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Dissemination of *Staphylococcus warneri* in the Hair of ICU Doctors

Ikue Shamoto[1], Yuka Ishihara[1], Akiko Sato[1], Hisae Usami[1], Naoyuki Matsuda[2], Michio Ohta[1*]

[1]School of Nursing, Sugiyama Jogakuen University, Nagoya, Japan
[2]Department of Emergency and Critical Care Medicine, Graduate School of Medicine, Nagoya University, Nagoya, Japan
Email: [*]mohta@med.nagoya-u.ac.jp

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Abstract

This study reports a case of *Staphylococcus warneri* dissemination in the hair of ICU/ER doctors. Eleven of 23 doctors in the first survey and 11 of 24 doctors in the second survey, which were conducted after 6 months, carried *S. warneri*, while hair contamination of other coagulase-negative staphylococci was only sporadic. Chromosomal DNA analysis with PFGE revealed that 7 *S. warneri* strains isolated in the first survey and 7 strains isolated in the second survey showed similar PFGE patterns, suggesting that a cross-infection with a *S. warneri* strain continued over 6 months among the ICU doctors.

Keywords

***Staphylococcus warneri*, ICU Doctor, PFGE**

1. Introduction

Coagulase-negative staphylococcal species (CNS) constitute a major component of the normal flora of the human skin. Their role in causing opportunistic infections, however, has been recognized in recent years. Infections caused by CNS involve indwelling catheters and artificial devices and are sometimes life-threatening. Kawamura identified 1230 staphylococcal strains from human clinical specimens with DNA-DNA hybridization, the most reliable method for the determination of staphylococcal species, and found that the isolation frequencies of CNS species were in the order of *Staphylococcus epidermidis*, *S. haemolyticus*, *S. caprae*, *S. simulans*, *S. hominis*, *S. capitis*, *S. saprophyticus*, *S. warneri*, and *S. lugdunensis* [1]. CNS colonizing on the hair and in the

[*]Corresponding author.

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skin niche of patients is probably the source of infection and those colonizing on the hair and the skin of doctors and nurses can also be the source of infection in compromised patients. We therefore investigated the colonization of staphylococci on the hair of doctors working in the surgical ICU/ER of a university hospital, and found the dissemination of a *S. warneri* strain among the ICU doctors.

2. Materials and Methods

2.1. Samplings and Identification of Bacteria

A bunch of head hair was removed without contamination from each of the 36 ICU doctors at a university hospital in July 2011, January 2012, and April 2012. The second survey was conducted without informing the examinees of the results of the first survey; however, the third survey in April 2012 was conducted 2 weeks after informing the examinees of the previous results.

A 10-cm-long hair from each examinee was used for bacterial examination. The hair was homogenized with a glass homogenizer (Iwaki Sci Tech, AGC, Japan) in 0.2 mL sterilized distilled water and 20 μL of the supernatant was inoculated on a mannitol salt agar plate (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan). After 48 h of incubation at 37˚C, colonies on the plate were gram-stained, and suspected colonies were applied to a VITEK 2 automated system with a GP card (bioMerieux Inc., Durham, NC, USA) for identification. Identified staphylococci were then amplified in Tryptic Soy Broth (BD Japan, Tokyo, Japan) and stored at −80˚C until use. This study was approved as the approved number 59 by the institutional ethics committee, Sugiyama Jogakuen University School of Nursing, Nagoya, Japan. All subjects provided informed consent prior to enrollment in the study.

2.2. DNA Fingerprinting

DNA fingerprinting of *S. warneri* chromosomal DNA was performed by pulsed-field gel electrophoresis (PFGE) as described previously, with some modifications [2] [3]. In brief, the bacteria were spread from the −80˚C stock on LB agar and cultured overnight at 37˚C. The colonies were collected and suspended in TE8 buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0). The suspension was mixed with an equal volume of 1.2% Seaken Gold Agarose (Cambrex Bio Science Rockland, Inc., Rockland, ME, USA) and allowed to solidify in a CHEF disposable plug mold (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The gel block was incubated for 24 h at 37˚C in a lysis solution [1 M NaCl, 0.1 M EDTA, 6 mM Tris-HCl, pH 8.0, 0.2% deoxycholate, 0.5% Na-N-dodecylsarcosine, supplemented with lysozyme (3 mg/mL, Sigma-Aldrich Japan, Tokyo, Japan) and lysostaphin (60 IU/mL, Wako Pure Chemical Industries, Ltd., Osaka, Japan)]. The block was then incubated at 55˚C for 20 h in a proteinase K solution [0.5 M EDTA, 3 % Na-N-dodecylsarcosine, pH 8.0, supplemented with proteinase K (1 mg/mL, Wako Pure Chemical Industries, Ltd.)] and washed with TE8 buffer for more than 8 h. A thinly sliced section of the block was digested with 10 units of *Sma*I (Takara Bio Inc., Shiga, Japan) overnight at 30˚C and then electrophoresed at 6 V/cm for pulse times ranging from 1 to 40 s through 1% agarose gel in TBE buffer (50 mM Tris-HCl, 50 mM boric acid, 1 mM EDTA, pH 8.0), supplemented with 50 μM thiourea (Wako Pure Chemical Industries, Ltd.) at 14˚C for 20 h with the CHEF-DRII system (Bio-Rad Laboratories, Inc.). Thereafter, the gel was stained with ethidium bromide, washed with distilled water, and photographed. A yeast chromosome PFGE marker (New England Biolabs Japan, Inc., Tokyo, Japan) was used as the size standard. Chromosomal patterns were digitally analyzed with Finger Print II (Bio-Rad) to generate a dendrogram based on Dice coefficients.

3. Results and Discussion

An aliquot of the supernatant of the hair-homogenate was cultured on mannitol salt agar plates and bacterial colonies on the plates were gram-stained. Colonies of probable staphylococci were further identified with a VITEK 2 automated system/GP card. As shown in **Table 1**, most identified species of the strains that colonized on the hair of the ICU doctors were CNS, and among these, *S. warneri* was dominant. Eleven of 23 doctors in the first survey and 11 of 24 doctors in the second survey carried *S. warneri*. Other identified species were *S. aureus*, *S. epidermidis*, and *S. kristinae*. None of the examined doctors in the third survey carried staphylococci in their hair.

Chromosomal DNA fragment analysis with PFGE presented in **Figure 1** and **Figure 2** revealed two lineages

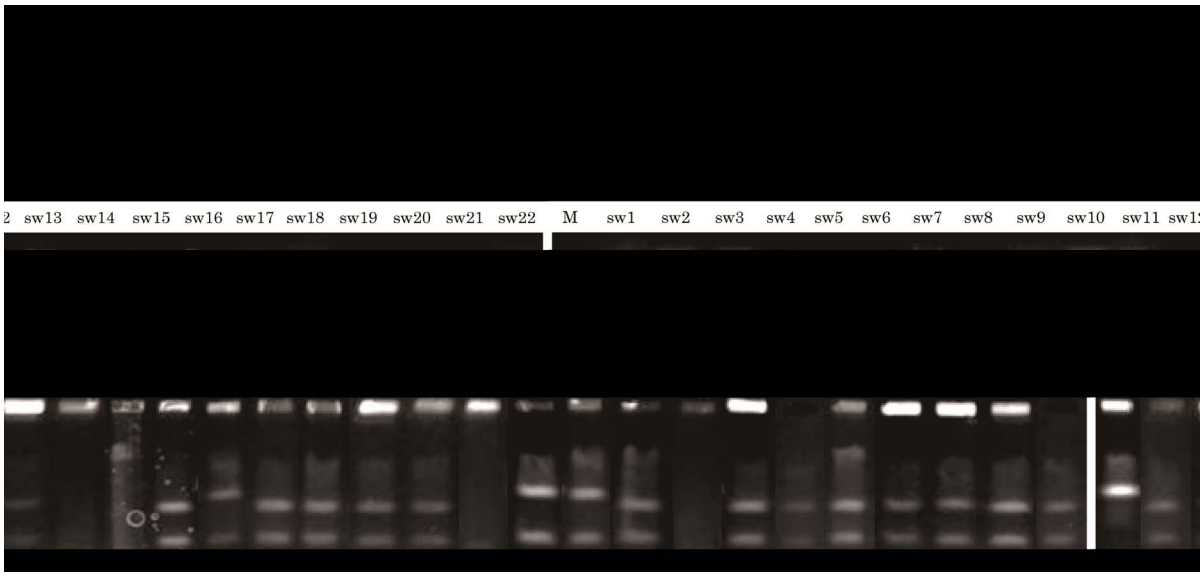

Figure 1. PFGE analysis of *Staphylococcus warneri* strains. Chromosomal DNA of *S. warneri* strains sw1-sw22 digested with *Sma*I was analyzed with PFGE. M, Yeast chromosome size standard.

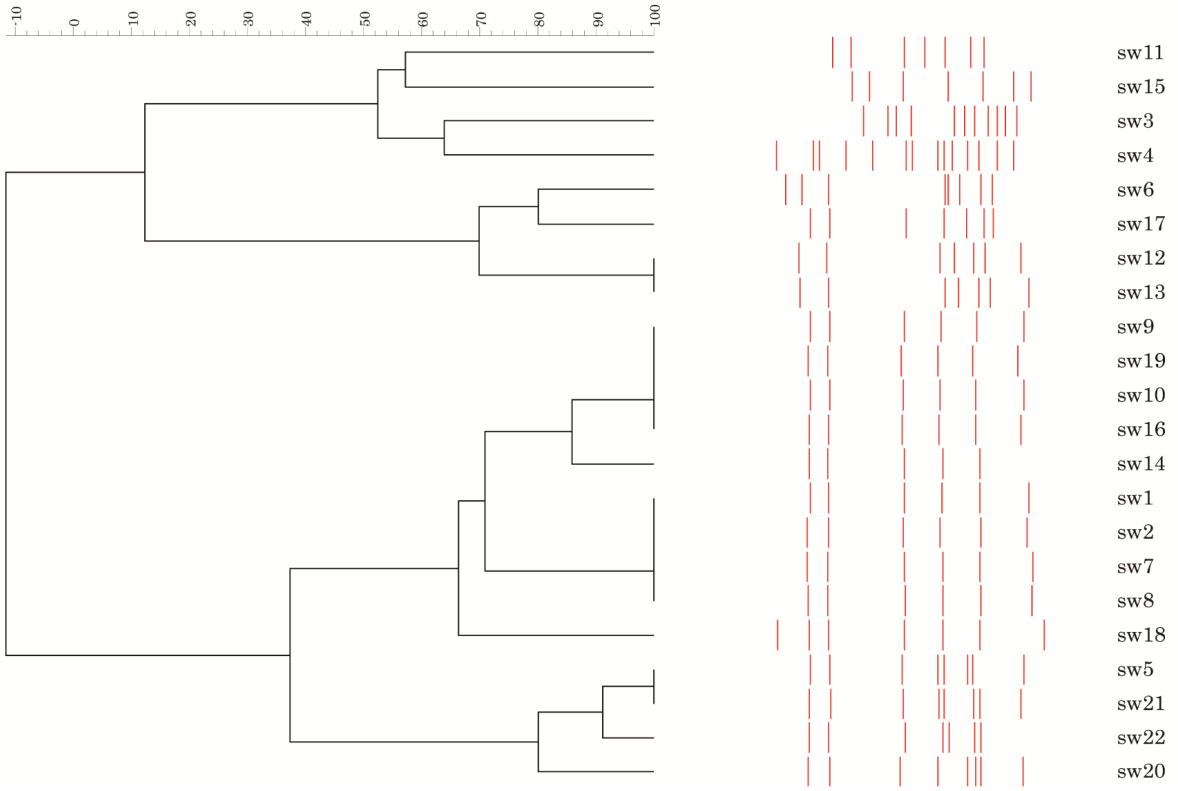

Figure 2. Dendrogram of PFGE results based on the Dice coefficients and schematic representation of the *Sma*I restriction pulsotype of *S. warneri* strains.

A and B of *S. warneri* strains. A group of sw9, sw19, sw10, and sw16 showed an almost identical pattern and the patterns of a group of sw1, sw2, sw7, and sw8 were almost identical, suggesting that cross-infections with *S. warneri* strains existed among the ICU doctors. *S. warneri* sw1, sw2, sw5, sw7, sw8, sw9, and sw10 isolated from the hair of different doctors in the first survey, and sw14, sw16, sw18, sw19, sw20, sw21, and sw22 iso-

Table 1. Isolation of staphylococci from the hair of ICU/ER doctors.

Examinee[*1)]	First survey (July 2011)	Second survey (January 2012)	Third survey (April 2012)
1	*S. warneri* (sw1)	ND[*2)]	ND
2	*S. aureus*		
3	*S. warneri* (sw2)		ND
4	*S. epidermidis*	ND	ND
5	*S. warneri* (sw3)	ND	ND
6	*S. kristinae*	ND	ND
7	*S. epidermidis*	ND	ND
10	*S. warneri* (sw4)	ND	ND
11	ND	ND	ND
13	*S. warneri* (sw5)	*S. warneri* (sw12)	ND
14	*S. warneri* (sw6)	*S. warneri* (sw13)	ND
15	ND	*S. warneri* (sw14)	ND
16	ND	*S. warneri* (sw15)	ND
17	ND	*S. warneri* (sw16)	ND
18	*S. warneri* (sw7)	*S. warneri* (sw17)	ND
19		ND	ND
20	*S. warneri* (sw8)	*S. warneri* (sw18)	
21	*S. warneri* (sw9)	*S. epidermidis*	ND
22		ND	ND
23	ND	ND	ND
24	*S. warneri* (sw10)		ND
25		*S. warneri* (sw19)	ND
26			ND
27			ND
28	ND		ND
29	*S. warneri* (sw11)		ND
30	ND		ND
31	ND		ND
32		ND	ND
33		*S. warneri* (sw20)	ND
34		*S. warneri* (sw21)	ND
35		ND	ND
36		*S. warneri* (sw22)	ND

[*]1) ICU/ER doctor. [*]2) ND, no staphylococci was isolated.

lated in the second survey belonged to the lineage B (**Figure 2**). Moreover, PFGE patterns of sw6 and sw17, and sw12 and sw13 were almost similar and these strains belonged to the lineage A. According to the criterion of Tenover *et al.* (1995) that an isolate with a 2 - 3-fragment difference was probably part of the same outbreak, it was determined that the strains of the lineage B were closely related to each other, because the differences among the patterns of the lineage B were only the positions of 3 fragments [4]. It is therefore likely that an en-

demic infection with *S. warneri* among the ICU doctors was present for over 6 months. *S. warneri* seems to have a high affinity for human hair compared with other staphylococci such as *S. aureus*, *S. epidermidis* and *S. kristinae*, since these staphylococci were isolated only sporadically. The ICU doctors had shared a bed and a blanket as well as a pillow, the cover of which was left unwashed for a long time, in the nap room for both men and women. In the third survey conducted 2 weeks after informing the examinees of the previous results, no staphylococci were detected in their hair. According to the interview with the doctors, they had washed their hair vigorously and had not used the nap room bed before the third survey, suggesting that the nap room bed was probably the site of transmission.

Although *S. warneri* represents only 1% of the skin staphylococci in normal individuals [5], it may be associated with various human infections such as septicemia, endocarditis, meningitis, conjunctivitis, and wound infections [5]-[8]. According to previous reports, *S. warneri* infections were most frequently associated with indwelling devices in patients with underlying immunosuppressive illnesses [7] [8]. All postoperative patients in ICU are in high-risk conditions with indwelling catheters after severely invasive treatments. *S. warneri* may cause serious infections, including blood stream infections and endocarditis, in these patients. This study therefore emphasizes the importance of the eradication of *S. warneri* from the hair of ICU doctors.

Conflict of Interest Statement

The authors have no conflict of interest to report.

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