

## Comparison of Efficacy of Two Disinfectants Against Foodborne *Staphylococcus aureus*

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### Article Type:

Short Communication

### Article History:

Received: 14 Des 2020

Revised: 20 Jan 2021

Accepted: 26 Jan 2021

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DOI: [10.29252/jorjanibiomedj.9.1.9](https://doi.org/10.29252/jorjanibiomedj.9.1.9)

### Abstract

**Background and Objective:** Foodborne illness is a common public health problem. Surfaces and equipment used in food production are involved in the spread of foodborne pathogens. The aim of this study was to evaluate and compare the antimicrobial effect of Vinoxide and Sanisept against *Staphylococcus aureus* strains isolated from food production lines.

**Material and Methods:** In this descriptive study, 110 samples were taken from various equipment and surfaces used in food workshops and local kitchens. After identification of *S. aureus* by microbiological tests, the antimicrobial effect of two disinfectants (Vinoxide and Sanisept) on the isolates was assessed using the dilution-neutralization test according to the protocols of Iranian National Standards No 2842 and 9899.

**Results:** Of 110 collected samples, 21 (19.1%) were contaminated with *S. aureus*. The results showed that 19% and 38% of *S. aureus* isolates were able to grow after treatment with Vinoxide and Sanisept, respectively. Although Vinoxide had better bactericidal effect than Sanisept, both disinfectants could significantly reduce the number of live *S. aureus* isolates ( $P < 0.05$ ).

**Conclusion:** Sanisept and Vinoxide have significant inhibitory effects on *S. aureus* isolates, but due to the unpleasant odor of Vinoxide, the use of Sanisept is recommended in food industry.

**Keywords:** Disinfectant; Foodborne disease; *Staphylococcus aureus*

**Highlights**

Two disinfectants for control of foodborne *Staphylococcus aureus* were identified. Both disinfectants were able to significantly reduce the number of live *S. aureus* isolates. Due to the unpleasant odor of Vinoxide, the use of Sanisept was recommended in food workshops and local kitchens.

**Introduction**

For decades, foodborne diseases have been considered as a serious public health threat around the world. *Staphylococcus aureus* is one of the main human pathogens that is of great importance. These strains are gram-positive bacteria that are widely present in the environment and can be isolated from various sources such as humans, air conditioners, foodstuff and sometimes food production lines. These bacteria have the ability to produce enterotoxins that can result in food poisoning (1,2). The risk of foodborne illness can be reduced by taking some simple precautions such as preventing cross-contamination and applying good hygiene practices. One of the main reasons for the high prevalence of foodborne illnesses is thought to be the inadequate sanitation of equipment and surfaces in food production and storage processes. Microorganisms form biofilms on the surface of materials commonly used in food processing, such as stainless steel, so that these surfaces become a potential source of contamination that can cause food spoilage, transmit diseases, damage equipment and endanger food hygiene (3,4). Hazard analysis critical control point (HACCP) system is a critical safety control system so that international and executive organizations for food control strongly emphasize the continuity and advancement of this method in the process of human food preparation. Although

determining HACCP points in the production line is not a problem, it is difficult to prevent consumer health risks in these points (5). Therefore, in order to prevent spread of contamination, in addition to understanding disinfection, additional disinfection measures should also be considered. In this regard, the selection of disinfectants and the use of products according to their formulation and manufacturer's instructions play an important role in the surface disinfection quality (6,7). On the other hand, due to the dissemination of microorganisms in environment, the demand for the production of new antimicrobial compounds has increased. There are multiple types of disinfectants with different properties (8). Silver ion (Ag<sup>+</sup>) has a wide range of antimicrobial effects against gram-positive and gram-negative bacteria as well as fungi. Because of their low toxicity to human tissues, silver-based chemicals and pharmaceutical compounds are widely used in industry and medicine. Sanisept S4 is a synthetic disinfectant with a wide spectrum of antimicrobial effects due to the synergistic effect of hydrogen peroxide and silver. The silver in this disinfectant breaks down the outer membrane of bacteria and destroys cellular respiration (9,10). Vinoxide is a peracetic acid-based disinfectant used for disinfection of surfaces in contact with food such as tanks, pipelines and pasteurizers. Peracetic acid is a safe disinfectant and an optimal biocide with limited side effects. It is effective against a variety of microorganisms such as bacteria, fungi, yeasts and viruses at different temperatures (7 to 40 oC). It is also effective against resistant microbial contaminations such as biofilms compared to other disinfectants (11). Due to the important role of food in the spread of infectious diseases, we aimed to compare and determine the antibacterial effects of Sanisept S4 and

Vinoxide on *S. aureus* strains isolated from food production processes.

## Materials and Methods

In this descriptive-analytical study, samples were taken from surfaces and production equipment of several workshops and kitchens for food production using sterile swabs moistened with sterile physiological saline. The samples were cultured in cooked meat medium containing 8% sodium chloride (Sigma, USA) at 37 °C for 48 hours. Then, 0.1 ml of the resulting suspension was incubated in Baird-Parker agar (Sigma, USA) for 24 hours at 37 °C. Black colonies with clear halos were cultured in mannitol salt agar (Merck, Germany). *S. aureus* strains were identified by examining mannitol positive colonies, colony morphology examination, gram staining, hemolysis, catalase, coagulase, and DNase tests and finally confirmed by PCR. Specific primers for *S. aureus* genomic DNA (forward: 5'-AAAACACTTGTCGATATGG-3'; reverse: 5'-GTTTCAATACATCAACTGC-3') were designed using the Oligo5 software. *S. aureus* isolates were confirmed by detecting a 950 bp band in the 1.5% agarose gel electrophoresis.

### Determining the bactericidal effect of the disinfectants

According to protocols No. 2842 and 9899 of Iranian National Standards Organization (INSO), the bactericidal activity is defined as the ability of an agent to cause at least 105 reduction in the number of living reference bacterial cells. In this study, the dilution-neutralization method was used. First, 2-3 bacterial colonies from the 24-hour culture were added to a 100 ml erlenmeyer containing 10 ml of Ringer's solution (diluent) to prepare a bacterial suspension containing  $1.5 \times 10^8$  to  $5 \times 10^8$  live bacteria (18-hour enriched

bacterial suspension). Then, 1 ml of the interfering substance (30 g/l skimmed milk) was added to 1 ml of the test suspension and mixed with 8 ml of the test solution. The mixture was kept in water bath (20 °C) for 15 minutes in case of Vinoxide and for 40 minutes in case of Sanisept S4. After 15 minutes, 1 ml of the test mixture was mixed with 8 ml of neutralizer (containing polysorbate, lecithin and thiosulfate) and 1 ml of sterile distilled water. After preparation of dilutions, they were placed in water bath (20 °C) for 5 minutes.

To determine bacteria counts according to the national standard No. 8923-1, 1 ml from each dilution was transferred to a Petri dish containing 15-20 ml of TSA medium (Merck, Germany) that was preheated to 45 °C. After thorough stirring, the plate was incubated for 24 hours and bacteria were counted based on the standard No. 9899 using *S. aureus* ATCC25923 as the standard strain. Finally, for each product concentration and test condition, the logarithmic reduction was calculated separately using the following formula:  $\text{Log R} = \log N_0 - \log N_A$ .

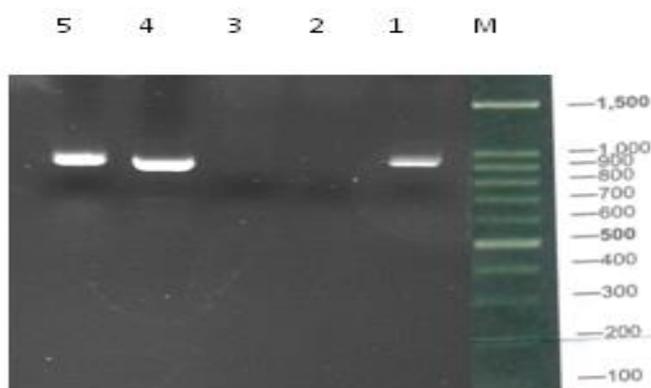
After confirming normality of data distribution using the Kolmogorov-Smirnov test, quantitative and qualitative data were analyzed using independent t-test and Chi-Square test, respectively. All data analyses were performed at 95% confidence level.

## Result

Of 110 samples collected from surfaces and equipment used in food preparation and production, 21 samples (19.1%) were positive for *S. aureus* contamination ([Figure 1](#)). All isolates were grown in -1 to -8 dilutions before adding disinfectants. In case of Vinoxide, only four *S. aureus* isolates (19%) grew in the -1 dilution. However, eight *S. aureus* isolates (38%) grew in -1 Sanisept

dilution, while no growth was observed in other bacterial suspensions, indicating the

higher bactericidal effect of Vinoxide compared to Sanisept S4 (Table 1).



**Figure1:** The polymerase chain reaction fragment length profile of *S.aureus* isolates. Lanes 1, 4, 5: positive *S.aureus* isolates

**Table 1:** Bactericidal effect of disinfectants in-1 dilution on *S. aureus* isolates

Disinfectant	Log (number of Isolates ( $\frac{CFU}{ml}$ ))		P value
	Before treatment	After treatment	
Vinoxide	17(80.1%)	4(19%)	0.034*
Sanisept	13(61.9%)	8(38.1%)	0.02*

$p < 0/05$ (\*significant)

According to standard No. 2842 and dilution-neutralization test, both disinfectants were able to significantly reduce the number of live *S. aureus* isolates (Figure 2,  $P < 0.05$ ).



**Figure 2:** Antimicrobial effect of Sanisept on *S.aureus* isolates (in -1 dilution)

## Discussion

Most foodborne pathogens and spoilage bacteria are able to adhere to food production line surfaces and survive even after cleaning and disinfection. Such bacterial resilience may lead to food contamination during the production process, which can significantly affect the food quality and safety (12). Pathogens usually remain on lifeless surfaces for weeks or even months, so it is necessary to observe hygienic principles and disinfect the surfaces in order to prevent the spread of contaminants. Based on our results, the frequency of *S. aureus* contamination in samples taken from surfaces and equipment used in food production centers was 19%. It has been demonstrated that food preparation surfaces could be a microbial hotspot (13). In a study in Iran (2014), the rate of food contamination in hospitals through cooking tools and utensils was reported to be 73% (14). A study in Spain showed that the rate of bacterial contamination of food line workers' gloves was high (15). In another study, frequency of *S. aureus* isolates in dishcloths, chopping board and kitchen drawers was 42%, 24% and 28%, respectively (16).

In a study in India, 77.7% of foodstuff samples from a garrison were contaminated with *S. aureus* (17). Tools and equipment used in food production play a very important role in food contamination, which emphasizes targeted disinfection, especially in the case of contact surfaces. The results of our study showed that both disinfectants were effective against microbial agents isolated from food. However, Vinoxide showed a better bactericidal effect against *S. aureus* isolates which could be due to the formation of free radicals, induction of microbial autocidal activity and cytoplasmic coagulation or increased membrane permeability (18). Sen et al. believe that the reaction of disinfectant

solutions with some conventional culture compounds may affect the results. Therefore, the results of in vitro studies on a single strain isolate should not be generalized directly to clinical conditions with microbial infections (19). The combination of such disinfectants with chemical agents may also increase their antimicrobial effects, as some researchers have confirmed the synergistic effects of peracetic acid and ultraviolet radiation in reducing the growth of *Escherichia coli* and *Staphylococcus epidermidis* in wastewater (11).

## Conclusion

Based on the results, the mean rate of *S. aureus* contamination decreases significantly after disinfection with both Vinoxide and Sanisept. Although Vinoxide has an unpleasant odor, it is cheap and does not require rinsing. Periodic sampling and cultivation of equipment and contact surfaces for accurate estimation of microbial contamination is a critical strategy for controlling contamination spread through local kitchens and food factories.

## Acknowledgments

The authors would like to express their gratitude to all colleagues who helped conduct the present research.

## Funding sources

This research has been accomplished by the support of Microbiology lab, I.A.U, Gorgan branch and it is extracted from the MSc thesis of Zahra Tajari.

## Conflict of interest

The authors declare that there is no conflict of interest.

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**How to cite:**

Tajari Z, Fozouni L. Comparison of Efficacy of Two Disinfectants Against Foodborne *Staphylococcus aureus*. Jorjani Biomedicine Journal. 2021; 9(1): 9-15.