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ORIGINAL ARTICLE

Evaluation of the Susceptibility of Methicillin-Resistant *Staphylococcus aureus* Isolates to Selected Common Disinfectants

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Abstract

Staphylococcus aureus bacteria cause a wide range of infections, from simple skin infections to life-threatening diseases. This bacterium is one of the five most common causes of hospital-acquired infections, particularly post-surgical wound infections. Methicillin-resistant *Staphylococcus aureus* (MRSA) refers to strains resistant to most antibiotics, predominantly observed in hospitals and currently spreading. Controlling these bacteria necessitates strict management of hospital disinfectants and antiseptics by healthcare personnel. This study evaluates the efficacy of four disinfectants — Cetrimide-C, Vico Science, Intra Hydrocare, and Benzalkonium Chloride — at manufacturer-recommended dilutions on isolated strains of *Staphylococcus aureus* over different time periods. A standard strain and eight MRSA serotypes were isolated and subjected to verification experiments. Each disinfectant was prepared at the manufacturer's recommended concentration and tested on all nine isolates. The number of viable bacteria was determined by culturing and counting colonies at 5, 10, and 15-minute post-exposure. The results indicated that Cetrimide-C was ineffective at all time intervals. In contrast, Intra Hydrocare, Benzalkonium Chloride, and Vico Science disinfectants were effective at 5, 10, and 15-minute intervals. The efficacy of disinfectants was assessed using the micro-dilution technique. Cetrimide-C at a 1:200 concentration was the least effective disinfectant across all time intervals, while the other three disinfectants were completely effective according to the manufacturer's protocol for all three intervals.

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Introduction

The *Staphylococcus aureus* is a gram-positive, catalase-positive, aerobic, and facultative anaerobic bacterium that does not form spores. It is widely distributed and is often found on the skin and mucous membranes (1). Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant subspecies of Staphylococcus, notable for its resistance to conventional antibiotics (2, 3). This bacterial strain is highly contagious, primarily spreading through direct contact with infected individuals or contaminated objects (4, 5, 6). Effective control of environmental microbial contaminants and hand hygiene, key factors in the transmission of these pathogens, has led to a notable reduction in the risk of infection transmission. This approach also minimizes the reliance on antibiotics, preserving their effectiveness and helping to prevent the emergence of new antibiotic resistance. As a result, both illness and mortality rates have been significantly reduced, yielding substantial cost savings (7).

Understanding the fundamental principles of sterilization and disinfection is crucial for improving the methods employed in both clinical and veterinary medicine (8). Vicoscience is a disinfectant characterized by its peroxygen structure and is available in a stimulating powder form, demonstrating high efficacy against viruses, bacteria, and fungi. Notably, this disinfectant is environmentally friendly (9, 10, 11). Cetrimide-C, commonly known as Savlon, is recognized for its rapid disinfectant properties and is suitable for medical and surgical instruments, as well as for washing surgeons' hands and cleaning wounds. This formulation combines Cetrimide and Chlorhexidine gluconate and offers a broad spectrum of antimicrobial activity without toxic effects (12, 13). Additionally, Benzalkonium Chloride is widely utilized to enhance hygiene in hospitals, veterinary clinics, food products, and various environmental settings (14, 15, 16).

It is crucial to exercise caution when selecting the appropriate disinfectants, as manufacturers often make exaggerated claims regarding their products' effectiveness against various microorganisms. Additionally, both exposure time and the specific type of microorganism significantly influence the efficacy of these disinfectants in reducing microbial populations. With this in mind, the present study was designed and conducted to evaluate the effectiveness of four disinfectants — Vicoscience, Cetrimide-C, Intra Hydrocare, and Benzalkonium Choloride — at the manufacturer-recommended dilutions on MRSA isolates, considering common disinfection practices across varying time intervals.

Materials and Methods

In order to conduct the present research, a standard strain and eight different intermediate isolates were obtained from the Department of Mycology, Tehran University, Iran.

Sample Revival

To revive the samples, they were removed from the freezer and briefly exposed to a flame, while adhering to aseptic principles. Each sample was extracted using needles and cultured in a streak pattern on *Mueller-Hinton agar* culture medium. The plates were then inverted and incubated at 37°C for 24 to 48 hours. After this incubation period, small and clear colonies appeared, indicating the presence of *Staphylococcus aureus*.

Sample Purification

To purify the colonies at the initial stage, one or two identical colonies were selected from the plate during the revival phase and cultured on fresh *Mueller-Hinton agar* medium, which was incubated at 37 °C. After approximately 24 to 48 hours, pure colonies were successfully obtained.

Investigating the Effect of Disinfectants on Methicillin-Resistant Strains of *Staphylococcus aureus*

The effect of disinfectants on MRSA isolates was investigated according to the following steps: 1. The MRSA strains were cultured and incubated in Tryptic Soy Broth (TSB) for 8 hours prior to testing, aiming to develop 3 to 4 colonies. The bacterial concentration was assessed by measuring turbidity and visually comparing it to the 0.5 McFarland turbidity standard. If the turbidity of the bacterial suspension exceeded that of the standard tube, additional bacterial colonies were introduced into the Brain Heart Infusion (BHI) medium until its turbidity matched the 0.5 McFarland standard (2.5×10^8 CFU/mL). 2. 100 microliters of each strain, adjusted to a concentration of 2.5×10^6 CFU/mL, was added to 9 ml Yeast extract tubes containing disinfectant stock, mixed thoroughly, and the desired time was immediately recorded. 3. After 5, 10, and 15 minutes (from time zero), 1 ml from the tube in step 2 was added to the tubes containing 3 ml of BHI broth and mixed. 4. The tubes were incubated at 37°C for 4 days. 5. After incubation, for each time point, one loopful of culture was lawn plated onto *Nutrient agar* and incubated again at 37°C for 24 hours. 5. The number of colonies present at each time point was counted and recorded.

Table 1. The effect of the disinfectants tested on *Staphylococcus aureus* serotype isolates

Disinfectant	5-minute	10-minute	15-minute
Vicoscience	100% lack of growth	100% lack of growth	100% lack of growth
Intra Hydrocare	100% lack of growth	100% lack of growth	100% lack of growth
Benzalkonium Chloride	100% lack of growth	100% lack of growth	100% lack of growth
Cetrimide-C	90% lack of growth	90% lack of growth	90% lack of growth

Results

The assessment of the concentrations recommended by the disinfectant manufacturers for the elimination of staphylococcal agents involved four disinfectants: Vicoscience, Intra Hydrocare, Benzalkonium Chloride, and Cetrimide-C. The effectiveness of these disinfectants against MRSA isolates was evaluated, and the results are presented in Table 1.

According to the results presented in Table 1, no growth was observed for Vicoscience, Intra Hydrocare, and Benzalkonium Chloride disinfectants at any time interval. The data indicate that Cetrimide-C failed to eliminate one of the nine tested isolates on three separate occasions, during which a significant number of colonies were noted. Even with extended exposure times, no improvement in the efficacy of Cetrimide-C was observed, allowing the bacteria to proliferate despite its presence.

Thus, Cetrimide-C is deemed unsuitable for sensitivity testing, as it cannot effectively eliminate *Staphylococcus aureus* isolates. In contrast, the other three disinfectants demonstrated a more adequate and comparable ability to control bacterial growth.

Discussion

In workplace environments, various infectious disease agents, including bacteria, viruses, and fungi, pose significant health risks. These pathogens can lead to outbreaks and illnesses, particularly among individuals who are more vulnerable due to compromised immune systems or underlying health conditions. Many of these agents are opportunistic, exploiting specific weaknesses in their hosts to establish infections.

One particularly concerning bacterium is *Staphylococcus aureus*, which is notorious for its role in hospital-acquired infections. This pathogen can lead to a wide range of diseases in both human and veterinary medicine, ranging from skin infections to severe conditions like pneumonia and sepsis. A key factor in the virulence of *Staphylococcus aureus* is its ability to acquire antibiotic resistance, a process mediated by genetic elements located on both chromosomes and plasmids.

The rampant and often unwarranted use of antibiotics, particularly without medical supervision, has exacerbated this problem. Such overuse contributes to the emergence of resistant strains, effectively diminishing the efficacy of existing antibiotics. As resistance levels increase, treatment options for infections caused by *Staphylococcus aureus* and similar pathogens become increasingly limited, posing a significant challenge for healthcare providers. Addressing this issue necessitates a multifaceted approach, including improved antibiotic stewardship, enhanced infection control measures, and ongoing research into alternative treatment strategies.

The present study, which focused on three specific disinfectants — Vicoscience, Intra Hydrocare, and Benzalkonium Chloride — found no significant differences in their effectiveness at the concentrations specified by their manufacturers when targeting *Staphylococcus aureus* bacteria. However, this finding underscores the need for further investigation. It is vital to explore a broader range of disinfectants and to apply the knowledge gained from successful companies and institutions that have excelled in this field.

Despite the extensive and long-term use of disinfectants, most of the *Staphylococcus aureus* strains are still sensitive to some of these substances. The MRSA strains may be resistant to some antiseptics such as Acriflavine, benzalkonium Chloride, and Cetrimide-C. The common mechanism of resistance to antiseptics is related to the reduction of the permeability of the bacterial cell wall, in which some of the resistance factors are located on plasmids (17).

In a study conducted across two public hospitals in Brazil (2011), 74 cases of MRSA were isolated to investigate the effects of disinfectants. Among these, 80 isolated samples exhibited the presence of the qacA/B gene. The findings indicated that not all MRSA exhibit resistance to disinfectants; rather, only those MRSA strains possessing the qacA/B gene have developed this resistance (18).

Telchik et al. (2024) assessed chlorhexidine resistance in MRSA isolates from hospitals in Cleveland, OH, and Detroit, MI. The goal of this study was to determine the prevalence of chlorhexidine resistance and to identify the resistance-associated genes in MRSA samples using whole

genome sequencing (WGS). The qacA gene was detected in only one MRSA sample from the Cleveland-area hospital. In the samples from Detroit, 14 out of 287 exhibited disinfectant resistance genes. The qacA, qacB, and qacD were present in 1, 6, and 7 samples, respectively. The prevalence of any qac gene in the Cleveland-area samples was 0.5%. Meanwhile, the prevalence of any qac gene in Detroit-area samples was 4.9%. Among the seven samples carrying the qacD gene, six samples contained more than one copy of qacD (19).

In Malaysia, Winarti et al. (2024) conducted a study to compare the efficacy of disinfectants against *Staphylococcus aureus* at Universiti Sains Islam Malaysia. Nine operators were enrolled to collect 19 environmental samples from the glove-dominant hand following non-surgical extraction in the Oral Surgery Clinic. Staphylococcus aureus was identified, and an antibiotic susceptibility test was performed to determine the presence of the MRSA strain. Two different levels of disinfectants for the disinfection of dental chairs were tested on rough and smooth surfaces experimentally contaminated with *Staphylococcus aureus*. The number of colonies before and after disinfection was counted, and the percentage reduction was calculated and analyzed. *Staphylococcus aureus* was detected in 68.42% (n=13) of the samples. 5.26% (n=1) of the samples were Staphylococcus sp. 26.32% (n=5) showed no bacterial growth. No MRSA strain was identified. There was no statistically significant difference ($p>0.05$) in the efficacy of the three disinfectants at two different levels used in USIM Dental Polyclinic on rough and smooth surfaces against *Staphylococcus aureus* (20).

A study (conducted in 2012) investigating the effectiveness of Virkon S disinfectant on the survival of *Staphylococcus aureus* and *Salmonella enterica* revealed that increased contact surface area between Salmonella and the disinfectant resulted in reduced bacterial survival. It was found that horizontal surfaces significantly contribute to the deactivation of Salmonella by Virkon S. In contrast, for *Staphylococcus aureus*, both horizontal and vertical surfaces, as well as the composition of these surfaces and the dynamics of airflow, play crucial roles in the efficacy of the disinfectant (21).

In the study conducted by Rokoci et al. (2008), results from the Handsept and Decosept plates indicated no growth of any of the 10 MRSA strains after exposure times of 15 seconds, 30 seconds, 60 seconds, and 3 minutes. Consequently, when comparing disinfectants, it is essential to consider additional factors beyond efficacy, such as economic efficiency, skin compatibility, and the longevity of the antibacterial effects on the skin. Given the findings of this study and its limitations, it appears that the concentrations of the substances may not have been accurately observed by these two companies (22).

John et al. (2018) investigated the impact of disinfectants in a contaminated environment, focusing on certain microbes, including MRSA and aerobic bacteria (AB) colonies. Their study collected 70 samples and revealed that the level of disinfection is significantly effective in reducing pathogenic agents. Notably, the presence of MRSA was diminished (23).

Kitagawa et al. (2010) examined the effect of ultraviolet light on MRSA on high-contact surfaces in a Japanese hospital. A total of 306 samples were collected. The PX-UV disinfection led to a significant reduction in the abundance of AB and MRSA. In addition, the PX-UV disinfectant significantly enhanced hand hygiene and the reduction of AB and MRSA contamination on high-contact surfaces in hospital rooms (24).

Khalid et al. (2010) conducted an analysis of the anti-biofilm efficacy of hand washes and disinfectants against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Among the eight hand washes and disinfectants tested, 25% demonstrated antimicrobial activity against both bacterial strains, while 50% exhibited no controlling effects. These findings have significant implications for application in hospitals and laboratories (25).

Although the results obtained from various antimicrobial products have been generally satisfactory, it is crucial to understand that the application of antiseptic substances and solutions, regardless of their specific forms, primarily addresses visible or likely contaminations. This limited efficacy is particularly relevant in light of the ongoing evolution of common infection strains within medical treatment environments. Over time, these strains can become increasingly complex, and the emergence of highly resistant and pathogenic species adds another layer of concern. Consequently, the development of resistance to both antibiotics and disinfectants poses a significant challenge in infection control.

Given the pivotal role of *Staphylococcus aureus* in both human and veterinary medicine, ongoing research and innovation in the development of new disinfectants are essential. Continued efforts in this area will enhance our capacity to address the challenges posed by resistant bacterial strains, ultimately improving health outcomes across medical fields.

Conclusion

In the conducted studies, determining the most effective disinfectant proved challenging, as all three disinfectants, Intra Hydrocare, Vicoscience, and Benzalkonium Chloride, demonstrated equal inhibitory strength. The dilutions

recommended by the manufacturers for eliminating MRSA were both accurate and completely effective. However, the Cetrimide-C disinfectant did not exhibit effectiveness across all three exposure durations for certain *Staphylococcus aureus* strains, based on the dilution specified in the manufacturer's protocol.

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Author Contributions

Mina Payan: investigation, methodology, writing the original draft. **Azizollah Ebrahimi:** investigation, methodology, supervision, **Somayeh Shahrokh Shahraki:** Conceptualization, investigation, methodology, supervision, validation, visualization, writing – review & editing.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical Approval

Ethical approval was not required for this study according to institutional guidelines.

Conflict of Interest

There is no conflict of interest.

Consent for Publication

Not applicable.

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