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# Resistances to benzalkonium chloride of bacteria dried with food elements on stainless steel surface

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**Abstract**

To confirm the importance of washing food sediments from the surface of food-related environments, we examined resistances against benzalkonium chloride of pathogenic bacterial (*Escherichia coli* O26, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) cells dried and adhered on stainless steel dishes with milk, beef gravy or tuna gravy. Suspensions (0.1 ml) of these bacteria (8–9 log cfu/ml) were put on a 5 cm $\varphi$ stainless steel dish and dried at room temperature (20–24 °C) for 90 min in a bio-clean bench with ventilation. Though these bacteria suspended with distilled water decreased 30–40 fold during the drying period, milk and the gravies protected the bacteria. Without the food elements, the adhered *E. coli* and *Stap. aureus* were decreased from 6 to $<2$ log cfu/dish by 0.5 mg/ml benzalkonium chloride (BKC) for 10 min treatment. Although *Ps. aeruginosa* showed resistance to BKC, the adhered cells were inactivated by 2.0 mg/ml BKC. However, the bactericidal effect disappeared by the food elements, particularly with milk, even at 1.0 and/or 2.0 mg/ml BKC levels. The protective efficiency of milk on bacteria disappeared if washed with water.


*Keywords:* Food sediment; Stainless steel; Benzalkonium chloride; *Escherichia coli*; *Pseudomonas aeruginosa*; *Staphylococcus aureus*

## 1. Introduction

Adhesion of microorganisms to equipment surface have the potential to transmit pathogens to food, and this is apparent in the food processing industry (Barnes, Lo, Adams, & Chamberlain, 1999; Giaouris & Nychas, 2006) and in the domestic environment (Humphrey, Martin, Slader, & Durham, 2001). There are many kinds of disinfectants for food utensils, such as alcoholic solutions and hypochloric solutions. Quaternary ammonium compounds are cationic biocides that are commonly used as disinfectants in food production environments (Krysinski, Brown, & Marchisello, 1992). Benzalkonium chloride (BKC) is a quaternary ammonium compound that is widely used for sanitation in food-processing environments (Mustapha & Liewen, 1989). BKC acts on general membrane permeability, causing the cytolytic leakage of cytoplasmic materials at low concentrations. At high concentrations, they target the carboxylic groups and cause general coagulation in the bacterial cytoplasm (To, Favrin, Romanova, Mansel, & Griffiths, 2002). Previous reports showed that these bacteria have different resistivities (*Pseudomonas aeruginosa* > *Escherichia coli* > *Staphylococcus aureus*) against BKC in suspensions (Reuda, Amigot Lázaro, & Ducha, 2003).

It is known that the microorganisms on the inner surfaces of food and medical apparatuses and equipments often forms biofilm, and it is reported that the tolerance of the biofilms to various stresses is different from the planktonic cells in the test tube (Carpentier & Cerf, 1993). Particularly, there are many reports about the resistances of biofilms of *Ps. aeruginosa* (Ishikawa & Horii, 2005; Landry, An, Hupp, Singh, & Parsek, 2006), *Stap. aureus* (Shanks et al., 2005; Valle et al., 2003) and *Listeria monocytogenes* (Carpentier & Chassaing, 2004; Chemielewski & Frank, 2006) because of their strong resistances against disinfectants and for serious medical reasons, such as nosocomial infections.

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On the other hand, it is considered that the food elements protect microbial cells when the adhered cells are dried on the surface of food utensils and equipments (Leslie, Israeli, Lighthart, Crowe, & Crowe, 1995), and maybe the microorganisms achieve resistances to disinfectants and biofilms. Although there are many reports about the effect of dirty conditions determined by the European Standard EN-1276: 1997 (Payne, Babb, & Bradley, 1999; Taylor, Rogers, & Holah, 1999), these studies were carried out in suspensions in tubes.

In this study, to determine the importance of washing food sediments from the surface of food utensils before chemical disinfection, the protective efficiency of milk, beef gravy and tuna gravy on three pathogenic bacteria (*E. coli* O26, *Ps. aeruginosa*, *Stap. aureus*), they were dried and adhered on stainless steel dishes were examined.

## 2. Material and methods

### 2.1. Bacterial culturing

*E. coli* O26:HNM (VT1), *Ps. aeruginosa* IAM1514 and *Stap. aureus* IAM 12544 were employed to investigate attachment and disinfection treatments on stainless steel surfaces. To produce cultures, the bacterial cells were inoculated into 5 ml of Trypton-Soya Broth (TSB, Nissui Pharmaceutical Co., Tokyo, Japan) and incubated at 37 °C for 18 h with shaking (120 rpm). The culture reached the stationary phase.

### 2.2. Chemicals and food materials

Ten percent BKC was purchased from Wako Pure Chemical (Osaka, Japan). Alkyl ether sulfuric acid ester sodium (AES), a neutralized detergent, was purchased from Kao Corporation (Tokyo). Ultra high temperature-treated (UHT) milk, frozen beef (outside round) meat and yellowfin tuna *Thunnus albacares* meat were purchased from retail shop. Beef gravy and tuna gravy were prepared from same volumes of the meat and distilled water (50% gravies). These were minced and centrifuged (2000*g* for 10 min at room temperature).

### 2.3. Preparing of stainless steel dishes

Commercial stainless steel dishes (sus304, 5 cm$\varphi$, As-one Co., Tokyo) were used in this study. In advance, the dishes were carried out the twice treatment of ultrasonication for 15 min, brushing for 60 s and autoclaving at 121 °C for 15 min.

### 2.4. Bacterial adhesions

Bacterial suspensions were prepared from stationary phase cultures (5 ml). These were centrifuged at approximately 2000*g* for 5 min at room temperature (Decleva, Menegazzi, Busetto, Patriarca, & Dri, 2006) and resuspended in 0.31 mmol/l phosphate-buffered saline (PBS, pH 7.2). This washing process was carried out twice. The cells were finally resuspended in 5 ml of distilled water, milk, 50% beef gravy or 50% tuna gravy. The cell concentration was 9.0–9.3 log cfu/ml. Bacterial suspension (0.1 ml) was placed on the center of the stainless steel dish ($n = 3$) and dried for 90 min at room temperature (20–24 °C) in a bio-clean bench (SCB-1300B, Shimadzu Rika Instrument, Tokyo) with ventilation (20 $m^3$/min). After drying, the cells adhered as a dried scale with about a 10 mm-diameter circle.

### 2.5. BKC treatment and enumeration of survival cells

To determine the bactericidal effect of BKC, the dried and attached cells were covered with 0.1 ml of 0, 0.5, 1.0 and 2.0 mg/ml BKC solution. After 10 min at room temperature, 5 ml of TSB was added. Then, the attached cells were brushed and suspended well for 60 s using cotton swab (for microbial test, Nissui Pharmaceutical Co., Tokyo) and this suspension (0.1 ml) was plated immediately on a Tripton Soya Agar (TSA, Oxoid, Basingstoke, UK). The incubation was carried out at 37 °C for 24 h.

Data of the survival cells on stainless steel dish were expressed as the mean and SD or SE of log cfu/dish ($n = 3$). Statistical analysis was performed using the software EXCEL Statistic 5.0 (Esumi Co., Ltd., Tokyo). One-way ANOVA was used to assess the differences. Then, individual means were compared by Duncan's multiple-range test or Student's *t*-test. Significant differences were accepted at $p < 0.05$.

### 2.6. Effect of water washing on adhered cells with milk before the BKC treatment

To determine the effect of water washing, after the cell adhesion with milk on a stainless steel surface in the same way as above, water washing treatment was carried out as

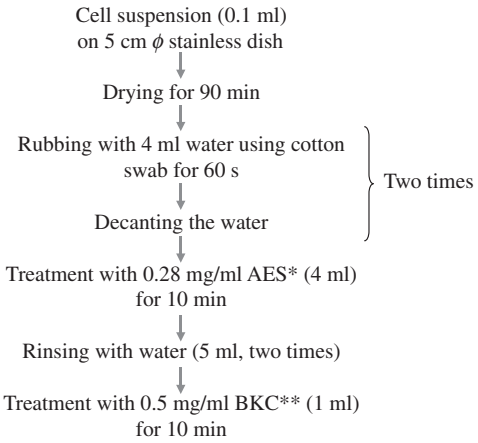


Fig. 1. Scheme of the method for the effect of water washing on adhered cells with milk before benzalkonium chloride treatment. *Alkyl ether sulfuric acid ester sodium, **benzalkonium chloride.