【Original Article】

Survival of MRSA attached to materials used in medical facilities at different humidity levels

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Summary

Methicillin-resistant *Staphylococcus aureus* (MRSA) attached to materials and equipment used in medical facilities reportedly survives for several weeks under normal conditions. According to our survival studies on pieces of silicone rubber, silver, aluminum, and stainless steel at 20°C, methicillin-sensitive *Staphylococcus aureus* [MSSA (ATCC29213)], MRSA (ATCC43300), and environment-origin MRSA (L1) all survived after 30 days. On silver, however, there have been conflicting reports concerning the survival in environments at different humidity levels. In this study, we evaluated the survivability of *S. aureus* on the bed railing and linen used at medical facilities at 20°C under different humidity conditions.

The survival rates of bacteria at 88.7% RH (83.3-94.7% ; high humidity) and 47.9% RH (38.1-57.6% ; low humidity) were compared after culturing for 1, 2, 3, 7, 15, and 30 days on some metal or non metal materials.

On pieces of copper and brass, MSSA, MRSA, and MRSA (L1) did not survive longer than 1 day at either the high or low humidity. On pieces of silver, aluminum, and stainless steel, MSSA, MRSA, and MRSA (L1) survived for longer at the low compared to the high humidity. On silver, which is considered to exhibit antimicrobial activity, *S. aureus* is believed to survive for a long period in seasons with a low relative humidity, and appropriate anti-infection measures should be practiced without overconfidence in their antimicrobial effects.

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Keyword: MRSA, humidity levels, survivability, materials, nursing environment

I. Objective

Methicillin-resistant *Staphylococcus aureus* (MRSA) attached to materials and instruments used at medical facilities reportedly survives for several weeks under normal conditions. We previously showed that methicillin-sensitive *Staphylococcus aureus* [MSSA (ATCC29213)], MRSA (ATCC43300), and environment-origin MRSA (L1) can survive for 30 days or longer on some materials.[1)] MRSA and MRSA (L1) survived until after 30 days at room temperature (20°C) on pieces of silicone rubber, aluminum, silver, and stainless steel. Michels et al.[2)] on the other hand, showed that the survival of MRSA attached to a silver ion-coated material is affected by the humidity, with bacterial counts of Log_{10} 0.2 at <20%RH but Log_{10} 5.5 at >90%RH, employing a film contact test.[3)] In this study, we evaluated the survivability of *S. aureus* on a number of materials at room temperature (20°C) at different humidity levels.

II. Materials and Methods

1. Bacterial strains used

ATCC 29213, a standard strain of MSSA, MRSA ATCC 43300, and environment-origin MRSA (L1) were used as test strains. The environment-origin MRSA (L1) is a strain that showed resistance to oxacillin (MPIPC) based on the dilution method according to the sensitivity testing standards of the Clinical and Laboratory Standards Institute (CLSI) among those isolated and identified from the patient-care environment of a medical facility. This strain is also PBP2′-positive on the latex slide agglutination test (MRSA-LA Seiken® : Denka Seiken Co., Ltd.).

2. Materials to which test strains were attached

The test strains were attached to a single layer of a square piece of 100% cotton cloth 0.40 mm thick, ultra-fine polypropylene unwoven cloth 0.25 mm thick used for disposable gowns, etc., silicone rubber 0.50 mm thick used as a material for bag valve mask, etc., and pieces of 99.9% copper 0.05 mm thick, brass 0.10 mm thick, 99.9% silver 0.05 mm thick, aluminum with a purity of 99.0% or higher 0.05 mm thick, and SUS304 stainless steel 0.05 mm thick used for antimicrobial coating.

3. Attaching test strains to various materials

The above materials were cut into 2×2-cm pieces, sterilized by autoclaving, and treated with 50 μl [10^4 colony forming units (cfu)] of a suspension of *S. aureus* in phosphate-buffered saline. Then, the materials to which the test strains were attached were placed in a desiccator containing diphosphorus pentaoxide and were dried rapidly by aspirating the air in the desiccator. These pieces were used as samples.

4. Evaluation of the survival of test strains

Sets of 3 pieces to which the test strains were attached were placed in incubators adjusted to the high and low humidity levels at 20° C and incubated for 1, 2, 3, 7, 15, or 30 days. The temperature and humidity in the incubators were monitored with ultra-small thermo-hygrometers (Hygrocron® : KN Laboratories, Inc.) placed in them. After incubation, the pieces to which the test strains were attached were cultured in Triptic soy broth (Eiken) and observed for 7 days to judge whether the test strains had survived or died.

The pieces to which the test strains were

Table.1 Survival of MSSA（ATCC29213）attached to materials used in medical facilities at different humidity levels at 20℃

material	humidity	after the attachment	immediately after drying	1days after	2days after	3days after	7days after	15days after	30days after
cotton cloth	low	3/3	3/3	3/3	3/3	3/3	3/3	3/3	1/3
	high	3/3	3/3	3/3	3/3	3/3	2/3	0/0	0/0
polypropylene cloth	low	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	high	3/3	3/3	3/3	3/3	3/3	3/3	1/3	0/0
silicone rubber	low	3/3	3/3	3/3	3/3	3/3	3/3	3/3	1/3
	high	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/0
copper	low	3/3	0/0	0/0	0/0	0/0	0/0	0/0	0/0
	high	3/3	0/0	0/0	0/0	0/0	0/0	0/0	0/0
brass	low	3/3	0/0	0/0	0/0	0/0	0/0	0/0	0/0
	high	3/3	0/0	0/0	0/0	0/0	0/0	0/0	0/0
silver	low	3/3	3/3	3/3	3/3	3/3	3/3	1/3	1/3
	high	3/3	3/3	3/3	3/3	0/0	0/0	0/0	0/0
aluminum	low	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	high	3/3	3/3	3/3	3/3	2/3	0/0	0/0	0/0
stainless steel	low	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	high	3/3	3/3	3/3	3/3	3/3	3/3	0/0	0/0

MSSA : methicillin-sensitive *Staphylococcus aureus*
the number：the number of test tubes could grow/the number of test tubes were cultivated
low: 47.9% RH（38.1 ～ 57.6% RH）, high：88.7% RH（83.3 ～ 94.7% RH）

attached were similarly examined immediately after the attachment of the test strains and immediately after drying.

5. Relative humidities

The means of the high and low humidity levels were 88.7 % RH (83.2-94.7% RH) and 47.9% RH (38.1-57.6% RH), respectively.

III. Results

Among non-metal materials, MSSA and MRSA survived on cotton cloth until after 30 days at the low but not at the high humidity. MRSA (L1) survived until after 15 days at the low humidity but not at the high humidity.

On polypropylene sheets and silicone rubber plates, MSSA, MRSA, and MRSA (L1) all survived until after 30 days at the low but not at the high humidity.

Concerning metals, survival of MSSA, MRSA, and MRSA (L1) was not observed on copper or brass plates from immediately after drying after the attachment of the test strains at either the high or low humidity level, and they did not survive even for 1 day.

On silver, aluminum, and stainless steel plates, MSSA, MRSA, and MRSA (L1) all survived until after 30 days at the low humidity, but none of them survived until after 15 days at the high humidity. On aluminum, none of them survived until after 7 days at the high humidity. On silver, none of the strains survived until after 3 days at the high humidity.

On both metals and non-metals, the survival period of *S. aureus* tended to be longer at the low compared to the high humidity level.

The survival of *S. aureus* tended to be affected by the humidity as well as the material to which they were attached.

IV. Discussion

On polypropylene sheets and silicone rubber plates, MSSA, MRSA, and MRSA (L1) all survived for 30 days at the low but not at the high humidity. On cotton cloth also, their survival periods tended to be shorter at the high compared to the low humidity. On non-metal materials, the test strains were more likely to die at the high humidity.

Table.2 Survival of MRSA (ATCC43300) attached to materials used in medical facilities at different humidity levels at 20℃

material	humidity	after the attachment	immediately after drying	1days after	2days after	3days after	7days after	15days after	30days after
cotton cloth	low	3/3	3/3	3/3	3/3	3/3	3/3	3/3	2/3
	high	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/0
polypropylene cloth	low	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	high	3/3	3/3	3/3	3/3	3/3	3/3	0/0	0/0
silicone rubber	low	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	high	3/3	3/3	3/3	3/3	3/3	2/3	1/3	0/0
copper	low	3/3	0/0	0/0	0/0	0/0	0/0	0/0	0/0
	high	3/3	0/0	0/0	0/0	0/0	0/0	0/0	0/0
brass	low	3/3	0/0	0/0	0/0	0/0	0/0	0/0	0/0
	high	3/3	0/0	0/0	0/0	0/0	0/0	0/0	0/0
silver	low	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	high	3/3	3/3	2/3	2/3	0/0	0/0	0/0	0/0
aluminum	low	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	high	3/3	3/3	3/3	2/3	2/3	0/0	0/0	0/0
stainless steel	low	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	high	3/3	3/3	3/3	3/3	3/3	3/3	0/0	0/0

MRSA：methicillin-resistant *Staphylococcus aureus*
the number：the number of test tubes could grow/the number of test tubes were cultivated
low: 47.9% RH（38.1 ～ 57.6% RH）, high：88.7% RH（83.3 ～ 94.7% RH）

By directing attention to the humidity, Mcdade et al.[4] examined the survival rate of S aureus after 7-day incubation on silk cloth and rubber plates at 25°C under 11, 33, 53, and 85% RH, and showed their survival at 11 and 33% RH but death at 52 and 85% RH. Lindwall et al.[5] compared the survival of *S. aureus* attached to dust under different humidity conditions after 1-day incubation in terms of the mean log survival rate, and reported that it was 0.88 in a dry state but low at 0.08 at 84% RH. The humidity is considered to affect the survival of *S. aureus*, and its survival time is considered to shorten with elevation of the relative humidity.

On aluminum, silver, and stainless steel plates, MSSA, MRSA, and MRSA (L1) all survived until after 30 days at the low but not at the high humidity. They were likely to die in a high humidity environment.

As for silver, Michels et al.[2] compared the survival of MRSA on a silver ion-coated surface at 20°C under different relative humidity conditions, and showed that the death rate was higher at RH >90% than at RH <24%. Silver was reported to exhibit an antimicrobial activity[7,8] according to the results of the film contact test (JIS Z2801).[3] Also, the infection rate-reducing effects of silver-coated central venous catheters[9] and silver-containing urethral catheters to be inserted into the body[10] are considered to be related to the high humidity conditions due to contact with blood and body fluids. On the other hand, the silver-containing stethoscope diaphragm cover was reported not to show antibacterial activity,[11] probably because MRSA was sampled from stethoscopes used in a daily low-humidity patient care environment. Since the survival time of MRSA on silver is markedly affected by the relative humidity, overconfidence in its antimicrobial activity should be avoided, and appropriate infection control measures should be taken.

MSSA, MRSA, and MRSA (L1) attached to copper and brass were likely to die at both the high and low humidity levels. On the copper plates, the test strains were confirmed to be alive immediately after their attachment, but they died thereafter under both humidity conditions. The results were the same on the brass plates. In the reports of

Table.3 Survival of MRSA (L1) attached to materials used in medical facilities at different humidity levels at 20℃

material	humidity	after the attachment	immediately after drying	1days after	2days after	3days after	7days after	15days after	30days after
cotton cloth	low	3/3	3/3	3/3	3/3	3/3	3/3	1/3	0/0
	high	3/3	3/3	3/3	3/3	3/3	3/3	0/0	0/0
polypropylene cloth	low	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	high	3/3	3/3	3/3	3/3	3/3	2/3	2/3	0/0
silicone rubber	low	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	high	3/3	3/3	3/3	3/3	3/3	2/3	1/3	0/0
copper	low	3/3	0/0	0/0	0/0	0/0	0/0	0/0	0/0
	high	3/3	0/0	0/0	0/0	0/0	0/0	0/0	0/0
brass	low	3/3	0/0	0/0	0/0	0/0	0/0	0/0	0/0
	high	3/3	0/0	0/0	0/0	0/0	0/0	0/0	0/0
silver	low	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	high	3/3	3/3	2/3	2/3	0/0	0/0	0/0	0/0
aluminum	low	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	high	3/3	3/3	3/3	3/3	3/3	0/0	0/0	0/0
stainless steel	low	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	high	3/3	3/3	3/3	3/3	3/3	3/3	0/0	0/0

MRSA (L1)：environmental-origin MRSA
the number：the number of test tubes could grow/the number of test tubes were cultivated
low: 47.9% RH（38.1 ～ 57.6% RH), high：88.7% RH（83.3 ～ 94.7% RH）

Michels et al.[2] concerning the survival of MRSA on copper and brass, the bacterial count after 24 hours was log10 6.1 at RH >90% but was reduced to log10 5.9 at RH <24%. The use of medical materials or equipment made of materials with an antimicrobial activity in a patient care environment may well contribute to infection control. However, Tolba et al.[12] reported that the survival time of MRSA prolonged to 2 weeks when sterilized blood was added to a copper-containing coin. Even if copper-containing materials have bactericidal activity, the hospital environment is likely to undergo invisible contamination by the patients' body fluids and blood, and so periodic cleaning employing an appropriate method is considered necessary.

The survival time of *S. aureus* attached to materials in a hospital environment tended to be prolonged in a low-compared with a high-humidity condition. Also, silver, which is considered to have antimicrobial activity, is not expected to be effective depending on the relative humidity. Therefore, overconfidence in such materials should be avoided, and appropriate infection control measures should be practiced.

Conflict of interest disclosure

This study was carried out with a Grant-in-Aid for Scientific Research [(C) General], 2009-2011. There is no conflict of interest.

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湿度の異なる条件における医療施設で使用される資材や器材に付着したMRSAの生存性

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要　旨

室内環境に近い20℃における綿布、ポリプロピレン布、シリコーンゴム片、銅片、真鍮片、銀片、アルミニウム片、ステンレス片に付着したmethicillin-sensitive Staphylococcus aureus〔MSSA（ATCC29213）〕、及びmethicillin-resistant Staphylococcus aureus〔MRSA（ATCC43300）〕、環境由来MRSA（L1）の湿度の異なる条件［高湿度：88.7%RH（83.3-94.7%）、又は低湿度：47.9%RH（38.1-57.6%）］での1、2、3、7、15、30日後の生存性を比較した。その結果、銅片、真鍮ではいずれの菌株も湿度に関わらず1日も生存しなかった。銀片、アルミニウム片、ステンレス片においてはいずれも、「高湿度」より「低湿度」において生存期間の延長が見られた。抗菌効果があるとされる銀片は、相対湿度が低い季節において*S.aureus*の長期の生存が考えられるため抗菌効果を過信すべきではなく、適正な感染予防策を講じるべきであると考える。

キーワード：MRSA、湿度条件、生存性、材質、療養環境

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