existed. Most (>90%) of the strains were susceptible to KM, EM, AIV-TS, and LCM (MIC, 0.1 to 0.78, 0.2 to 0.78, 0.78 to 1.56, and 0.39 to 0.78 \( \mu g/ml \), respectively). Only 64.4% were susceptible to OTC (MIC, 0.39 to 0.78 \( \mu g/ml \)).

Overall, 33 (36.7%) of the 90 strains were resistant to at least one of the drugs tested. Resistance patterns of these 33 resistant strains are shown in Table 2. In the strains isolated in 1982 to 1985, the frequency of resistance was 41.7% and 91.7% in strains from healthy and diseased dogs, respectively. Rates of resistance in 1999 were 22.6% in healthy dogs and 28.6% in diseased dogs. The incidence of resistant strains isolated in 1982 to 1985 was significantly higher than that of 1999. The reason for the resistance ratio between the former isolates and the latter ones is unclear, but the discrepancy might be due to the regions of isolation.

OTC resistance (n=32) was the most frequent, followed by KM (n=9), EM (n=7), AIV-TS (n=7), and LCM (n=7). The most frequently occurring resistance pattern was OTC KM EM AIV-TS LCM (n=1). There were no significant differences between isolates from healthy dogs and those from diseased dogs with respect to their resistance patterns. Grenne and Schwarz [7], and Pedersen and Wegener [11] found no differences in antibiotic resistance patterns between clinical isolates and isolates from healthy dogs, a finding confirmed by our results.

Three tested \( \beta \)-lactam antibiotics, ABPC, DMPPC, and CEX, were highly effective against \textit{S. intermedius} strains. In the United States, however, ABPC resistance was
Antimicrobial resistance pattern | Healthy dog (n=12) | Diseased dog (n=12) | Healthy dog (n=31) | Diseased dog (n=35) | Total (n=90)
--- | --- | --- | --- | --- | ---
OTC | 4<sup>b</sup> | 9 | 5 | 6 | 24
OTC KM | 0 | 0 | 0 | 2 | 2
OTC KM EM AIV-TS LCM | 1 | 2 | 2 | 1 | 6
KM EM AIV-TS LCM | 0 | 0 | 0 | 1 | 1
Total | 5/12<sup>c</sup> | 11/12 | 7/31 | 10/35 | 33/90
(41.7%) | (91.7%) | (22.6%) | (28.6%) | (36.7%)

a) No. of strains tested.
b) No. of drug resistant strains.
c) No. of drug resistant strains/No. of strains tested.

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Table 2. Antimicrobial resistance patterns of S. intermedius strains isolated from healthy dogs and diseased dogs

Detected in 39.4 to 82.7% of S. intermedius isolates from dogs in 1982 to 1984 [4, 9, 13].

Generally, β-lactamase open the β-lactam ring of penicillins and cephalosporins and abolish their antimicrobial activity. In the present study, we found that 42 (46.7%) of the 90 strains tested were β-lactamase positive. These β-lactamase-producing strains showed highly susceptible to ABPC and CEX. Wegener et al. [22] also found Staphylococcus hyicus showing highly susceptibility (MIC, <0.03–2.0 µg/ml) to ABPC, although about 41% of their strains (41 out of 99) were β-lactamase-producers. According to the NCCLS standard for antimicrobial susceptibility tests (M100) [10], a susceptible criterion for MIC of penicillin is given less than 0.12 µg/ml. However, it also notes that β-lactamase may or may not be produced by staphylococci with penicillin MICs between 0.06 to 0.12 µg/ml. Although penicillin MIC was not examined in the present study, remarkable relations may not exist between β-lactamase production and MICs of penicillins.

Tetracycline (TC) resistance has been characterized in S. intermedius strains from dogs [7, 17]. The frequency of TC resistance was 33.3 to 52.8% in the United Sates [3, 4, 9,13], and 20% in Denmark [11]. Also, resistance to doxycycline has been reported in isolates from a patient with bacteremia [21]. In the present study, about 36% of the strains tested were resistant to OTC. These three antibiotics are tetracyclines sharing a similar chemical structure, indicating the occurrence of cross-resistance. The increasing resistance of strains of S. intermedius to tetracyclines may be a reflection of tetracyclines’ much wider use than other antimicrobial agents for veterinary use in many countries including Japan. Piriz et al. [15] stated that OTC cannot be recommended for treatment of canine staphylococcal dermatitis, due to the high percentage (over 25%) of strains that were found to be resistant. In Japan, the frequency of resistance to KM (9 out of 90, 10%) and EM (7 out of 90, 7.8%) was slightly low compared with those in the United States (KM resistance, 7.6 to 25.7%; EM, 10.5 to 23.9%) [3, 4, 9, 13].

Methicillin-resistant Staphylococcus aureus has recently been isolated from dogs [6, 20]. All the 90 S. intermedius strains tested in this study were susceptible to methicillin. However, methicillin-resistant S. intermedius strains have been isolated from dogs [6, 14, 15], but the frequency of isolation is of a low level [6].

The data from this study might serve as a guideline in selecting drugs to be used for treating dogs with staphylococcal infections. Also, these results could be used to compare the sensitivities of isolates in future years to determine any changes in resistance to drugs commonly used for treating canine staphylococcal infections.

REFERENCES