A STUDY OF THE EFFECTS OF DIFFERENT DISINFECTANTS USED IN RIYADH HOSPITALS AND THEIR EFFICACY AGAINST METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)

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Abstract:
Methicillin resistant Staphylococcus aureus (MRSA) and the means of controlling it, continue to be of major interest to the healthcare community. The bactericidal activity of some disinfectants which are in common use in seven major tertiary care hospitals in Riyadh was tested against two control strains of S. aureus, namely MRSA ATCC 33591 and Methicillin sensitive Staphylococcus aureus (MSSA) ATCC 29213. The disinfectants tested in this study were a group used for hand antisepsis (Purell, EZ-clean, Cida stat and Manorapid Synergy) and another group used for environmental disinfection (Combi spray, Tristel fusion, Alphadine, Isopropanol, Presept and Diesin). Presept, Diesin and tristel fusion had a remarkable effect on the tested strains, both methicillin sensitive and methicillin resistant. There was hardly any noticeable difference between the effects on either (P> 0.05). On the other hand, Purell and EZ-clean and Manorapid Synergy hand rubs had a relatively weak action after 15 and 30 minutes while their effect was better after 1 and 2 hours. There were no observable differences between their effects on MRSA or MSSA, P>0.05. Cida stat had a remarkably pronounced effect against both MRSA and MSSA. Contrary to some previous reports, this study has also proven that chlorhexidine and quaternary ammonium compounds show comparable efficacy against both MRSA and MSSA.

Introduction

Staphylococcus aureus is the leading cause of human infections in the skin and soft tissues, bones and joints, abscesses and normal heart valves. It flourishes in the hospital setting, producing bloodstream and surgical wound infections, including methicillin-resistant Staphylococcus aureus (MRSA). MRSA is now a huge burden in addition to methicillin sensitive Staphylococcus aureus (MSSA) for most healthcare institutions around the world and is by far the most significant antibiotic-resistant hospital acquired pathogen we have ever encountered (1).

MRSA is usually spread by direct physical contact with those already infected or through indirect contact by touching objects (towels, clothes, sports equipment, etc.) that infected skin has contaminated. Consequently, any heavily trafficked area can be a source of infection. Many previous studies have evaluated MRSA contamination of various items such as computers and computer keyboards (2), pens (3), television sets (4), stethoscopes (5), tourniquets (6), uniforms and gowns (7), blood-pressure cuffs (8), and mattresses, pillows, chairs, bed-frames, and over-bed tables (9). Even all of the surfaces sampled on chiropractic adjusting tables in one study carried microorganisms. Most of these were harmless skin bacteria or environmental fungi. Some of the surfaces did harbor MRSA. Armrests and uncovered portions of headrests had the highest bacterial counts. Disinfection protocols must therefore address removal of staphylococci from these surfaces to prevent horizontal transmission in the outpatient settings (10). Strange enough, a study has shown that a hairdresser is a possible source for cross-contamination of MRSA, and that currently this is not a widely recognized potential route. Analysis of the 49 patients visited by a hairdresser over a four-week period in that study showed eight (16.3%) patients colonized with MRSA at the time of her visit (11). In another study, The door handles in 53 (27%) of 196 rooms were contaminated by S. aureus (MSSA and/or MRSA). MSSA was detected on door handles of 41 rooms (20.9%) and MRSA on those of 17 (8.7%), and both MSSA and MRSA

were detected on the same door handles of five rooms (2.6%) (12). Sextona et al (15), have shown that the environment of isolation rooms with patients who are colonized or infected with MRSA is often positive for MRSA, and that patient and environmental isolates are usually indistinguishable. Environmental reservoirs may therefore be a significant contribution to endemic MRSA. Interventions to reduce potential environmental sources may then be shown to be effective in reducing the clinical burden of environmental reservoirs of MRSA. It is thus essential that all perceivable surfaces be disinfected and that the effect of the disinfectants be monitored to ensure adequacy.

MRSA can spread within hospitals via the transiently colonized hands of staff (13). Barrier precautions and hand disinfection are the main methods used to break the chain of transmission (14). Thus, antiseptic soaps and scrubs containing chlorhexidine (CHX) are mainly available for this purpose.

Antiseptic is defined as a chemical that either inhibits the growth of microorganisms or destroys them; this term refers to agents used on living tissue while a disinfectant is defined as a chemical that kills or destroys nearly all disease-producing microorganisms, with the exception of bacterial spores; this term refers to agents used on inanimate objects.

Previous studies have shown the minimum inhibitory concentrations (MICs) of the commonly used biguanide disinfectant, CHX, for MRSA to be 4-fold, (16) 8 to 16-fold (17), or 2 to 4-fold (18) greater than for MSSA strains. In contrast, other studies have failed to show a difference in CHX susceptibility between MRSA and MSSA using MIC testing. Reduced susceptibility, albeit slight, of MRSA to the cationic surface-active disinfectants, quaternary ammonium compounds (QACs), has also been reported, (19) the determinant of which is thought to be located on plasmids that also confer resistance to ethidium bromide, pro-pamidine isothionate, and some antibiotics. In general, Gram-positive bacteria are more susceptible to CHX than Gram-negative bacteria.

The bactericidal activity of some disinfectants and antiseptics was tested against two strains of *S. aureus*, namely MRSA ATCC 35391 and MSSA ATTC 29213.

**Materials and Methods**

This study was carried out using two strains of *S. aureus*. The *S. aureus* strains were strain ATCC 29213 which is methicillin sensitive and ATCC 33591 which is methicillin resistant. Two days prior to the study, a pure culture of each bacterial type was plated on to tryptone soya agar (TSA) and incubated at 30 to 35°C. A bacterial suspension to 5 McFarland units (equivalent to 10^9 cfu/mL) was prepared and serial 10-fold dilutions were made in phosphate buffered saline (PBS). The 10^5 to 10^7 dilutions were plated on to TSA, incubated for 48 h, colonies were counted and the initial bacterial concentration was calculated. Ceramic tiles measuring 3 x 4 cm were cleaned using detergent followed by 70% isopropyl alcohol, wrapped in aluminium foil and autoclaved at 121°C for 15 min. One hundred microlitres of each disinfectant at its use dilution were spread on the surface of each tile. They were left to dry at room temperature for 2 h. Thirty microlitres of bacterial suspension [10^7 colony-forming units (cfu)/mL] were evenly distributed on to the tiles (positive tiles), and on to the surface of tiles without disinfectant (negative tiles) as controls and kept in a humidity chamber to exclude the effect of dryness on death of bacteria. After 15, 30 min, 1 and 2 hours, one tile from each type of disinfectant was tested for each type of bacteria. Each tile was placed in a sterile Petri dish containing 20 mL Dey-Engley neutralizing broth (Sigma) to neutralize the effect of the disinfectant. The tiles were then vortexed for 1 min, and serial 10-fold dilutions were made in PBS. Since each tile was eluted into 20 mL PBS, and 100 µL of this was plated in triplicates on TSA for counts, the limit of detection was 200 cfu/tile. This process was repeated for the positive controls, and the negative controls. The plates were then incubated at 35°C for two days and colonies were counted (with no growth being equivalent to <200 colonies on the tile; the limit of sensitivity). Recovery of the organisms from the negative controls demonstrated that the organism remained viable once dried on to the ceramic tiles. Experiments were run in duplicates. All disinfectants were freshly prepared prior to testing and used according to manufacturer's instructions. Sterile distilled water was used as a diluent and as a disinfectant control.
The bactericidal activities of the disinfectants were expressed as logarithmic reductions in viable organisms and were calculated by:

\[ RF = \log_{10} \text{cfu (negative tile)} - \log_{10} \text{cfu (positive tile)} \]

in which \( RF \) is the reduction factor (20).

\( \log_{10} \) Reductions (where a \( \log_{10} \) reduction of \( N \) indicates viable counts fell by a factor of \( 10^{N} \)). \( \log_{10} \) reductions of >5 were taken as indicative of satisfactory bactericidal activity.

The disinfectants tested in this study were a group used for hand antisepsis (Purell, EZ-clean, Cida stat and Manorapid Synergy) and another group used for environmental disinfection (Combi spray, Tristel fusion, Alphadine, Isopropanol, Presept and Diesin).

### Results

The results of the tests performed are tabulated in table 1 showing the effects on MRSA ATCC 33591 and MSSA ATCC 29213. From the table, no pronounced difference was noted between the effects of the tested disinfectants on MRSA versus MSSA (\( P>0.05 \)).

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>( \log_{10} ) initial count</th>
<th>Mean ( \log_{10} ) of MRSA reductions at</th>
<th>Mean ( \log_{10} ) of MSSA reductions at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 min</td>
<td>30 min</td>
<td>1hr</td>
</tr>
<tr>
<td>Cida stat</td>
<td>9.60</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Combi spray</td>
<td>8.27</td>
<td>2.929</td>
<td>3.302</td>
</tr>
<tr>
<td>Alphadine</td>
<td>9.87</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Presept</td>
<td>9.32</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Tristel</td>
<td>9.94</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

### Discussion

The transfer of pathogenic bacteria via the hands of staff in health care centers is known as a source of outbreaks of nosocomial infections (21) and might be significantly reduced by the use of antiseptics. The College of Nurses of Ontario(22) emphasizes that "hand washing" is the single most important infection control practice. There are several studies that demonstrate that efforts to control MRSA are cost-effective (23). Another study found that a poster campaign in conjunction with the introduction of hand decontamination with an alcohol hand-rub solution increased hand washing from 48% in 1994 to 66% in 1997 (\( P < .001 \)), and episodes of MRSA acquisition decreased from 2.16% to 0.93% per 10,000 bed days (\( P<0.005 \)) (24).

Alcohol hand antiseptic appears to be effective in controlling the transmission of MRSA. In a previous study (25) the incidence of MRSA colonization or infection decreased from 1.26 cases/1,000 patient-days before the application of alcohol based antiseptic handrub to 0.75 cases/1,000 patient-days after the intervention, for a 1.46-fold decrease (95% confidence interval, 1.04-2.58; \( P=0.037 \)).

In the present study, surface disinfectants (presept, diesin and tristel fusion) had a favourable effect on the tested isolates, both methicillin sensitive and methicillin resistant (\( P>0.05 \)).

The hygienic hand disinfection products (mainly alcohol-based hand rubs) claim (i) to reduce or inhibit the growth of microorganisms with maximum efficacy, a fast acting and broad-spectrum activity, some excellent microbiocidal characteristics, a lack of potential emergence of resistance, (ii) to overcome the lack of accessibility to sinks or other facilities (including clean running water or towels in some poor and remote areas), (iii) to perform hand cleansing actions that require the...
use of water (hand washing and hand antisepsis using a formulation different from a waterless agent), (iv) to improve compliance with hand hygiene by reducing the time required to perform it and the convenience of the method, and (v) to reduce costs (26). In the present study, Purell and EZ-clean hand rubs had a relatively weak action after 15 and 30 minutes while their effect was better after 1 and 2 hours. There were no observable differences between their effects on MRSA or MSSA, \( P > 0.05 \).

For decades alcohol-based hand rubs were available as liquids. Since the first commercially available liquid products introduced in the early 1960s (e.g., Sterillium) they were regarded to be the standard for hand disinfection mainly because of their fast and immediate antimicrobial activity. During the last years alcohol-based gels have been found in many countries. Hygienic hand disinfection with an alcohol-based product requires only about 30 seconds because a dispenser may be available next to the patient's bed or a pocket bottle may be available so that no further walking away from the patient is necessary. The hand disinfection maybe done during a conversation with the patient, which is only possible if the health care worker does not have to leave the patient. The use of alcohol-based hand rubs solves two problems often associated with hand washing: lack of facilities (no sinks required) and lack of time (27). Both aspects are particularly important in community medicine especially due to the unique environment during home care activities.

Sodium dichloroisocyanurate is stable until dissolved, rapidly effective and slightly less damaging to surfaces and instrument components than sodium hypochlorite which was formerly used. However, like all other chlorine-releasing agents, the use of NaDCC in hospitals is usually limited to environmental surfaces because of its corrosiveness. In the present study, presept, the NaDCC was quite effective with acceptable bactericidal activity against the tested strains.

Irizarry et al. (18) and Suller & Russell (20) showed MRSA strains to be less susceptible than MSSA to CHX and QACs. In contrast, Al-Masaudi et al. (28) have described MRSA and MSSA as equally sensitive to CHX, phenols and esters but MRSA strains were more resistant to QAC. On the other hand, in some experiments, no differences between CHX, triclosan and povidone iodine susceptibility of MRSA and MSSA strains were found (29). In the present study, Diesin, a QAC was found to be almost equally effective against MRSA and MSSA as were all of the other tested disinfectants. The effect of Diesin was equally strong against tested strains similar to a previous study that found Diesin to belong with substances with strong inhibitory effect against Salmonella (30). Koljalg et al (31), reported cross-resistance between CHX and several antibiotics such as beta-lactams, quinolones and aminoglycosides. Combined resistance of antibiotics and biocides can be presumed by a similar kind of action of both types of agents. CHX acts through the damage of the outer cell layers and crosses the cell wall or outer membrane, then attacks the bacterial cytoplasmic or inner membrane causing leakage of intracellular constituents (32). Changes in outer or inner membranes, but also efflux pumps have been implicated in bacterial resistance to biocides and antibiotics (27). Cida stat had a remarkably pronounced effect against both MRSA and MSSA tested strains, the effect was evident at all sampling times, namely 15min., 30 min., 1hr. and 2 hrs.

In conclusion, this study highlighted a strong effect of the disinfectants used for environmental and equipment surfaces (except for isopropanol) which were found to exert a recognizable bactericidal effect without an evident difference between their effects on methicillin sensitive or resistant \( S. aureus \). However, the hand rubs in current use in the study hospitals except for Cida stat (namely, EZ-clean, Manorapid Synergy and Purell for hygienic disinfection on the wards) were less effective in reducing both MRSA and MSSA. However, these products are reputed to be well tolerated (33). This study has also proven that CHX and QACs show comparable efficacy against both MRSA and MSSA.

References


2- Devine J, Cooke RPD, Wright EP. Is methicillin resistant \( Staphylococcus aureus \) (MRSA) contamination of ward-based computer terminals a surrogate marker for nosocomial MRSA transmission and handwashing compliance? J Hosp Infect 2001; 48: 72-5.
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