Evaluation of Antimicrobial Activity of Surface Disinfectants by Quantitative Suspension Method

Dr. K.Prasanthi, M.D. (Assistant Professor)
Department of Microbiology, Gandhi Medical College, Secunderabad - 500003, Andhra Pradesh, India
Academic Qualification : M.D. Microbiology

Dr. D.S.Murty, M.D. (Assistant Professor)
Department of Microbiology, Osmania Medical College, Hyderabad – 500 095, Andhra Pradesh, India
Academic Qualification : M.D. Microbiology

Dr. Nirmal Kumar Saxena, M.D. (Professor)
Department of Microbiology, Gandhi Medical College, Secunderabad - 500003, Andhra Pradesh, India
Academic Qualification : M.D. Microbiology

Address of Corresponding author :
Dr. K.Prasanthi, M.D.
1-7-1046/30, Flat No. 203, Shiridi Sai Enclave, Azamabad, Ram Nagar, Hyderabad – 500020, Andhra Pradesh, India,
Ph : 09490119539 Land : 040 27612804
Email : bebold_p@yahoo.co.in

Received 12 August 2012; accepted 28 August 2012

Abstract

Background : Testing the efficacy of disinfectants plays an important role in ensuring the infection control in health care settings. The study was undertaken to evaluate the antimicrobial activity of various disinfectants. Methodology and Principal Findings : The study carried on three surface disinfectants : 1% Poly hexa methylene guanidine hydrochloride (PHMG), 1% Sodium Hypochlorite (Hypo) and 1% Non ionic surface active iodine complex. They were tested for their activity against Pseudomonas aeruginosa, Candida albicans, spore strips of Bacillus subtilis using quantitative suspension method. 1% PHMG showed good antibacterial activity at a contact time of 15 sec while 1% Sodium hypochlorite showed similar activity at a contact time of 30 sec. Both showed fungicidal activity at a contact time of 30 sec and sporicidal activity at a contact time of 1 min. But, 1% Iodine solution did not show any antibacterial, fungicidal or sporicidal activity even after a contact time of 15 min. To conclude 1% PHMG and 1% Sodium Hypochlorite showed good antimicrobial activity while 1% iodone solution was not effective. Conclusions : This study stress the need to establish a disinfection policy, periodical check of the efficacy of disinfectants and strict vigilance over unauthorized local products.

INTRODUCTION

Disinfectants are used extensively in the health care settings, playing an important role in the control of infections. Surface disinfectants are routinely used for decontamination of a variety of work areas in the hospitals including laboratories which minimizes the contamination of samples and media. The disinfectant activities are relied on the consistent efficacy of the disinfectants, which is of great significance in ensuring the final result of infection control. Hence testing the efficacy of disinfectants is very important component in hospital infection control, but largely overlooked by many hospitals.

Disinfectant testing dates back to 1881, first compiled by Robert Koch, using silk threads submerged in liquid culture of Bacillus anthracis for testing. Over the years many conceptual mile stones were reached and much knowledge is gained and methods have developed so as to standardize the testing conditions, to yield a quantitative result by reduction in the microbial growth, which shows the performance of a particular product tested. First suspension test was developed by Geppert; 10 years after Koch initiated disinfectant testing [1]. After several modifications quantitative suspension tests are specifically designed to evaluate the disinfectant activity. Organisms are tested
in certain important conditions like concentration, time, presence of organic material etc. In countries like Netherlands, Suspension tests are still the only mandatory tests in official regulatory procedures [2]. Unfortunately in India, the guidelines and standardized procedures are not being implemented on a regular basis because of their complexity. With this background the present study was under taken to evaluate the antimicrobial activity of three commonly used surface disinfectants by quantitative suspension tests.

MATERIALS & METHODS
The study was carried out in Clinical Microbiology Laboratory of our hospital, which is a tertiary care centre and a referral hospital. Three surface disinfectants namely, 1% Sodium Hypochlorite and 1% Non ionic surface active iodoine complex (commonly used for topical application as they are cheap and readily available) and 1% Poly hexa methane guanidine hydrochloride, a newly introduced nonalcoholic surface disinfectant and skin antiseptic, were chosen for the study. As per the manufacturers’ instructions, the three disinfectants are effective at a concentration of 1%. Poly hexa methane guanidine hydrochloride (1% PHMG, Bios agri corp), is a cationic polymer based on guanidine salts as its active substance, a newly marketed product. 1% Sodium Hypochlorite (Merck) is widely used in health care settings because it is cheap, less toxic, water-soluble, and has a broad spectrum of activity. The 1% Non ionic surface active iodoine complex is an iodophore which is widely used, cost effective (purchased from Local manufacturers). Microorganisms selected to test the biocidal activity of disinfectants were Pseudomonas aeruginosa (ATCC 27853), Candida albicans (Hospital isolate) and spore strips impregnated with spores of Bacillus subtilis (10⁶/strip, Himedia), as these are frequent surface contaminants among the health care settings. Sterile normal saline was used for the preparation of microbial suspensions. A mixture of equal volumes of 1% Sodium thiosulphate and 0.1% Tween 80 was used as neutralization solution [3] to neutralize the residual disinfectant activity. Nutrient broth was used to revive the organisms resistant to disinfection and nutrient agar (NA) plates to demonstrate visible microbial colonies. Sabouraud’s dextrose agar (SDA) plates were used for isolation Candida albicans. Specific contact times tested were 15sec, 30sec, 1min, 5min, 15min for bactericidal activity and fungicidal and sporicidal activity.

McFarland Standard No. 0.5 suspensions of Pseudomonas aeruginosa and Candida albicans were prepared in normal saline. Under sterile conditions, 1ml of this suspension was added to 9 ml of disinfectant in a sterile test tube. From this, at specific contact times of 15sec, 30sec, 1 min, 5min and 15 min, 1ml of microbial disinfectant mixture was transferred to 9ml of neutralizing solution. Approximately after 5 minutes, 1ml of suspension from each neutralizer tube was added to nutrient broth tubes. All the nutrient broth tubes were incubated at 37°C for 24 hrs. After 24 hrs incubation, a loopful of inoculum (using calibrated loop, to deliver 0.01ml) from each tube was streaked on NA plates. SDA plates were used for isolation of Candida albicans. All the plates were incubated at 37°C for 24hrs for isolation of Pseudomonas aeruginosa and 48 hrs for Candida albicans.

The method used for testing Bacillus subtilis spore strips was a carrier based technique where a known concentration of spores, coated on the filter paper strips (10⁶/strip) were directly placed in disinfectant solution. Each strip was placed in separate disinfectant tubes for different contact times. At specific contact times (15sec, 30sec, 1min, 5min, 15min), strips were transferred to neutralizer solution, thence to nutrient broth tubes and further processed as described above. Controls were put up for all the three organisms to show the activity of the neutralizer. For control, 1ml each of 0.5 McFarland broth of Pseudomonas aeruginosa and Candida albicans were mixed with 9ml neutralizing solution in separate tubes, then transferred to nutrient broth, as the procedure described with disinfectants. Similarly Bacillus subtilis spore strip were placed in neutralizer, then to nutrient broth. Later all the three controls are streaked onto NA and SDA plates. Presence of growth indicated that neutralizer was not inhibiting the microbes tested. Similarly 1ml of each disinfectant was mixed with 9ml of neutralizer, then 0.1ml suspension of Pseudomonas aeruginosa (0.5 McFarland standard) added to each tube, later directly transferred and incubated in nutrient broth and streaked on NA plates. Growth on NA plates shows effective neutralization of the disinfectant activity.

RESULTS
After incubation, the nutrient agar plates were observed for any visible growth (microbial colonies). The colonies were counted, multiplied with factor hundred and expressed as colony forming units (CFU) per milliliter. The results were evaluated by calculating the decimal log reduction or microbicidal effect by subtracting the logarithm of the survivors after disinfectant contact from the logarithm of the original inoculum in control plates, using the following formula:

\[
\text{Logarithmic Reduction Factor (RF)} = \log N_c - \log N_d
\]

Where:
- \(N_c\): Number of colonies from control plates
- \(N_d\): Number of colonies from test plates (after contact with disinfectant)

Log₁₀ reductions of 5 or more were taken as an indication for satisfactory microbicidal activity ie atleast 99.99% of the germs killed [1, 4].

The 1% solution of PHMG (1% Poly hexa methane guanidine hydrochloride) showed good activity against Pseudomonas aeruginosa even at a contact time of 15 sec, whereas 1% Sodium Hypochlorite was effective at 30 sec (Table 1).

**Table 1:** Bactericidal activity of surface disinfectants (against Pseudomonas aeruginosa)

<table>
<thead>
<tr>
<th>Contact time</th>
<th>15 sec</th>
<th>30sec</th>
<th>1min</th>
<th>5min</th>
<th>15min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%PHMG</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>1%Hypo</td>
<td>G</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>1% Iodine complex</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
</tr>
</tbody>
</table>

G: Growth NG: No growth
1% PHMG and 1% Sodium Hypochlorite showed good fungicidal activity (Table 2) and sporidial activity (Table 3) at a contact time of 30sec and 1 minute respectively.

Table 2: Fungical activity of the surface disinfectants (against Candida albicans)

<table>
<thead>
<tr>
<th>Contact time</th>
<th>15 sec</th>
<th>30 sec</th>
<th>1 min</th>
<th>5 min</th>
<th>15 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%PHMG</td>
<td>G</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>1%Hypo</td>
<td>G</td>
<td>G</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>1% Iodine complex</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
</tr>
</tbody>
</table>

G:Growth  NG: No growth

DISCUSSION

The effective use of disinfectants constitutes an important factor in preventing hospital-acquired infections.[5] Quantitative suspension tests are developed to yield quantitative results on the performance of a particular product tested under standard conditions. The organisms tested were known to be common contaminants and colonizers of patients, intensive care units, operation theatres, laboratory surfaces etc. Generally, the testing was carried out at three stages. First stage concerns laboratory tests in which antimicrobial activity of disinfectant is verified. The second is also carried out in laboratory, but in conditions simulating real life conditions. The third phase comprises the field tests. The Present study was a first stage laboratory based study for which, quantitative suspension tests are considered. [1]

Among the three disinfectants tested, the 1% PHMG is very effective bacterial surface disinfectant at the lowest contact time of 15 sec, followed by 1% hypochlorite which is effective in 30 sec (Table 1). But locally purchased 1% non ionic iodine complex was poor in its bacteridial activity. In addition, 1% PHMG and hypochlorite were showing successful fungidical activity ( Table 2) at the lowest contact time of 30 sec and 1 minute respectively where as locally purchased non ionic iodine complex surface disinfectant could not inhibit the growth at the concentration of 1 percent. Also PHMG and Hypochlorite were sporidical at the concentration of 1 percent but nonionic iodine complex at that concentration failed in doing so (Table 3).

Table 3: Sporicidal activity of the surface disinfectants (against Spores of Bacillus subtilis)

<table>
<thead>
<tr>
<th>Contact time</th>
<th>15 sec</th>
<th>30 sec</th>
<th>1 min</th>
<th>5 min</th>
<th>15 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%PHMG</td>
<td>G</td>
<td>G</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>1%Hypo</td>
<td>G</td>
<td>G</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>1% Iodine complex</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
</tr>
</tbody>
</table>

Awodele et al from Nigeria [6] did a study using similar organisms and methylated spirit, sodium hypochlorite, Savlon, kerosene as disinfectants and revealed that Savlon was 100% effective microbicide. Miles R Majcher et al from Canada [7] studied sporidical activity of Hypochlorite, Accelerated Hydrogen peroxide (AHP), Chlorine dioxide, peracetic acid (PAA) and found PAA acting fastest, followed by Hypo and AHP.

In the present study, we have chosen 1% PHMG, 1% Hypo and 1% non ionic surface active iodine complex for testing. Among these PHMG, which is an aqueous, alcohol free, odourless, nonirritant, nontoxic newer disinfectant, was found to be readily active (within seconds), followed by 1% Hypochlorite, a fast acting, inexpensive, readily available chemical. Least activity was by the locally purchased iodine preparation. But, based on this we can not conclude that all the iodine preparations are equally ineffectve as the standard of manufacture, whether he comes under chemical regulation authority guidelines is also to be counted. Neutralizers utilized did not have any growth inhibition on test organisms [3, 8].

CONCLUSIONS

Disinfectants used in hospitals and laboratories must be tested periodically to ascertain its potency and efficacy. Otherwise, disinfectants of questionable quality and efficacy will continue to be used and abused in clinical settings where their microbicidal activity is wrongly assumed to be effective against all organisms that contaminate surfaces. There were times when no disinfection policies in place for the use. The situation has changed markedly in the recent era and many hospitals do have such policies, but implementation is unsatisfactory. The poor activity of iodine preparation at a given concentration, suggested the need for strict vigilance by the authorities over the local products. Our study emphasizes that there is a need to test the quality of disinfectants routinely supplied to the laboratory or hospital to ensure proper control of infections by using right disinfectant in right concentration for a right contact time.

REFERENCES


Source of support: Nil; Conflict of interest: None declared