Infectious diseases pose a significant threat to public health, and their prevention and control are global challenges. Since the outbreak of coronavirus disease 2019, the role of disinfection in breaking the transmission chain of infectious diseases has been increasingly recognized, and disinfection has been practiced in various settings, including the healthcare system, public places, and residential buildings.

Chemical disinfection is the most common disinfection method, which is effective in quickly killing or inactivating microorganisms on abiotic surfaces. Hydrogen peroxide (H₂O₂) disinfection technology has received considerable attention since the 1980s. The decomposition products of H₂O₂ are water and oxygen, which do not pose any threat to human health or the environment. It has been widely used in hospital wards, laboratories, biopharmaceutical factories, and various biosafety equipment. Chlorine dioxide (ClO₂) and chlorine-containing disinfectants are commonly used high-efficiency disinfectants. They effectively destroy bacteria, spores, fungi, viruses, and protozoans. Chlorine dioxide yields chloride and eventually chloride ions, and chlorine-containing disinfectants produce hypochlorous acid when dissolved in water, and both of them have microbicidal activity. Chlorine dioxide and chlorine-containing disinfectants have strong bactericidal and detoxifying abilities as well as irritability to humans and corrosiveness. They are suitable for the disinfection of contaminated surfaces of objects and fabrics as well as water, fruits and vegetables, and food and

OBJECTIVE
The disinfection efficiency of disinfectants differs in specific conditions. This study aimed to investigate the disinfection efficiency of commercial hydrogen peroxide, chlorine dioxide, and chlorine disinfectant on real field surfaces and provide data for precise disinfection.

METHODS
Simulated field disinfection and field disinfection methods were conducted to quantitatively evaluate the disinfection efficiency of hydrogen peroxide, chlorine dioxide, and sodium dichloroisocyanurate. The log₁₀ reduction of biological indicators, Escherichia coli (ATCC 8099) and Staphylococcus aureus (ATCC 6538), was calculated. Next, the reduction in natural bacteria on the surfaces of a food production and processing workshop and a biosafety laboratory was determined.

RESULTS
The 3 commercial disinfectants evaluated were effective against E coli and S aureus, with a reduction of more than 3.00 log₁₀ colony-forming units/mL tested for an exposure time of 15 minutes with 3.5% hydrogen peroxide, 100 mg/L chlorine dioxide, and 250 mg/L sodium dichloroisocyanurate. The natural load in the food production and processing workshop decreased by more than 90% using 10.5% hydrogen peroxide with an exposure time of 30 minutes. The same disinfection level in the biosafety level 2 laboratory was achieved by 500 mg/L chlorine dioxide at an exposure time of 60 minutes and 450 mg/L sodium dichloroisocyanurate at 60 minutes.

CLINICAL RELEVANCE
This study provides a reference for precise disinfection of surfaces in the food industry and biosafety laboratories.

Keywords: hydrogen peroxide, chlorine dioxide, chlorine disinfectant, disinfection, surface
beverage utensils; however, the corrosive effect of these compounds on metals and the bleaching and discoloration effect on fabrics are unavoidable. Therefore, the application of these compounds for the disinfection of metal objects and colored fabric should be approached with careful consideration.

Spraying, fumigation, immersion, and wiping are the commonly used methods of chemical disinfection. Among the novel methods, gas disinfection is currently popular as it produces submicron-level disinfectant droplets by vaporization devices and is effective in inactivating a broad spectrum of pathogens, including spores. These devices typically rely on electrostatic spray disinfection systems, which are expensive and complex for use. For places that are not suitable for whole-room disinfection, such as those with poor air tightness and only require partial disinfection, spraying is more suitable. Spraying is relatively simple to perform, and the equipment used has technically low requirements, which makes it easy to apply to effectively counter the spread of infectious diseases and for daily disinfection by grassroots disinfection professionals and most of the public.

Disinfectant efficiency is influenced by the microorganism species, soiling, material and texture of the surface to be disinfected, disinfectant susceptibility of the microorganism, temperature, toxicity, and economic constraints. With the widespread application of disinfection, a series of problems must be addressed, including inappropriate disinfection methods, improper operation of disinfection by personnel, excessive disinfection, and other problems resulting in the wastage of resources, health hazards, and environmental pollution. Therefore, it is necessary to evaluate the effectiveness of disinfection and fully understand the disinfection level at different locations, which plays a fundamental role in guiding standardized disinfection.

Accurate scientific evaluation plays a fundamental role in product promotion and technical guidance. Moreover, field studies reveal the need to implement efficient protocols; therefore, it is of great practical significance to conduct a field evaluation of the effectiveness of disinfection in key places, identify problems, avoid blind and excessive disinfection, improve disinfection effects, and reduce economic burden.

Methods

The study was conducted according to the Chinese Standard for Evaluating the Efficacy of Disinfection on Site (WS/T 797-2022), with minor adjustments. A portable hand-pressure constant-spray sterilizer (type 409; Solo) was used to perform spraying disinfection. Overall, 3 commercial disinfectants, 35% H2O2 solution, 9% ClO2 tablets, and chlorine disinfectant tablets (main component, 45% sodium dichloroisocyanurate; DCCNa), were used to prepare solutions according to the manufacturer’s instructions. A neutralizer containing 0.5% sodium thiosulfate and 0.5% Tween-80 was used to neutralize the test before disinfection using indicator cultures of *Escherichia coli* (ATCC 8099) and *Staphylococcus aureus* (ATCC 6538), which were obtained from the China General Microbiological Culture Collection and Management Center. *E coli* serves as an indicator of gram-negative bacteria and enteric infection, whereas *S aureus* is an indicator of gram-positive bacteria and supplicative infection.

The bacteria were isolated from freeze-dried cultures in vials. They were subcultured to the fifth to seventh generations by slant nutrient agar medium for 18 to 24 hours and diluted with sterilized PBS to a concentration of 1.0 X 10^8 to 1.0 X 10^9 CFU/mL using the plate-counting method.

### Evaluation of simulated field disinfection

A simulated field disinfection evaluation was conducted in a biosafety level 2 laboratory. Spraying and bacterial contamination were applied on surfaces of horizontal smooth, flat ceramic tiles without any visible organics or prior treatment. First, the flat surfaces were swabbed in a defined area (5 X 5 cm) using a sterile cotton swab immersed in a bacterial suspension, and the amount of collected bacteria was recovered as 1.25 X 10^7 to 1.25 X 10^8 CFU/sample; that is, 5 X 10^5 to 5 X 10^6 CFU/cm². The stained areas were allowed to dry for 10 minutes. The positive control samples were collected without disinfection. The contaminated surfaces, after drying, were disinfected with any one of the 3 disinfectants. For disinfection, spraying was performed at a flow rate of approximately 0.7 L/min and a spray volume of approximately 200 mL/m² until the surface was moist and water droplets were not formed. Samples were collected from each of these surfaces 15, 30, and 45 minutes after spraying according to the following method.

**Sampling and detection:** PBS containing 0.1% Tween-80 was used as the positive control before disinfection, and the test groups postdisinfection were sampled using the neutralizer, which has been described earlier. Each surface was wiped by a sterilized cotton swab immersed in the sampling solution within a defined area (5 X 5 cm). The cotton swab was wiped back and forth horizontally 8 times with rotation, ensuring thorough coverage. Thereafter, the swab was vortexed in 10 mL PBS/neutralizer for more than 20 seconds, followed by inoculating 1 mL in duplicate on nutrient agar. Finally, the plates were incubated at 37 °C for 48 hours, and the number of bacterial colonies (CFU/cm²) was determined. Two replicate samples were set for *E coli* and *S aureus* at each time point, and the experiment was performed 3 times. The bacterial log₁₀ reduction was calculated.
Evaluation of field disinfection

The effectiveness of field disinfection was evaluated separately in a food production and processing workshop and a biosafety level 2 laboratory. The sampling floor in the food production and processing workshop was rough, with some fruit crumbs on the ground. The laboratory had been relocated and vacant for more than 1 year, which made it old, dirty, humid, and cluttered, and contained a large number of microorganisms and dust. The smooth ceramic tiles and plastic desktops were chosen for sampling in the laboratory. Natural bacteria and molds were used as microbial indicators and were collected from each surface before and after disinfection. The doors and windows were closed, and nobody remained in the room. A total of 15 sites were set at each location.

Sampling, detection of natural bacteria and molds, and the disinfection strategy were the same as for the evaluation of simulated field disinfection; however, the cotton swab was wiped back and forth vertically and horizontally 5 times with rotation, and continuous sampling of several plate areas was conducted to collect the appropriate bacteria. An untouched area was swabbed for reference counts before disinfection, and the area next to the reference was sampled after disinfection. Finally, the average killing percentage of the natural bacterial surfaces was calculated.

Benchmark and statistical analysis

The log10 reduction value of all samples had to be ≥ 3.00 in the simulated field disinfection test, and the average killing percentage of natural bacteria and molds had to be ≥ 90% in the field disinfection test to qualify as effective disinfection.22

Data were transferred to Excel (Microsoft Corp), GraphPad Prism software v6.0 (GraphPad), and SPSS Statistics, version 23.0 (IBM Corp), and the log10 reduction value of bacteria were statistically compared using nonparametric tests (Kruskal-Wallis and Mann-Whitney U tests, \( P < .05 \)). The killing percentage of natural bacteria and molds was analyzed using a paired t test (\( P < .05 \)).

Results

Bactericidal efficacy of simulated field disinfection

The log10 reductions of \( E \) coli and \( S \) aureus were above 3.00 for the 3 disinfectants and exposure times of 15, 30, and 45 minutes, respectively (Figure 1), which were qualified according to the benchmark. Besides, the log10 reductions increased with time, and 250 mg/L of DCCNa was the most effective against \( S \) aureus among the tested groups (Figure 1).

The \( E \) coli and \( S \) aureus groups in each of the 3 disinfectants were analyzed using the Mann-Whitney U test, and the results showed that there was no significant difference in the disinfection efficiency between the 2 bacteria (\( H_2O_2: E \) coli vs \( S \) aureus, \( P = .700 \); \( ClO_2: E \) coli vs \( S \) aureus, \( P = .700 \); DCCNa, \( E \) coli vs \( S \) aureus, \( P = .400 \)).

Multiple comparison analyses of each disinfectant to individual bacteria at different exposure times using the Kruskal-Wallis test indicated that there was no difference between the 15-minute vs 30-minute and 30-minute vs 45-minute groups, with \( P > .05 \) in each group; however, there was a significant difference between the 15-minute and 45-minute groups (\( H_2O_2: E \) coli, 15 minutes vs 45 minutes, \( P = .0219 \); \( H_2O_2: S \) aureus, 15 minutes vs 45 minutes, \( P = .0219 \); \( ClO_2: E \) coli, 15 minutes vs 45 minutes, \( P = .0219 \); \( ClO_2: S \) aureus, 15 minutes vs 45 minutes, \( P = .0219 \); DCCNa: \( E \) coli, 15 minutes vs 45 minutes, \( P = .0219 \), which indicated that the disinfection efficacy was significantly affected by the exposure time.
Disinfection efficiency of field disinfection

Hydrogen peroxide: The disinfection efficacy of H₂O₂ was evaluated in a food production and processing workshop. Three concentrations (3.5%, 7.0%, and 10.5%) and exposure times of 30 and 60 minutes were tested (Figure 2). The average killing percentage of natural bacteria was only 30.80% with 3.5% H₂O₂ at an exposure time of 30 minutes, and the average total number of bacteria was 7.07 X 10³ CFU/m² and 4.48 X 10³ CFU/m² before and after 30 minutes of exposure, respectively, indicating that the disinfection efficiency was not satisfactory. The average killing percentage increased to 78.14% and 84.70% when 7% H₂O₂ was used for exposure times of 30 and 60 minutes, respectively; however, this was still below the qualification standard. With an increase concentration to 10.5% of H₂O₂, the killing percentage was above 90% at exposure times of 30 and 60 minutes. This indicated that 10.5% H₂O₂ was qualified as an efficient disinfectant in experiments conducted at the workshop (Figure 2).

The paired t test revealed that there was no significant difference in the killing effect on natural bacteria when 7% H₂O₂ was used at an exposure time of 30 or 60 minutes or when 10.5% H₂O₂ was used at an exposure time of 30 minutes (P > .05); however, a significant difference was noted between 3.5% and 10.5% H₂O₂ when used at an exposure time of 30 minutes (P < .05).

Chlorine dioxide: The disinfection efficacy of ClO₂ was evaluated in a biosafety level 2 laboratory. A killing percentage of 73.15% and 86.42% was noted when 250 mg/L ClO₂ was used at exposure times of 30 and 60 minutes, respectively, indicating that the disinfection efficiency of natural bacteria was not ideal as the killing percentage was below 90%. Therefore, concentrations of 500 and 750 mg/L were tested at an exposure time of 60 minutes, and the average killing percentage was above 90%, indicating that the disinfection effect should be extended to 60 minutes to achieve a qualified disinfection efficiency (Figure 3).

Sodium dichloroisocyanurate: The disinfection efficacy of DCCNa was evaluated in a biosafety level 2 laboratory. The average killing percentage of natural bacteria was 81.62% when 450 mg/L DCCNa was used at an exposure time of 30 minutes, which did not meet the minimum qualification standard of disinfection. There were still molds that were difficult to disinfect. The average killing percentage of natural bacteria was above 90% at concentrations of 450 and 900 mg/L and an exposure time of 60 minutes, which met the qualification standard of disinfection (Figure 4).

Discussion

The results showed that H₂O₂, ClO₂, and DCCNa were effective in disinfecting indicator E. coli, S. aureus, and natural environmental bacteria and are promising for surface disinfection.
**Hydrogen peroxide**

The environmental surface of the food production and processing workshop selected in this study was rich in nutrients, with masses of microorganisms and an average bacterial concentration of 7.07 X 10^7 CFU/m², although daily cleanliness and spraying with alcohol once per week were conducted. Our studies revealed that only 10.5% H₂O₂ and exposure for 30 minutes and above was able to achieve a killing percentage above 90%. Hydrogen peroxide is used as an ideal disinfectant, being efficacy against a wide range of microorganisms, and the concentration ranges from 3% to 9% (w/w) in the form of an aqueous solution. It is applicable in specific environments for disinfecting surfaces of packaging and filling machines in the food industry and animal breeding fields. It is worth noting that 10.5% H₂O₂ in our study was relatively high compared to commonly used concentration of 3% to 9% (w/w) due to the mass load of organics and microorganism. Herein, cleanliness and microorganism level of the surfaces is to be considered in disinfection.

**Chlorine dioxide and DCCNa**

The use of ClO₂ at 100 to 250 mg/L and an exposure time of 30 to 60 minutes was evaluated for disinfection in some reports and showed efficiency. The killing percentage of ClO₂ disinfectant selected in this study was only 73.15% when the concentration was 250 mg/L at an exposure time of 30 minutes, which was far lower than the disinfection qualification requirements. Chlorine is a commonly used disinfectant for spraying; 250 to 500 mg/L is the commonly used concentration for preventive disinfection, and concentrations above 1,000 mg/L are recommended for disease terminal disinfection. Zhang et al. evaluated the disinfection efficiency in medical institutions, markets, airports, etc using 200 to 1,000 mg/L ClO₂ and 1,000 to 3,000 mg/L chlorine disinfectant, and the total killing percentage was 94.44%. In our study, the killing percentage of bacteria was 81.62% at 450 mg/L for 30 minutes. The disinfection efficiencies of both ClO₂ and DCCNa were below the recommended level, which may be due to the low killing efficiency of the mass load of microorganisms. According to the results, it is necessary to increase the concentration of disinfectant and prolong the exposure time for environments with high microbial load, especially molds and spores, as the components of the products are different, and the environmental microorganisms are complex. Therefore, it is necessary to evaluate the disinfection effect before formal disinfection.

Chlorine-containing disinfectants have a strong bactericidal effect, and the stability of the products is slightly better than that of ClO₂. Chlorine-containing disinfectants are highly irritating to the human body, whereas H₂O₂ and ClO₂ are relatively less irritating. Chlorine-containing disinfectants are suitable for environments with several microorganisms, complex components, and fewer personnel, such as open outdoor environments and waste-disposal sites. The drawbacks of chlorine-containing disinfectants are that they can cause damage to the surface of articles and cause discomfort to patients and medical staff because of their irritating odor. Chlorine dioxide is a potential alternative to chlorine because of its effectiveness in pathogen inactivation, low yields of organic halogenated disinfection by products, and low irritation, which are favorable for water disinfection.

Different disinfectants have different properties, and appropriate disinfectants should be selected based on the pathogenic bacteria, environmental state, and safety considerations. Clearly, cleaning to remove dirt is essential before chemical disinfection. Different places that are more accurately representative of real-world settings, such as homes, schools, markets, etc, should be used in future studies to obtain a comprehensive disinfection effect.

The killing percentages of spraying H₂O₂, ClO₂, and DCCNa in food production and processing workshops and laboratories were different. The disinfection efficiency was limited by factors such as the concentration of the disinfectant and exposure time, the environment, and the texture and cleanliness of the surfaces. Therefore, it is important to evaluate the disinfection efficiency when developing a disinfection strategy.

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**Disclosures**

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**References**

5. Ayub A, Cheong YK, Castro JD, Cumberledge O, Chrysanthou A. Use of hydrogen peroxide vapour for