

## **The Disinfecting Effect of Electrolyzed Water Produced by GEN-X-3**

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### **ABSTRACT**

**BACKGROUND:** The use of disinfectants is necessary for hygiene control to prevent food poisoning and for the control against infectious diseases. Evaluation was performed of the disinfecting effect of electrolyzed water produced from GEN-X-3 manufactured electrolysis equipment.

**METHODS:** Both the strains isolated from the specimens collected from hospitals and the reference strains were exposed to the electrolyzed water produced from GEN-X-3 for 0.5, 1, 2, 5, and 10 min. The mixture was inoculated onto tryptic soy broth/agar, and the bacterial growth and viable count were observed.

**RESULTS:** General bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE), and *E. coli* and trophozoites such as *Bacillus subtilis* were all killed within 1 min after the exposure.

**CONCLUSION:** The electrolyzed water produced from GEN-X-3 has strong disinfecting effect against general bacteria, and thus can be useful for hygiene control to prevent food poisoning and for control against infectious diseases.

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## BACKGROUND

Washing, disinfection, sterilization and pasteurization are all a procedure that reduce the number of or remove bacteria, and an important part of approaches for infection control. Washing is a method by which organic matter, soil or microorganisms are removed from certain objects using water, mechanical friction or detergents.

Disinfectant means an agent that can kill infection-causing fungi and trophozoites within 10 min, and does not mean killing of bacterial spores. Disinfectants kill can be divided into one that can kill bacteria, virus and some fungi but cannot kill tuberculosis bacilli or spores of bacteria (a low grade disinfectant), one that can inactivate tuberculosis bacilli and fungi but cannot kill spores of bacteria (medium grade disinfectant), and one that can even kill spores of bacteria if exposure time is sufficient time (high grade disinfectant).

Sanitizer means an agent that can reduce the number of microorganisms that exist on the surface of inorganic substance to the level at which the public health is considered safe. Sterilization means destructing all forms of microorganisms (bacteria, fungi, virus and spores of bacteria) via chemical or physical means.

Sanitizer/disinfectant is an agent that can inhibit the growth of or kills microorganism. Sanitizer/disinfectant for instruments based on the Food Hygiene Law [2] of South Korea is a substance that can be used for sterilization and disinfection of instruments, containers or packaging and can be indirectly transferred to food. This sanitizer/disinfectant can kill 99.999% of trophozoites (5 log<sub>10</sub> CFU/mL) within 5 min, and is widely used for food hygiene purposes such as instruments for food, and packaging instruments.

Due to the expansion of catering services, an increase in eating out, westernization of dietary life, global warming and increased indoor temperature, the incidence of food poisoning is on the rise and tends to be large scale involving large number of people.

As such, in August 2002, revising Food Hygiene Law, the Korea Food and Drug Administration (KFDA) designated the disinfectants for instruments and other miscellaneous things as the item to be controlled according to Food Hygiene Law to prevent food poisoning and enhance food hygiene. In December 2003, KFDA announced a temporary criteria and specification for disinfectants used for instruments and other miscellaneous things for the purpose of disinfecting instruments, containers and packaging, and established a criteria for certifying the disinfecting products.

The most common ingredients used for sterilization/disinfection agents in South Korea

include ammonium, ethanol, chloride, and peroxide families (class IV). Of these, chloride family disinfectants are most commonly used for problems regarding duplicate cost and ease of use.

As infection control becomes active, electrolyzed water, a novel disinfectant agent has been introduced, and its use is expanding. Hypochlorous acid (HOCL) that exerts strong disinfecting effect by electrolyzing water and elemental chloride and electrolyzed oxidizing water that contains active oxygen produced from generator are known to have good disinfecting effect.

In this study, assessment was performed of the sterilizing/disinfecting effect of electrolyzed water produced from GEN-X3 a electrolyzed water producing equipment.

## MATERIALS AND METHODS

### 1. Bacterial Species and Strains.

A total of 9 strains isolated from the specimen collected from patients hospitalized in a college hospital were used: 4 gram negative cocci; methicillin-susceptible *Staphylococcus aureus* (1 strain), methicillin-resistant *Staphylococcus aureus* (1 strain), vancomycin-resistant *Enterococcus faecium* (1 strain), *Enterococcus faecalis* (1 strain) and 9 gram negative bacilli; *Escherichia coli* (1 strain), *Salmonella Typhi* (1 strain), *Salmonella Enteritidis* (1 strain), *Shigella sonnei* (1strain), *Pseudomonas aeruginosa* (1 strain), *Acinetobacter baumannii* (1strain), *Klebsiella pneumoniae* (1 strain), *Enterobacter cloacae* (1 strain), *Stenotrophomonas maltophilia* (1 strain)

Reference strains included *Staphylococcus aureus* ATCC 29213, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Bacillus subtilis* ATCC 6633.

### 2. Preparation of Sterilizing/disinfecting Water (Electrolyzed Water)

Sterilizing/disinfecting water was produced using TOUCAN-ECO EU-7010 (KCTC Global, Hoengseong, South Korea), a electrolyzed water-producing equipment according to the instruction from the manufacturer: tap water 1.2 L and salt 2 g were put in the electrolyzing reactor and electrolysis was performed for 3 min. According to the instruction from the manufacturer of GEN-X-3, the chloride concentration of the electrolyzed water, if produced following the instruction, would be 100-200 ppm. At 50 ppm the solution can kill almost everything.

### 3. Assessment of Disinfecting Effect

Pure cultured strains were cultured in tryptic soy broth (TSB) for 18 hrs. The cultured colony was put into physiological saline, centrifuged at 3,000 rpm for 15 min, and then the supernatants were removed. Phosphate buffer 10 ml (pH 7.2) was added to the precipitated bacteria, suspended using mixer, and the turbidity was set at McFarland 3.

Each (0.5 ml) of the bacterial liquids was put in the physiological saline 4.5 ml, serial dilution was performed from 10 to 107 folds in TSB to achieve final bacterial count 107-108 CFU/mL. Each (0.5 ml) of the bacterial liquids was added to the electrolyzed water 4.5 ml, and left in the room temperature; 0.1 ml each was inoculated onto the TSB broth 5 ml with exposure time set at 0.5, 1, 2, 5 and 10 min and cultured in tryptic soy agar (TSA) using platinum loop 0.01 mL. Then the growth and viable count was calculated.

The criteria of disinfecting effect of the sterilizing/disinfecting agent was defined as viable count being decreased to 5 log 10 folds or less in 5 min after the treatment with sterilizing/disinfecting agent according to the temporary criteria and specification for qualification for food announced by KFDA (KFDA notice No. 2007-29).

## RESULTS

### 1. Disinfecting Effect Against Gram Negative Cocci

For all strains (methicillin-susceptible *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecium*, *Enterococcus faecalis*, and *Staphylococcus aureus* ATCC 29213), the viable count decreased to 5 log<sub>10</sub> within 30 sec after the exposure (Table 1).

### 2. Disinfecting Effect Against Gram Negative Bacilli

For all strains (*E. coli*, *Salmonella Typhi*, *Salmonella Enteritidis*, *Shigella sonnei*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Stenotrophomonas maltophilia*, *E. coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853) the viable count decreased to 5 log<sub>10</sub> within 1 min after the exposure (Table 1).

### 3. Disinfecting Effect Against *Bacillus subtilis*

Viable count of *Bacillus subtilis* ATCC 6633, a trophozoite decreased to 5 log<sub>10</sub> within 1 min after the exposure (Table 1).

Table 1. Biocidal activity of electrolyzed water produced by GEN-X3 against various microorganisms according to exposure time

Test microorganism	Initial count (CFU/mL)	Colonies remaining (CFU/mL)				
		0.5 min	1 min	2 min	5 min	10 min
methicillin-susceptible <i>Staphylococcus aureus</i>	1.5x10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
methicillin-resistant <i>Staphylococcus aureus</i>	1.3x10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
vancomycin-resistant <i>Enterococcus faecium</i>	1.1x10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
<i>Enterococcus faecalis</i>	1.2x10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
<i>Escherichia coli</i>	1.6x10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
<i>Klebsiella pneumoniae</i>	1.3x10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
<i>Enterobacter cloacae</i>	1.2x10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
<i>Salmonella Typhi</i>	1.4x10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
<i>Salmonella Enteritidis</i>	1.8x10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
<i>Shigella sonnei</i>	1.0x10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
<i>Pseudomonas aeruginosa</i>	1.2x10 <sup>8</sup>	> 10 <sup>3</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
<i>Acinetobacter baumannii</i>	1.4x10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
<i>Stenotrophomonas maltophilia</i>	1.5x10 <sup>8</sup>	> 10 <sup>3</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
<i>Staphylococcus aureus</i> , ATCC 29213	1.2x10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
<i>E. coli</i> , ATCC 25922	1.3x10 <sup>8</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>

<i>Pseudomonas aeruginosa</i> , ATCC 27853	1.5x10 <sup>8</sup>	> 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>
<i>Bacillus subtilis</i> , ATCC 6633 (vegetative form)	5.3x10 <sup>7</sup>	> 10 <sup>3</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>	< 10 <sup>2</sup>

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## CONCLUSION

The electrolyzed water produced from GEN-X-3 has a strong disinfecting effect against general bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE), *E. coli*, *Salmonella*, *Shigella*, and thus it is believed that it can be useful as a sterilizing/disinfecting agent for hygiene control to prevent the occurrence of food poisoning and for control against infectious diseases.