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Brief Communication

Evaluation of an Appropriate Replacement Cycle for Copper Antibacterial Film to Prevent Secondary Infection

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The use of copper antibacterial films as an effective infection prevention method is increasing owing to its ability to reduce the risk of pathogen transmission. In this study, we evaluated the bacterial contamination of the antibacterial copper membrane attached to a door handle at a university over time. Six mounting locations with high floating population were selected. In three sites, the door handles with the antibacterial film were exposed, while the remaining three were not attached with the antibacterial films. On days 7 and 14, isolated bacterial strains were inoculated in BHI broth and agar, respectively. Colony-forming units (CFU) were determined after incubation. Strain identification was performed using bacterial 16s rRNA PCR and sequencing. Results showed that the bacterial population on day 14 significantly increased from 6×10^9 CFU/mL (day 7) to 2×10^{10} CFU/mL. Furthermore, strain distribution was not different between the on and off the copper antibacterial film groups. In conclusion, although copper has an antibacterial activity, microbial contamination may occur with prolonged use.

Key Words: Antibacterial copper film, Contamination, Bacterial 16S rRNA

In the past two years, millions of people worldwide have died from infection with the new severe acute respiratory syndrome coronavirus (SARS-CoV-2) (Sharma et al., 2020; Hu et al., 2021). An antibacterial copper film was used as part of a method to prevent the spread of SARS-CoV-2 infection (Merkl et al., 2021; Lishchynskyi et al., 2022). Copper film prevents infection by reducing the risk of pathogen transmission (Noyce et al., 2006; Elguindi et al., 2009; Souli et al., 2013). Copper antibacterial films can be used in all public facilities, such as elevators, apartments, hospitals, and schools, especially when there is a large floating population. Among inorganic antibacterial agents,

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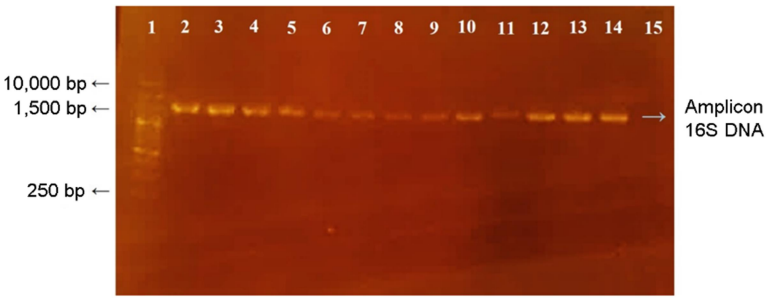

Fig. 1. Typical PCR results on the identification of bacteria isolated from door handles with/without copper antibacterial films. The amplified PCR product was loaded. Lane 1, 1 kb plus DNA ladder (Invitrogen); lane 2, *Bacillus subtilis*; lane 3, *Bacillus subtilis*; lane 4, *Staphylococcus hominis*; lane 5, *Bacillus* spp.; lane 6, *Bacillus* spp.; lane 7, *Staphylococcus aureus*; lane 8, *Bacillus subtilis*; lane 9, *Bacillus subtilis*; lane 10, *Bacillus velezensis*; lane 11, *Bacillus subtilis*; lane 12, *Bacillus subtilis*; lane 13, *Bacillus* spp.; lane 14, *Bacillus* spp.; lane 15, negative control.

copper, silver, and zinc have a reddish-brown surface, high electrical/thermal conductivity, and broad antibacterial ability. The antimicrobial activity of copper surfaces through contact has been well documented. Copper kills O-157, a representative food-poisoning bacterium, within 30 min and *Escherichia coli* within 90 min, and has an antibacterial effect against 90 malignant bacteria and 20 viruses (Faúndez et al., 2004; Kampf and Kramer, 2004; Elguindi et al., 2009; Grass et al., 2011). However, bacteria survive for a long time in masks and antibacterial films that prevent transmission and infection from existing pathogens, thus contaminating the surface, leading to secondary infection (Boyce, 2007; Otter et al., 2013). In order to prevent secondary infection of pathogens with high viability, it is necessary to systematically investigate the degree of bacterial contamination of the antimicrobial sinus membrane over time and to determine an appropriate antimicrobial membrane replacement cycle.

In Korea, studies on the degree of bacterial contamination in the hands of students attending schools have been conducted (Kim et al., 2012; Chong, 2016). However, a systematic investigation on the degree of bacterial contamination in antibacterial sinus membranes in public educational institutions has not been reported yet. In this study, we evaluated the degree of bacterial contamination in copper films over time.

Between April and May 2021, six-door handles of buildings of educational institutions in Busan were targeted. The antibacterial film with a high sales volume was selected among the antibacterial films (Topsafety Co., Ltd., Korea) temporarily approved by the Ministry of Food and Drug Safety and distributed in the domestic market. The selected target product was attached according to the manufacturer's instruction. Antibacterial films attached to each location were collected after 7 and 14 d, and cultured in Brain Hearth Infusion (BHI) broth (Hampshire, England) to observe bacterial contamination. To measure the degree of contamination of the antibacterial film, the collected test strains were inoculated in BHI broth at each time point and incubated at 36℃ in an incubator (MIR-253, Sanyo Electric Biomedical Co., Ltd.) for 18 to 24 h. For active culture of the test strain, one inoculation loop of the cultured test strain was inoculated onto BHI agar and incubated at 36℃ for 18~24 h. The strains that were active in secondary and tertiary cultures were used.

Next, DNA of the isolated bacteria was extracted, and the nucleotide sequence was analyzed. Briefly, one colony per type strain was suspended in 100 μL of Chelex-100 resin, boiled for 10 min, and then centrifuged at 13,000 × *g* for 10 min. The resulting supernatant was used as the DNA template. Polymerase chain reaction (PCR) was performed using 16S rRNA primers, including 27F (5'-AGAGTTTG-ATCMTGGCTCAG-3') and 1492R (5'-TACGYTACTTG-TTACGACTT-3'). The PCR conditions were set to 95℃ for 5 min (initial denaturation) followed by 30 cycles of 95℃ for 30 sec (denaturation), 60℃ for 1 min (annealing), and 72℃ for 1 min (extension). After the final cycle, sample were maintained at 72℃ for 10 min to complete strand synthesis. The PCR products were electrophoresed at 100 V for 30 min on a 1% agarose gel containing EtBr. The 16S rRNA gene sequencing was performed at Macrogen, and the sequence was compared with the sequences in the GenBank database of the National Center for Biotechnology Information (NCBI) for species assignment.

Fig. 1. shows typical PCR results on the identification of

Table 1. Number and species of bacteria isolates in door handles with and without the copper antibacterial film

Group	
With the copper antibacterial film	Without the copper antibacterial film
Species (No. of isolates)	
B. subtilis (6), *B. velezensis* (6), *Bacillus* spp. (2), *S. epidermidis* (1)	*B. subtilis* (6), *B. velezensis* (5), *Bacillus* spp. (5), *S. hominis* (4), *S. aureus* (1), *S. epidermidis* (1)

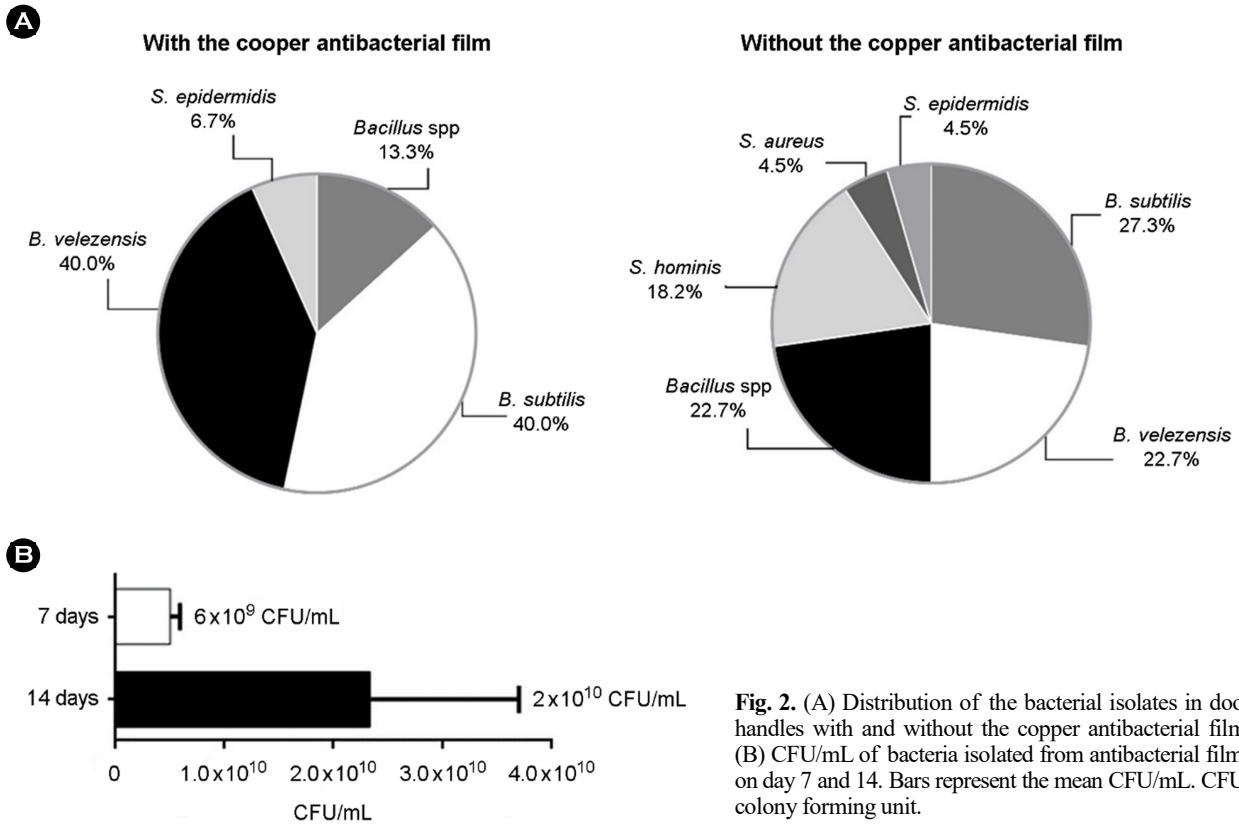

Fig. 2. (A) Distribution of the bacterial isolates in door handles with and without the copper antibacterial film. (B) CFU/mL of bacteria isolated from antibacterial films on day 7 and 14. Bars represent the mean CFU/mL. CFU: colony forming unit.

bacteria isolated from door handles with/without copper antibacterial films. Among the 37 bacterial isolates, *Bacillus subtilis* (40.0%), *B. velezensis* (40.0%), *Bacillus* spp. (13.3%), and *Staphylococcus epidermidis* (6.7%) were isolated from the copper antibacterial film-attached group, While *B. subtilis* (27.3%), *B. velezensis* (22.7%), *Bacillus* spp. (22.7%), *S. hominis* (18.2%), *S. aureus* (4.5%), and *S. epidermidis* (4.5%) were isolated from the copper antibacterial film-unattached film group (Table 1). These results revealed that the distribution of bacteria between the on- and off-copper antibacterial film groups was not different, with the exception of two isolate identified as *S. aureus* and *S. hominis* in the off-copper antibacterial film group (Fig. 2A). Next, to confirm the increase in bacterial population through time, bacteria were isolated from the antibacterial films on days 7 and 14. Results showed that 6×10^9 CFU/mL and 2×10^{10} CFU/mL of bacteria were isolated on days 7 and 14, respectively. This indicates that bacterial population on day 14 increased three times as that on day 7 (Fig. 2B).

The role of copper antibacterial films is to reduce the survival time of infectious agents. If the infectious agent survives for approximately 10 h in the normal environment, its survival time in the antibacterial metal environment is reduced by less than half. Recently, preventing the occur-

rence of copper antimicrobial films as reservoirs of potential pathogens has emerged as a potential solution (Boyce, 2007; Adlhart et al., 2018). Therefore, we investigated the appropriate replacement period by identifying the contamination level in the antimicrobial copper membranes used in domestic educational facilities. Our results detected various types of bacterial contaminants. These results suggest that despite the adhesion of antimicrobial copper membranes, the use of contaminated membranes may increase infection through the spread of pathogenic bacteria. The higher the concentration of contaminated bacteria, the more likely it is to spread to secondary contact areas. In this study, the population of bacterial contaminants was of 6×10^9~2×10^{10} CFU/mL. Previous studies have reported 3.72~7.51 log10 CFU/mL and 2.77~7.81 log10 CFU/mL (Chattman et al., 2011; Hong, 2020). It suggests the need for management and guidelines for bacterial contamination in public place and equipment including copper antibacterial film.

Bacillus subtilis (6), *B. velezensis* (6), *Bacillus* spp. (2), and *S. epidermidis* (1) were isolated from the six facilities. *B. subtilis* frequently causes laboratory contamination and sometimes causes lesions, such as conjunctivitis. *B. velezensis*, which was the most commonly isolated species in this study, is ubiquitous in nature. Although *S. epidermidis* is generally not pathogenic, patients with compromised immune systems are at risk of developing *S. epidermidis* infection.

In conclusion, antibacterial films that require reattachment have a high possibility of contamination by gram-positive bacteria, and the long-term use of antibacterial films in public facilities may cause transmission of infectious agents. Therefore, public facilities should comply with the replacement time of the antibacterial film, especially in places visited by people with weakened immunity.

Several limitations should be considered when interpreting the findings of this study. Further investigation to determine how much the number of bacteria has increased in the absence of a copper antibacterial film are necessary.

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CONFLICT OF INTEREST

The researcher claims no conflicts of interest.

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