Surface disinfection is essential for the prevention of infection in the households, hospitals, laboratories and other high-risk areas. The current surface disinfectants in use such as Phenolic compounds, Quaternary ammonium compounds, Aldehydes, Sodium hypochlorite etc., have associated health hazards. Clinister is an alcohol-free, citric acid based disinfectant routinely in use for disinfection in the Food Industry in Japan. In this study, the efficacy of Clinister for surface disinfection was assessed by contact plate method in a microbiological lab set-up in India. The Clinister powder was mixed with water at appropriate concentrations and applied over the surfaces of instruments, tables and the floor employing standard disinfection protocols. The microbiological sampling was done by applying the contact plate with the surfaces before and after application of Clinister, following which the plates were incubated. After incubation for six hours, there was a significant reduction in the number of colony forming units (CFUs) of the organisms isolated from these surfaces which were Staphylococcus spp., Bacillus spp., Micrococcus spp., Penicillium spp and A. fumigates. Thus, use of Clinister as a surface disinfectant is recommended in hospitals, laboratories, households, child care centres, public areas, poultry etc., wherever the risk of spread of infection is high.

Keywords: Clinister, surface disinfectant, antimicrobial agent

Introduction
Surfaces are considered “non-critical” as they are not directly in contact with intact skin and they are debated to be not commonly associated with transmission of infections in hospitals and other critical areas (1). However, they become important in situations of outbreak and in case of preventive measures against transmission of infection (2). Several studies have demonstrated the importance of surface disinfection (3-5). The common disinfectants employed for such surface disinfection are the following materials either individually or in combination: Phenol, Cresol, Benzalkonium chloride, Cetrimide, Isopropyl alcohol, Dichloroisocyanurates, Quaternary ammonium compounds like Didicyldimethylammonium chloride, Glutaraldehyde, Sodium bicarbonate, Hydrogen peroxide, Sodium hypochlorite etc.
However most of these disinfectants have disadvantages like dermatitis, urticaria and depigmentation of the skin associated with use of Phenol (which is commonly used in developing nations), reduced efficacy in the presence of organic matter, unpleasant smell, irritation to skin, eyes and mucous membranes and corrosiveness to metals associated with Chlorine, irritation to skin, eyes and air ways, allergic asthma and contact eczema with the use of Aldehydes. Even the widely used Quaternary ammonium compounds (9) have disadvantages like reduced efficacy in the presence of organic matter (10) and their widespread use has in fact increased the resistance of gram negative organisms (11). At this juncture, Clinister which is an alcohol- free citric acid based disinfectant developed by Kubota et al came into use for disinfection in food industry in Japan (12,13). Herein we report the efficacy of the Clinister as a surface disinfectant using appropriate microbiological testing methods in a microbiological lab set-up in India.

**Materials and Methods**

Clinister (supplied by GN Corporation Co. Ltd., Japan through Nichi Vision Life Sciences, Chennai, India) is a food additive based disinfection agent containing Anhydrous citric acid, Trisodium citrate; Dehydrate cetylpyridinium chloride and Lactose compounded in ratios described earlier by Kubota et al (12,13). The efficacy of Clinister in surface disinfection was analyzed by the contact plate method before and after application of the Clinister over the surfaces of instruments, tables and clean room floor in the Department of Microbiology, Aurolab, Madurai, India. Two grams of the Clinister powder was mixed with 3.5 liter of drinkable water and this solution was applied on the surface of instruments, tables and was used for mopping on epoxy floor surfaces in that lab. This application was performed under the supervision of a microbiologist following the standard surface disinfection protocols.

**Sampling device:** The surface sampling was accomplished by the use of Contact plate method. (Surface contact area 28 cm²). Contact plate is a plastic Petri dish (65 mm in diameter and 12 mm in height) pre -filled with Agar to give a convex surface with an area of 25cm² approximately and the diameter of the convex surface agar is 60 mm. The contact plate was filled with Soyabean casein Digest Agar with Lecithin and Polysorbate 80 (SCDA).

**Sample collection method:** The SCDA contact plate was applied to the surfaces with a uniform and steady pressure to the area to be sampled, without allowing circular or linear movement (Fig. 1). The contact plate testing was done before and after the exposure of the surfaces to Clinister. The sampled contact plates were closed and incubated at 30 - 35 °C. The growth in the SCDA plates was monitored after 3 days and further incubated for another 3 days at 20 - 25°C. This was done to revitalize the stressed bacteria which have been exposed to air and surface in order to achieve conditions where they can again form colonies. This temperature also favors growth and isolation of fungi. Visible colonies were counted after the incubation period manually.

![Fig. 1: Collection of floor samples by contact plate method](image_url)
Results

After six days of incubation, the colonies observed in the SCDA agar plates were counted and the respective genus was identified by staining methods like Gram stain and Lactophenol cotton blue. The average number of microbial colonies reduced from 31 CFU/Plate to <1 CFU/plate after application of Clinister (Table 1). The most common surface contaminants isolated were *Staphylococcus* spp., *Bacillus* spp., *Micrococcus* spp., *Penicillium* spp. *A. fumigates* which were observed to be significantly reduced within five minutes after application of Clinister on the floor in the media preparation room (Fig. 2), over the surface of the stainless steel table (Fig. 3) and on the floor in the wash room (Fig. 4).

Discussion

Surface disinfection becomes important in several situations like households (14), health care facilities like hospitals and laboratories (3-5), child care centres (15), poultry (16) and other public areas where the risk of spread of infection is high. Though the transmission of infection through surface contact is still debated (1), particular importance to surface disinfection is attached during times of outbreaks of infections (2). Though several surface disinfectants are in use, they have associated side effects like skin infections associated with Phenol, Chlorine (9) and resistance of gram negative organisms imparted by widespread use of Quaternary ammonium compounds (11). In case of uses in situations like poultry, it has been reported that most of the commonly used Phenol, Quaternary ammonium compounds and Sodium hypochlorite have reduced efficacy in the presence of organic matter (10, 16). The Clinister used in this study as it is food additive based, does not have the health associated adverse effects as with other surface disinfectants described above. Clinister has been found to be non-toxic even when ingested (12, 13). It is already widely used in the food industry in Japan. The present study demonstrates the efficacy of Clinister in surface disinfection as observed by the significant reduction in the number of CFUs before and after application of Clinister.

It was reported in a study that during the 2009 H1N1 outbreak in Thailand, the influenza virus RNA contamination could be documented on household surfaces and on the fingertips of ill children. Especially homes with younger children were more likely to have contaminated surfaces compared to older children (17). As Clinister has already been shown to be effective against the
Table 1: Bacterial colonies in the SCDA plates before and after Disinfecting with Clinister.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Sample locations</th>
<th>Result obtained (CFU/plate)</th>
<th>Before exposure</th>
<th>After exposure*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Media preparation room</td>
<td>34</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Media preparation room</td>
<td>21</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Microbial test room</td>
<td>21</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Microbial limit test room change room</td>
<td>69</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Instruments table</td>
<td>10</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Instruments table</td>
<td>21</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Laminar Air Flow</td>
<td>8</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Laminar Air Flow</td>
<td>11</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average number of colonies</td>
<td>31</td>
<td>&lt;1</td>
<td></td>
</tr>
</tbody>
</table>

*No growth denoted as <1 CFU per plate

HSN3 Avian Influenza virus (18), the routine use of Clinister in households, child care centres, hospitals, public areas with high risk of infection and in poultry is likely to decrease the spread of such pathogenic organisms. The Clinister will also be highly useful in situations of outbreak. As Clinister comes both in powdered and tablet forms (18), its use in a variety of situations becomes easier. Wet tissue wipes or cotton soaked with Clinister solution would be an ideal solution for wiping the instruments, gadgets, devices, surfaces etc in hospitals, laboratories, kitchens, tables, operating rooms, clean rooms for cell culture, microbiological laboratories and other domestic, professional set-ups, where disinfecting the surfaces is essential. Further studies are advocated to validate the use of Clinister in different situations of infection control.

Conclusion

Use of Clinister as a surface disinfectant effectively reduces the bacterial and fungal microbiological burden as proven by the reduction in the number of CFUs of the microorganisms after its application in a microbiological lab set-up in India. Use of Clinister is advocated for disinfection of a variety of surfaces in health care set-ups, pathological and microbiological laboratories, clean rooms, child care centres, old age homes, households, public areas, poultry etc. where the risk of spread of infection is high on a routine basis to prevent spread of infections and a possible outbreak.

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Potential conflict of interests: Dr. Nobuyuki Yamaji and Dr. Sunao Kubota are applicants to the patents which have been filed regarding the development of the Clinister - technology

References


