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Antibacterial Activity of Antibacterial Cutting Boards in Household Kitchens

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Received 30 December, 2006/Accepted 5 July, 2007

We examined antibacterial cutting boards with antibacterial activity values of either “2” or “4” in compliance with the JIS Z 2801 standard, and compared their findings with those of cutting boards with no antibacterial activity. These cutting boards were used in ten different households, and we measured changes in the viable cell counts of several types of bacteria with the drop plate method. We also identified the detected bacterial flora and measured the minimum antimicrobial concentrations of several commonly used antibacterial agents against the kinds of bacteria identified to determine the expected antibacterial activity of the respective agents. Cutting boards with activity values of both “2” and “4” proved to be antibacterial in actual use, although no correlation between the viable cell counts and the antibacterial activity values was observed. In the kitchen environment, large quantities of *Pseudomonas*, *Flavobacterium*, *Micrococcus*, and *Bacillus* were detected, and it was confirmed that common antibacterial agents used in many antibacterial products are effective against these bacterial species. In addition, we measured the minimum antimicrobial concentrations of the agents against lactobacillus, a typical good bacterium, and discovered that this bacterium is less sensitive to these antibacterial agents compared to more common bacteria.

Key words : Cutting boards in household kitchens/Antibacterial activity value/Bacteria flora/
Minimum antimicrobial concentration value.

INTRODUCTION

Since the 1990s, “amenity” has been a catchword to attract Japanese consumers, and this trend has brought many antibacterial products into the market. However, many of these so-called antibacterial products only contained some antibacterial agents and in many cases were not demonstrated to be “antibacterial” after testing.

In response to this finding, in June 1998 several manufacturers of antibacterial agents and products, as well as several academics in Japan, established the Society of Industrial-Technology for Antimicrobial

Articles (SIAA). This development was followed by the publication of “Guidelines of Antibacterial Processed Products” by the then Ministry of International Trade and Industry (now the Ministry of Economy, Trade and Industry), in May 1999 (Volume of The Ministry of International Trade and Industry 1999). These efforts called for new regulations to be defined for these products.

Later, “JIS Z 2801,” (Japanese Standards Association. 2000) a methodology to test antibacterial activity, was established to standardize the industry's own control of antibacterial products.

However, JIS Z 2801 is only a theoretical evaluation methodology and it has yet to prove effective for measuring antibacterial activity in the environments for which such products are designed to be used.

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This is particularly relevant given that antibacterial effects are invisible.

To date, no reports have been published on the antibacterial effects of products outside of Japan, though we do have some reports (Ojima et al., 2002a; Ojima et al., 2002b; Rusin et al., 1998; Josephson et al., 1997) on indoor microbial contamination written from the viewpoint of sanitary control.

Therefore, using "antibacterial food cutting boards," an antibacterial product of which use is comparatively widespread today, we conducted an investigation to assess the following:

- (1) Correlation between the antibacterial activity value "2" as defined by JIS Z 2801 and the value obtained by monitoring cutting boards actually used in ordinary homes.
- (2) Investigation of bacteria detected in the kitchen environment.
- (3) Effect of common, commercially available antibacterial agents upon the bacteria detected.

The results of the tests we conducted are described in this paper.

TEST METHOD

Measuring the antibacterial activity values of antibacterial cutting boards

Following the antibacterial activity testing method defined by JIS Z 2801, we measured the antibacterial activity values of the antibacterial cutting boards whose activity value was adjusted to be either 2 or 4. The antibacterial activity index obtained using the test method above can be defined as follows:

- Antibacterial activity index defined by JIS Z 2801

Conditions for the test to be verifiable include:

- (1) Examining the logarithm of viable cell counts on the untreated test samples immediately after inoculation:

$$(L_{\max} - L_{\min}) / (L_{\text{mean}}) < 0.2$$

L_{\max} : Maximum logarithm of the viable cell count

L_{\min} : Minimum logarithm of the viable cell count

L_{mean} : Average logarithm of the viable cell counts of three test pieces

- (2) The average viable cell count immediately after inoculation on the untreated test samples was between 1.0 and 4.0×10^5 .
- (3) The respective viable cell counts measured after 24 hours on all three untreated test samples was 1.0×10^3 [cfu/ml] or more.

The test is considered to be verifiable if these three

conditions are met.

The antibacterial activity value (R) is obtained as follows:

$$R = \{\log (B/A) - \log (C/A)\} = [\log (B/C)]$$

where

- A: Average viable cell counts of the unprocessed test pieces immediately after the inoculation.
- B: Average viable cell counts of the unprocessed test pieces 24 h after the inoculation.
- C: Average viable cell counts of the test pieces with antibacterial treatment 24 h after the inoculation.

In this test, we employed two bacterial species, *Staphylococcus aureus* (NBRC 12732) and *Escherichia coli* (NBRC 3972).

Measuring the effectiveness of antibacterial agents in the actual environment

Application of cutting boards.

In this test, we used untreated polypropylene cutting boards with antibacterial activity values of either 2 or 4. We asked 10 households (A to J), to use each of the three kinds of boards on successive days.

Sampling method.

Everyday, the households washed their cutting boards after use with a scrubbing brush and running water and then let them dry naturally. On the following day, before using the dry cutting board, they swabbed a 10×10 cm² area of the board surface with Q-tips moistened with phosphate buffered saline. These Q-tips were collected and examined at 1, 2, 4, and 6 weeks.

Measurement of the surviving bacteria.

Following the drop plate method, we inoculated 10 ml of phosphate buffered saline with the Q-tips that had been used to swab 10×10 cm² areas of the cutting board surfaces.

We then spread 0.1 ml of this saline solution on to agar plates that were then incubated in a temperature-controlled bath at 28 ± 2 °C for 5 days to culture the bacteria. We then counted the number of colonies to obtain the viable cell count.

The temperature of 28 ± 2 °C and incubation period of 5 days were used in order to find as many kinds of bacterial flora as possible.

For *Lactobacillus*, we conducted a catalase test with colonies cultured on APT agar media and counted those that were catalase-negative as *Lactobacillus*.

We determined viable cell counts of ubiquitous bacteria, such as coliform groups, *Pseudomonas aeruginosa*, various cocci (*Micrococcus* and *Staphylococcus*), and *Lactobacillus*, all of which were cultured using the following selective media:

General bacteria: CASO agar medium (Merck Ltd., Japan)
 Coliform groups: Deoxycholate agar medium (Merck)
Pseudomonas aeruginosa: Cefrimide agar medium (Nihon Pharmaceutical Co., Ltd.)
 Cocci: Salt egg agar medium (Nissui Pharmaceutical Co., Ltd.)
Lactobacillus: APT agar medium (Merck)+catalase test

Identification of the cultured bacteria.

We transferred the colonies grown on the CASO agar media before isolating the individual colonies. Next, we identified these individual colonies following the microorganism identification method (Kaneko, 1983).

Measurement of minimum inhibitory concentrations for antibacterial agents against the bacteria.

The minimum antimicrobial concentration of antibacterial agents commonly used in antibacterial products was measured using the minimum antimicrobial concentration measurement method I in the 2003 edition of the Antimicrobial Test Methods of the SIAA. We outsourced this work to the Japan Food Research Laboratories, an incorporated foundation.

Five antibacterial agents, most of which contained silver as the principal bactericidal agent, while one contained an organic substance as the main active constituent, and some contained both silver and organic substances, were evaluated:

- Silver and organic hybrid agents (used on the cutting boards)
- Silver-zeolite agents
- Silver-zirconium phosphate agents
- Silver-glass agents
- Organic (pyridine) agents

In our tests, the bacteria being tested were transferred to common agar media and cultured at 35 to 37°C for 24 h. Next, a platinum loopful of the cultured bacterium was transferred over to MHB culture media: Mueller-Hinton bouillon culture media, Oxoid Ltd.) culture media, where it was cultured at 35 to 37°C for 16 to 20 h. We diluted the culture solution with MHB culture media and adjusted the concentration to

where viable cell counts were within the range of 1.0 to 5.0×10^4 cfu/ml. This diluted solution was used as the test solution. The sterilized MHB media (10 ml) was then dispensed into sterilized L-shaped test tubes and 1, 2, and 1/2 portions respectively of an antibacterial agent were added to each (1 portion = 100 μ g/ml). Each of these test tubes were then inoculated with 0.1 ml of one of the test solutions before being cultured by shaking for 24 hours at 35 to 37°C at 100 to 200 rpm and at a shaking amplitude 40 to 60 mm. After the shaking-culturing, we visually inspected whether or not the test subject bacteria were growing. The minimum concentration for an antibacterial agent that prohibited bacterial growth was considered to be that agent's minimum antimicrobial concentration.

We used the following five test bacteria:

Micrococcus luteus (ATCC 9341)
Pseudomonas aeruginosa (NBRC 13275)
Enterobacter cloacae (IFO 3320)
Flavobacterium ferrugineum (NBRC 14992) and
Lactobacillus acidophilus (IFO 13951).

RESULTS AND DISCUSSION

Evaluation result of antibacterial activity of the antibacterial cutting boards

Tables 1 and 2 show the results of our evaluation of the cutting boards' antibacterial activity. The activity values of the boards with activity values of "2" were 2.24 against *Staphylococcus aureus* and 2.10 against *Escherichia coli*. The boards with activity values of "4" had activity values of 3.88 against *Staphylococcus aureus* and 3.68 against *Escherichia coli*. These results show that the cutting boards we examined were less effective for inhibiting *Escherichia coli* than for *Staphylococcus aureus*. These findings also indicate that our test method, which evaluated boards in the actual environment for different genera of bacteria, was appropriate and useful.

Antibacterial effectiveness for actual use

Table 3 and Figure 1 show the chronological changes in the viable cell counts on the respective cutting boards during six weeks of use in the 10 different households.

In households A to J the concentrations of common bacteria tended to be greater on untreated cutting boards used for the same periods. On boards with the antibacterial activities of "2", no chronological increase in the cell count, which remained around 10^3

TABLE 1. Results of antibacterial tests on cutting boards according to JIS Z 2801 against *Staphylococcus aureus*

Sample	Initial viable cell count [cfu/ml]		$(L_{max}-L_{min})/ L_{mean}$	Viable cell count after 24 hours [cfu/ml]		Antibacterial activity value
	Measurement value	Ave		Measurement value	Ave	
Cutting board of Antibacterial activity value 2	—	—	—	1.8×10^4	1.8×10^4	2.24
	—	—	—	1.9×10^4		
	—	—	—	1.8×10^4		
Cutting board of Antibacterial activity value 4	—	—	—	4.2×10^2	4.2×10^2	3.88
	—	—	—	4.3×10^2		
	—	—	—	4.0×10^2		
Blank	2.2×10^5	2.3×10^5	0.09 Satisfaction	3.1×10^6	3.2×10^6	—
	2.3×10^5			3.3×10^6		
	2.4×10^5			3.1×10^6		

TABLE 2. *Escherichia coli*

Sample	Initial viable cell count [cfu/ml]		$(L_{max}-L_{min})/ L_{mean}$	Viable cell count after 24 hours [cfu/ml]		Antibacterial activity value
	Measurement value	Ave		Measurement value	Ave	
Cutting board of Antibacterial activity value 2	—	—	—	6.9×10^3	6.9×10^3	2.10
	—	—	—	6.9×10^3		
	—	—	—	7.0×10^3		
Cutting board of Antibacterial activity value 4	—	—	—	1.8×10^2	1.8×10^2	3.68
	—	—	—	1.9×10^2		
	—	—	—	1.8×10^2		
Blank	1.4×10^5	1.3×10^5	0.15 Satisfaction	8.7×10^5	8.7×10^5	—
	1.2×10^5			8.8×10^5		
	1.3×10^5			8.7×10^5		

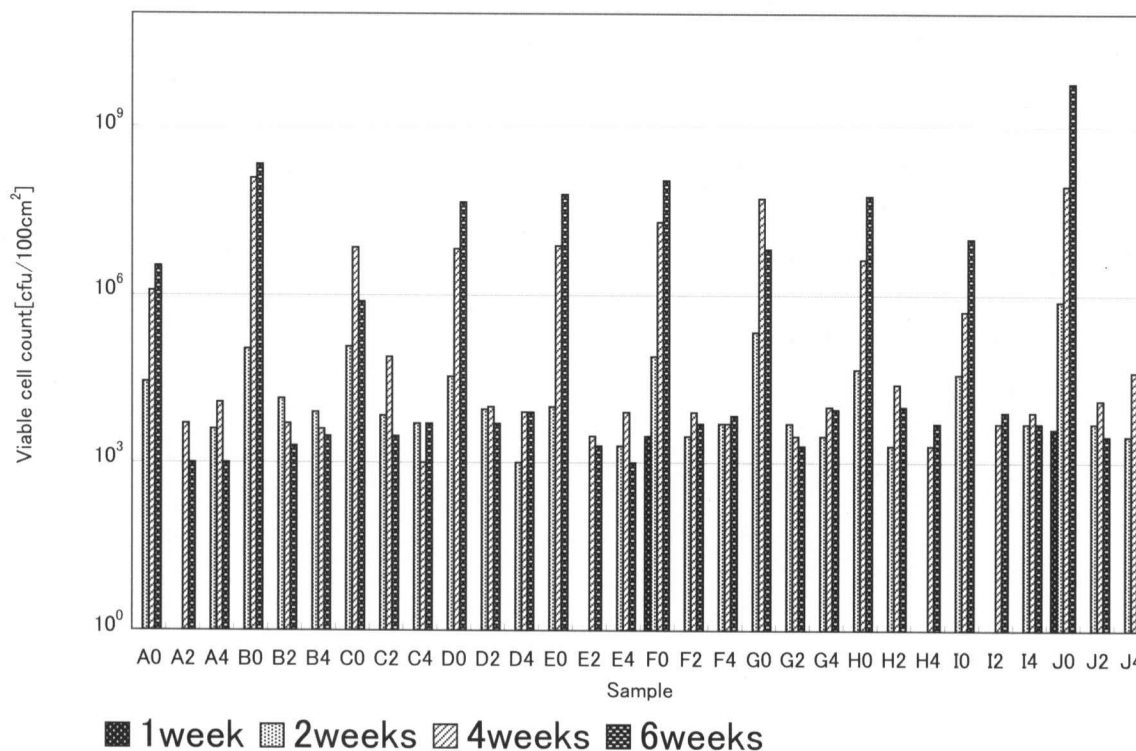


FIG. 1. Changes in the viable cell counts from cutting boards

TABLE 4. Bacteria-flora detected on cutting boards at each home

User	Antibacterial activity value	Bacteria Flora							
		<i>Pseudomonas</i> spp.	<i>Flavobacterium</i> spp.	<i>Micrococcus</i> spp.	<i>Enterobacterium</i> spp.	<i>Acinetobacter</i> spp.	<i>Bacillus</i> spp.	<i>Staphylococcus</i> spp.	<i>Lactobacillus</i> spp.
A	0	○	○	○	○			○	○
	2						○		
	4	○	○	○	○	○			
B	0	○	○	○	○		○		
	2	○	○	○		○	○	○	
	4	○		○			○		
C	0	○	○	○				○	
	2	○	○	○					
	4	○	○	○			○		
D	0	○	○						
	2	○	○				○		
	4		○				○		
E	0	○	○	○	○				○
	2		○				○		
	4	○	○						
F	0	○	○	○	○		○		
	2	○	○			○		○	
	4	○	○						
G	0	○	○	○					
	2	○		○					
	4	○	○	○			○		
H	0	○	○		○				
	2	○	○				○		
	4		○		○	○			
I	0	○	○	○	○			○	○
	2		○			○	○		
	4	○	○	○	○	○			
J	0	○	○	○				○	
	2	○	○	○					
	4	○	○	○		○			

to 10^4 [cfu/100 cm²] was observed over the six week period. This finding illustrated the effectiveness of the antibacterial cutting boards. We observed a similar trend on the cutting boards with activity values of "4", although this higher activity value had no marked difference on antibacterial effectiveness.

At households B, C, and G, cell counts of *coliform* bacteria tended to be greater on untreated cutting boards, but the antibacterial cutting boards showed some antibacterial activity. However, the difference in the set activity value did not result in any observable difference in their effectiveness. Some antibacterial activity was observed at households A, E, I and J.

Pseudomonas aeruginosa increased slightly on untreated cutting boards in households A, C, E, G, and I.

Cocci also increased over time on the untreated cutting boards in four households (B, C, F, and J), but showed no such increase on the antibacterial cutting boards, further confirming the antibacterial activity of the cutting boards.

We were not able to determine the effectiveness of the antibacterial cutting boards on members of the *Lactobacilli* as they could not be isolated on the untreated cutting boards.

Table 4 shows the bacteria flora detected in various households.

The bacteria genera detected most frequently at each household included those associated with water and/or soil, such as *Pseudomonas*, *Flavobacterium*, *Micrococcus*, *Bacillus*, with *Lactobacillus* was only detected at households A and I.

Our finding thus revealed that the antibacterial

TABLE 5. Minimum Inhibitory Concentrations of Biocides commonly used in Antibacterial Products

Strain		Biocide concentration [μ g/ml]				
		Ag-Glass	Ag-Zeorite	Ag-Zirconium	Ag+Organic biocide	Organic Biocide
<i>Micrococcus luteus</i>	ATCC 9341	50	50	50	≤ 25	≤ 25
<i>Pseudomonas aeruginosa</i>	NBRC 13275	200	200	100	> 3,200	≤ 25
<i>Enterobacter cloacae</i>	IFO 3320	400	400	200	> 3,200	≤ 25
<i>Flavobacterium ferrugineum</i>	NBRC 14992	100	100	100	200	≤ 25
<i>Lactobacillus acidophilus</i>	IFO 13951	800	800	200	50	50

cutting boards tested were capable of inhibiting the growth of most common bacteria, although no correlation was observed between their inhibitory effect and the antibacterial activity value. The antibacterial effectiveness of these cutting boards was less apparent against coliform spp., *Pseudomonas aeruginosa* and cocci, but it was still greater than that of untreated cutting boards in some households.

The differences among the households can be attributed to the different ingredients used, frequency of cooking, and several other related factors.

Measurement of minimum antimicrobial concentration for antibacterial agents against the different bacteria

Table 5 shows the minimum antimicrobial concentrations of the agents against the bacteria tested.

At minimum antimicrobial concentration values >3,200 μ g/ml, the antibacterial agent used in the antibacterial cutting boards (Ag + organic substance) proved to be only slightly capable of inhibiting *Pseudomonas aeruginosa* and *Enterobacter cloacae*. Consequently, *Pseudomonas* was detected on the cutting boards used in many of the households tested. The other agents appeared to have similar inhibitory effects on all of the bacteria tested.

Against *Lactobacillus* spp., all of the agents tested, except for the "Ag + organic substance" and "Organic (pyridine) agents" had a markedly lower effect than they did on general bacteria

From the test results, we can assume that antibacterial agents added to antibacterial products can be effective against microorganic bacteria. Based on the findings of this study and the bacteria isolated from the cutting boards, we propose that the most common bacteria found in and around the kitchen sinks of the households tested are *Flavobacterium* and *Micrococcus* spp.

CONCLUSION

Using cutting boards impregnated with antibacterial

agents, we evaluated the inhibitory effect of the active agents and confirmed the following:

- (1) Products with antibacterial activity values of "2" or above exhibit some inhibitory activity on the bacteria found in household environments.
- (2) Compared to common bacteria, lactobacilli are more resistant to antibacterial agents used in antibacterial products.
- (3) In the kitchen environment, the most common genera detected on cutting boards include *Pseudomonas*, *Flavobacterium*, *Micrococcus*, and *Bacillus*, although this could change depending on the food items being prepared.

In this study, we conducted our tests on cutting boards, one of many of household products containing antibacterial agents. Given that variation due to the environment and product type will encourage the growth of different bacterial species, future research should evaluate antibacterial activity of such products under situations of actual use. In addition, such findings should be correlated with the set antibacterial activity value assigned to such products.

ACKNOWLEDGMENTS

We are grateful for the assistance we received from the Society of Industrial-Technology for Antimicrobial Articles while conducting this research. In addition, we thank the staff and students at Kanagawa University of Human Services for their help with monitoring the evaluation.

REFERENCES

- Antimicrobial Test Methods (2003) Society of Industrial Technology for Antimicrobial Articles.
- Japanese Standards Association. (2000) JIS Z 2801 Test of Antimicrobial activity and efficacy.
- Josephson, K, L., Rubino JR., Pepper IL (1997) Characterization and quantification of bacterial pathogens and indicator organism in household kitchens with and without the use of a disinfectant cleaner. *J. Appl. Microbiol.*, **85**, 819-828.
- Kaneko, S. (1983) Microorganism identification method.

- Chapter 1: Identification of aerobic external bacteria, pp.1-94. Technology Association of Hygiene, Tokyo.
- Ojima, M., Toshima, Y., Koya, E., Ara, K., Tokuda, H., Kawai, S., Kasuga, F. (2002) Hygiene measures and actual distributions of microorganisms in Japanese households. *J. Appl. Microbiol.*, **93**, 800-809.
- Ojima, M., Toshima, Y., Koya, E., Ara, K., Kawai, S., Ueda, N. (2002) Bacterial contamination of Japanese households and related concern about sanitation. *Int. J. Environ. Health Res.*, **12**, 41-52.
- Rusin, P., Orosz-Coughlin P., Gerba C. (1998) Reduction of faecal coliform and heterotrophic plate count bacteria in the household kitchen and bathroom by disinfection with hypochlorite cleaners. *J. Appl. Microbiol.*, **85**, 819-828.
- Volume of The Ministry of International Trade and Industry (Consumer Goods Service Industries Bureau (1999) Guidelines of Antibacterial Processed Products.