eJBio

Electronic Journal of Biology, 2011, Vol. 7(3): 44-48

Sensitivity of Floor Microflora towards Various Disinfectants

Chavan Vaishali, Bathija Jivitesh, Rai Shalini, Dasani Sonal*

Department of Life Sciences, K.C. College, D.W. Road, Churchgate, Mumbai 400020, India.

* Corresponding author. Tel: +91-9820291092; E-mail: sonaldasani@yahoo.com

Abstract

Microorganisms are omnipresent. They may be useful (e.g. *Lactobacilli*) or harmful (e.g. *Pseudomonas, Salmonella* etc) for human beings. The warm and moist environment of our surroundings including floors provides optimal conditions for their growth. There are many microbial species commonly present on the floor surfaces (*Serratia marcescens, Staphylococcus aureus etc*), most of which are nonpathogenic or pathogenic at higher concentrations. In order to control growth of such organisms floor disinfectants like Lizol, Dettol, Domex are commonly used. According to the Indian Medical Association, Lizol and Dettol are claimed to be the most effective disinfectants. Reports from our laboratory using *in vitro* analysis to check efficacy of various floor cleansers suggested Dazzl to be equally effective disinfectant as Lizol. In practice, it is impossible to maintain the conditions of the floor as it is maintained *in vitro*. Therefore, in the present study, experiments were designed using *in vivo* analysis to confirm the findings. A survey was conducted to determine consumer's preference and preferred products were further analyzed for their effectiveness in inhibiting the growth of floor microorganisms (*in vivo).* Attempts were also made to study the longevity of these floor disinfectants using turbidometric analysis. Our results suggested Dazzl to be the more effective as compared to other products used for the investigation.

Keywords: Disinfectant, Floor Microflora, *in vitro*, *in vivo*, Pathogens, Turbidometric method.

1. Introduction

Microorganisms, the friend and foe of human being are present inside and outside the human body. Moisture present on the human body serves to be a perfect medium for the growth of numerous organisms [1]. It is necessary to maintain hygienic conditions for effective reduction in the potential infection caused by such organisms. Hygiene is defined as a science concerned with the prevention of illness and maintenance of health, whereas, personal hygiene is a condition promoting sanitary practices [2]. To keep a check on the growth of microorganisms, routine hygiene practices using commercially available or medicated personal hygiene products are generally followed.

There are innumerous microorganisms growing on our skin surface and even after use of soap or an antiseptic, these cannot be fully eradicated. Physical contact with any surfaces present in the surroundings leads to the transfer of microorganisms from the human body to the surrounding surfaces. Floors are one of the most important surfaces for establishment and growth of such microbes because of presence of uneven areas and crevices that can hold moisture. Most of the organisms present as common floor microflora, are opportunistic and can cause infections at higher concentration [3]. For example, *Serratia marcescens* is a human pathogen and is responsible for catheter-associated bacteremia, urinary tract infections and wound infections [4], *Staphylococcus aureus* can cause minor skin infections such as pimples [5]. Pseudomonas aeruginosa typically infects the pulmonary tract, urinary tract, burns, wounds, etc. *Aspergillus versicolor* can produce toxins [6], Cladosporium species cause infections of the skin, toenails, sinusitis and pulmonary infections. Children, aged individuals and patients are relatively more susceptible to such infection because of their low levels of immunity.

Earlier work in the laboratory has suggested that unhygienic places supported by moisture content facilitated maximum microbial growth [7]. It is practically impossible to eradicate microorganisms from the floor. Generally it is accomplished using phenyl as a disinfectant and deodorant, its prolong use is not safe for the health as is made up of chemicals that may damage the skin and cause skin diseases [8]. With the increase in the knowledge and research in this field, "disinfectants" were introduced. Disinfectants are the best weapons for fighting against germs. It is an agent, such as heat, radiation, or a chemical, that destroys, neutralizes or inhibits the growth of microorganisms [9].

Different disinfectants are presently available commercially which have pleasant smell along with the ability to inhibit the growth of microorganisms. There are a number of manufacturers who have launched various disinfectants commercially in the market. There is cut through competition for the market share of Fast Moving Consumer Goods (FMCG). To have an edge over others, all are

coming up with better and catchy advertising campaigns day by day. As a lay man, it is obvious to get biased and buy the disinfectants which have better packing and promotion campaign.

Indian Medical Association (IMA) states, a good disinfectant should be capable of killing the germs by 99.99% within 60 seconds and recommends only Lizol and Dettol. Earlier research from our laboratory using in vitro analysis showed Dazzl as the disinfectant that has maximum antimicrobial activity when compared with other floor disinfectants. Dazzl was found to be equally effective as Lizol. However, it is practically impossible to maintain the conditions in our surroundings that are comparable to in vitro conditions; therefore, the findings need to be confirmed using in vivo experimentations. Another important aspect of efficacy of any disinfectant from consumer's point of view would be the longevity of its effect. How long the floor can remain hygienic once cleaned with a particular disinfectant?

- To answer the question, with respect to aforesaid information, following objectives has been set forth the present study:
- To design a questionnaire for Consumer Survey Analysis.
- To identify most commonly used floor cleaners or disinfectants through a survey.
- In vivo analysis to check effectiveness of floor cleansers
- Standardization of methodology
- To select the optimum concentration of cleansers at which the growth of microbes is inhibited effectively
- To compare longevity of effect of the cleansers using in vivo and in vitro analysis
- Analysis of the results
- Preparing the guidelines for the consumers.

2. Methods

A questionnaire was designed keeping in mind our aim –'to know the preferences and awareness of consumers regarding floor disinfectants' that they use. The questionnaire forms were disributed amongst 500 people from the urban areas of Mumbai, India, consisting of domestic users, clinics, hospitals, laboratories etc. The filled forms were collected and subjected to statistical analysis.

To determine sensitivity of floor micro flora, the laboratory where the investigators work was selected as model to perform *in vivo* analysis using Turbidometric method where Nutrient broth was used as a medium. For collection of samples, three different locations in laboratory were marked 1 x 1 square feet and used for all the experimentation. Sterile cotton swabs of 2 × 2 inches were dipped in sterile distilled water and the marked floor location was wiped with it. This swab was suspended into a flask containing sterile saline (0.85% NaCl, pH 7.2) and the resultant solution was used as sample for preparing positive control. To nullify the effects of color imparted by nutrient broth, sterilized broth was used as negative control with each set.

All the commercially available disinfectants recommend minimum concentration to be used for its optimum effectiveness. To check the efficacy of disinfectant *in vivo,* 14 various concentrations were tested including the recommended and concentrated solution. The swab was dipped in the particular concentration of a disinfectant and the marked location was wiped and the swab was then discarded. After two to three minutes the same location was wiped with another swab dipped in sterilized distilled water. This swab, with the organisms not removed by disinfectant, was then suspended in a flask containing 20 ml sterile saline and mixed thoroughly to make the test sample for respective disinfectant and concentration. In the same manner the samples were collected for each of the concentration of all the disinfectants used in the present study.

All these samples of different concentrations were inoculated under aseptic conditions to the test tube containing 9 ml pre-sterilized broth. The tubes were then incubated at 37ºC for 24 hours and optical density (OD) was recorded at 470 nm. All the experiments were performed twice in duplicate with positive and negative controls.

To check the longevity of the efficacy of disinfectants, the disinfectant sample (recommended concentration) was added to the nutrient broth and then inoculated with inoculums (*in vitro* analysis). The inoculums were prepared by taking the sample after cleaning the floor with recommended concentration of the disinfectant. The tubes were then incubated at 37ºC and OD was recorded at 470 nm after every 24 hours interval. The entire experiment was twice conducted in duplicate along with positive and negative control.

To confirm the findings samples were collected after every 2 hours interval after cleaning the floor with respective disinfectant using recommended concentration (Considered as 0 hour). One ml of the samples were inoculated in the nutrient broth, incubated for 24 hours at 37ºC and read at 470 nm. All the experiments were performed twice in duplicates and mean along with ± standard deviation is represented in the graph.

The mean value was calculated along with ± standard deviation and used for calculation of % inhibition in the growth. The value of OD of negative control was subtracted from all other readings to nullify the effect of color imparted by nutrient broth. The OD of positive control was considered as 100% growth and compared with OD obtained using test samples to calculate % growth of floor microorganisms after the use of particular disinfectant. To calculate % inhibition, the values of % growth was subtracted from 100 and plotted on the graph against concentration or time of incubation.

To determine the significance of difference of various disinfectants used, the values of % inhibition obtained using various concentration (fourteen) of different disinfectants (five) were subjected to single factor analysis of variance. To determine the significance of difference between two sets of data, unpaired t-test assuming equal variance was used. Microsoft Excel – 2007 was used for performing all the statistical analysis.

3. Results and Discussion

It is practically impossible to keep our surroundings (including floor) free from microorganisms because they can grow in abundance in the presence of moisture. Cleaning of the floor will not be able to dislodge the microbes completely, but use of suitable disinfectant will help to decrease the growth of organisms to avoid potential infection that can be caused by higher concentrations of the same. For the very reason the floor with which one is in direct contact needs to be cleaned regularly with suitable disinfectant and cleansers.

Sinha *et al*. [7] from our laboratory have isolated and identified the most frequently occurring bacterial species as *E. coli, Micrococcus luteus* (Nonpathogenic) and *Serratia marcescens, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus cereus* (Pathogenic). They have used the floor disinfectant that are sold maximum and tried to compare their efficacy using *in vitro* analysis (Floor samples were collected and inoculated in the broth along with floor disinfectant and then incubated under controlled conditions). Dazzl was found to be as effective as Lizol in controlling the growth of floor microorganisms. However, in practice, at places like home, laboratory etc. maintaining controlled conditions is impossible, therefore, to confirm the effect of floor disinfectant *in vivo* experiments were designed in the present study.

Earlier work suggests no difference in various methods like Agar Ditch, Paper Disk and Turbidometric analysis that can be used for these kinds of experiments [7]. However, paper disk and agar ditch is difficult to perform with respect to floor micro flora as some of the samples need to be concentrated. Therefore, in the present study, to determine the effectiveness of products Turbidometric analysis is performed.

The results of basic survey conducted amongst 500 consumers are represented in Figure 1.

It revealed that Lizol, Domex and Dettol (antiseptic) are most frequently used products for domestic purpose. Majority of the clinics and hospitals prefer Dettol (antiseptic) as disinfectant. More then 90% of the people do not follow the standard concentration to be used, as recommended by the manufacturer.

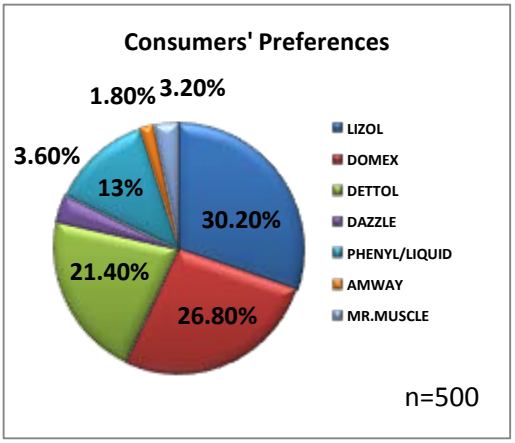

Figure 1. Consumer preferences (based on survey results)

People clean/swipe their floor once (~88%) or at the most twice a day (~12%) which indicated the importance of analysing the longevity of a particular disinfectant. People are happy with what they use and only a few are open to try new products launched. Instead of the composition and inhibition factor, to select a specific disinfectant, odour and cost are major criteria.

With respect to the above stated information obtained by survey analysis and to confirm the earlier work done in our laboratory, in the present study commonly used floor disinfectants like Lizol, Domex, Dazzl, Dettol and Phenyl are used.

Figure. 2 represents the results obtained for % inhibition in the growth of floor microorganisms using *in vivo* analysis by turbidometric method. Manufacturers of all the disinfectants used in the present analysis recommend use of 3µl/ml of water. Most of the disinfectants used showed ~25% inhibition in the growth of floor microbes with recommended concentration, except Dazzl and Phenyl, which are able to inhibit 47% and 44% growth respectively. All the disinfectants used showed steady increase in the % inhibition in accordance with increase in concentration except Sunny Phenyl which showed lots of variation while Dazzl showed consistent increase in %inhibition with increase in the concentration. Maximum inhibition (> 90%) was observed when concentrated samples were used for all the disinfectants. The correlation analysis performed between the two parameters (i.e. Concentration and % inhibition) for all the disinfectants shows r>0.9, suggesting dose dependent pattern of inhibition or in other words for these disinfectants recommended concentration is not sufficient enough. These disinfectants are not able to inhibit the growth of floor microorganisms efficiently at recommended concentration.

To support the observation, statistical analysis was performed for % inhibition in the growth obtained using various disinfectants. Single factor ANOVA performed amongst various floor disinfectants shows that there is no significant difference in % inhibition amongst the five floor

disinfectants tested (P≤ 0.354). When Lizol and Dazzl are compared (using t-test for two samples assuming equal variances) the difference was found to be statistically significant as ≤P 0.05, proving Dazzl to be more effective than Lizol. Dazzl was more effective *in vivo* as compared to Lizol.

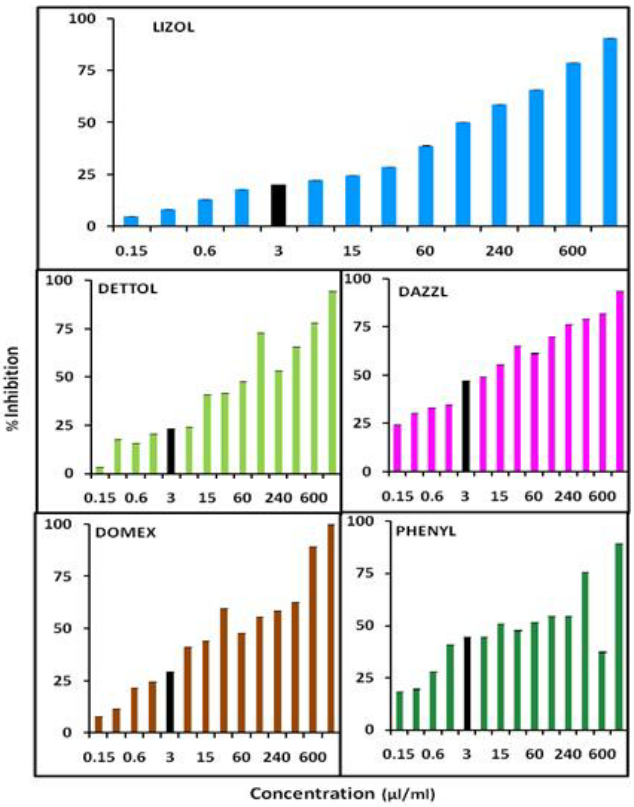

Figure 2. % Inhibition in growth of total floor microorganisms after cleaning with various concentrations of different disinfectant. Vertical bars denotes ±SD or are with in the symbol.

Sinha *et al*. [7] from our laboratory suggested using *in vitro* analysis that Dazzl is as effective as Lizol. However, in the present study Phenyl and Domex are also found to be very effective. There is no significant difference when Dazzl, Phenyl and Domex were analyzed by Single factor ANOVA (P<0.4). Dazzl is found to be little more effective then Phenyl (P<0.2) and Domex (P<0.26).

To determine comparative longevity of effect of floor disinfectants, the samples were collected from the floor and incubated along with the disinfectant for 24, 48 and 72 hours. According to our survey analysis majority of people (~88%) clean their floor once a day therefore in the present study interval of 24 hrs is chosen for the analysis.

Figure 3 represents the result obtained using five different floor disinfectants after 24, 48 and 72 hours of incubation after inoculation. Dazzl was found to be most consistent and most effective followed by Domex and Lizol. Phenyl was able to inhibit only approx 25% of microbial growth after 24 hours as compared to >90% by Dazzl. ANOVA performed shows highly significant difference (P< 0.001) in efficiency of disinfectants. The mean value of % inhibition by Dazzl was 97.42% as compared to 49.37% of Domex, 38.5% of Lizol and 24.72% of Phenyl. Highly significant difference in the ability of Dazzl to inhibit the growth of floor microbes as compared to Lizol, Phenyl and Domex was recorded using ANOVA (P<0.001). Dazzl is far more effective as compared to any other disinfectants used in the present analysis.

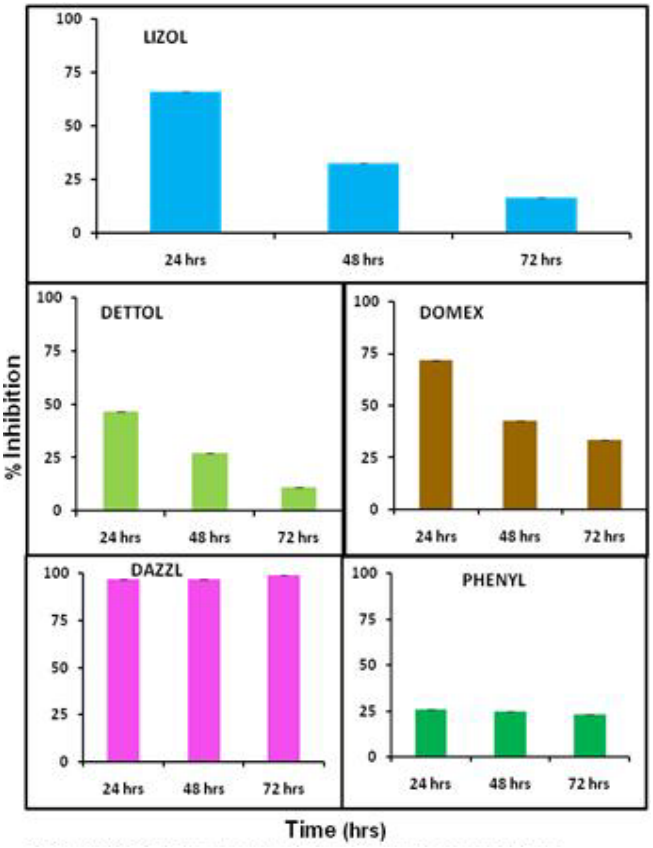

Figure 3.Inhibition in growth of total floor microorganisms cultured with standard concentration of Lizol,Dazzl and Phenyl.Vertical bars denotes±SD or are within the symbol.

To confirm the findings of longevity of Dazzl, the time interval was reduced to 2 hours and *in vivo* experiment was designed. Again here Dazzl was found to be the most effective in inhibiting the microbial growth at 3 consecutive time intervals followed by Domex and Phenyl (Figure. 4).

As the time increases % inhibition decreases, may be because of movement of individuals in the area and reestablishment of air flora. Though ANOVA performed shows no significant difference (P< 0.24), the mean value of % inhibition by Dazzl (51.62) is more as compared to any other disinfectant used. The % inhibition obtained by Lizol and Dazzl is compared using t-test and was found to be statistically less significant (P<0.16), but Dazzl showed more inhibition in the growth at any time period as compared to Lizol. Again Dazzl has proved itself.

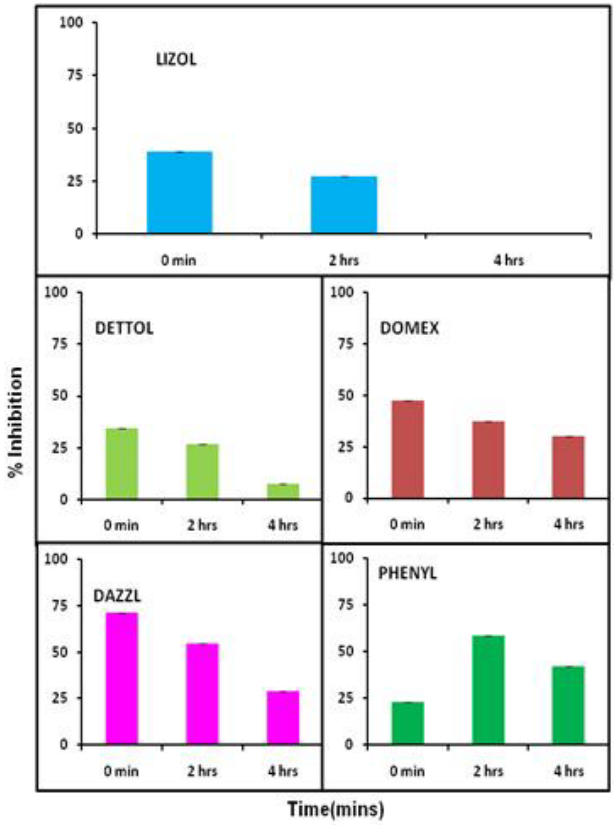

Figure 4. % Inhibition in growth of total floor microorganisms collected after every 2 hours after cleaning with respective disinfectant. Vertical bars denotes±SD or are within the symbol.

4. Conclusions

According to the survey Lizol, Domex and Dettol (antiseptic) were high on the charts, whereas, phenyl and Dazzl were amongst less preferred brands as floor disinfectant. Lizol is the only disinfectant that is recommended by the Indian Medical Association, but from the above study it was found that Dazzl showed maximum inhibition of microorganisms at the recommended concentration. Concentrated solution of Dazzl gave maximum inhibition as compared to concentrated solutions of any other disinfectant used. Dazzl also proves to be the one to give consistent and long lasting effect minimizing reoccurrence of the inhibited microbes. Domex proves to be more effective disinfectant as compared to Lizol after Dazzl.

Thus, *in vivo* (present study) as well as *in vitro* analysis (6) from our laboratory using various disinfectants with various concentrations and various time intervals suggests that Dazzl is most efficient and long lasting amongst the floor cleansers used. Dazzl's ability to inhibit growth of floor microorganisms is better than Lizol which is recommended by Indian Medical Association. We highly recommend use of Dazzl as floor disinfectant.

5. Future Prospects

We would like to continue the studies with Dazzl to determine its efficacy in various locations apart from laboratory.

Would try to make this piece of research work as popular science article that can provide guidelines regarding floor disinfectants for the consumers.

Acknowledgements

Authors are highly thankful to University of Mumbai and Science Honors Program of K.C. College for providing financial help and laboratory facilities.

References

[1] Nene Y.L. (2007) A glimpse at viral diseases in ancient period. *Asian Agri-History*, **11**(1): 35-46.

[2] Pelczar, M.J., Chan, E.C.S and Kreig, N.R., 1993. Microbiology- 5th Edition, New-York, Tata Mac Graw Hill Publishing Co. Ltd, pp. 137-386.

[3] www.wordnetweb.princeton.edu/perl/webwn

[4] Stainer, R.Y., Ingraham, J.L., Wherlis, M.L., Painter, P.R., 1987. General microbiology- 5th edition, MacMillan Press Ltd, London. pp- 2.

[5] Auwaerter, P., 2007. "Serratia species". Point-of-Care Information Technology ABX Guide. Johns Hopkins University. Retrieved on December 13, 2008.

[6] Daftary, V.G., Banker, D.D., Daftary, G.V., 1996. Clinical Microbiology-First Print, Bombay, Universal Publishing Corporation, pp. 12-55.

[7] www.healthand energy.com/aspergillis.htm

[8] Sinha, A.S., Dasani S.H., Rai S.A., 2009. Germ Invasion and a Comparative Analysis of various Floor Cleaners, Electronic Journal of Biology. 5(1): 17-21.

[8] www.lizol.co.in

[9] www.answers.com/topic/disinfectant.