Original Article

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Superoxidised water: A Promising Disinfectant Against Bacterial and Fungal Pathogens

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ABSTRACT

Background: Disinfectants are frequently used in hospital settings as prophylaxis. Superoxidised water is one of them, which is claimed to have disinfectant property against different microbes.

Aim: This study aims to assess the in vitro efficacy of different dilutions of superoxidised water against Candida spp. and filamentous fungi apart from several pathogenic bacterial isolates.

Material and Methods: The freshly grown clinical isolates of selected bacteria and fungi including Candida spp.,Aspergillus sp., Fusarium spp., Acremonium sp., Curvularia sp, and Bipolaris sp. were subcultured on culture plate with and without exposure of undiluted, five and ten times diluted, freshly generated superoxidised water.

Results: There was complete inhibition of all bacteria and Candida species in undiluted as well as five and ten times diluted freshly generated superoxidised water whereas filamentous fungi used in the present study were inhibited only with the use of undiluted superoxidised water.

Conclusion: Undiluted superoxidised water should be used to prevent the occurrence of molds infection as nosocomial pathogens.

Keywords: *Superoxidised Water, Filamentous Fungi, Candida, Aspergillus, Pseudomonas*

Introduction

In the era of antibiotic resistant microbes, preventive measures play an important role in the prevention of hospital acquired infection.[1]Antiseptic and disinfectants are commonly used to reduce the transmission of these nosocomial pathogens. Commonly lysol, alcoholic compounds, sodium hypochlorite, gluteraldehyde, *ortho*-phthalaldehyde (OPA), hydrogen peroxide with and without peracetic acid are being used as disinfecting agents. Recent studies suggest the emergence of microbial pathogens that are resistant to these common disinfectants. [2]

Superoxidised water (SOW) is a new disinfectant that readily kills the microorganisms within minutes of exposure. SOW is generated by oxidation-reduction process, also known as Electro-Chemical Activation. SOW contains a mixture of oxidizing species, predominantly hypochlorous acid and sodium hypochlorite with pH 5.0-6.5 and oxidation- redox potential of >950 mv. These oxidizing agents destroy the cellular activity of proteinsresulting in impaired transport of solutes and salt balance of bacterial cells, resulting in cell lysis. [3]

Available disinfectants are costly that necessitate a need of cheap disinfectant especially in developing countries which can't afford to invest huge money in health care system. SOW can be alternative to these costly disinfectants as it is cheap, easy to generate with broad activity. Recent data shows that SOW is effective against wide range of pathogenic bacteria namely *Methicillin* resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*. [4,5,6]

The present study has been undertaken to observe the effect of SOW in different dilutions against several pathogenic bacteria and fungi.

Materials and Methods

Disinfectant property of freshly generated SOW (Sterisol) generated by Steri-Gen® disinfectant generating system was assessed against bacteria, yeast and filamentous fungi. Frequently encountered microorganisms in hospital ecosystem were selected including bacteria (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, Enterococcus faecalis, Klebsiella pneumoniae*), Yeast (*Candida albicans, Candida krusei, Candida tropicalis, Candida parapsilosis) and* filamentous fungi *Aspergillus*sp. *Fusarium* sp., *Curvularia* sp., *Bipolaris* sp.

Freshly generated SOW was used undiluted, and after 5 and 10 times dilution. Bacteria and yeast suspensions were made in normal saline to have turbidity matched with 0.5 McFarland. This had a concentration of $5x10^8$ CFU/

ml (bacteria) and 5x10^6 CFU/ml (*Candida sp.)*. 50 µl of bacterial and yeast suspension was exposed to 500 µl each of undiluted and five and ten times diluted SOW for 1 minute.

Filamentous fungi were cultivated on potato dextrose agar. Conidia of filamentous fungi i.e. *Aspergillus* sp., *Fusarium* sp., *Curvularia* sp, and *Bipolaris* sp. were separated with Tween 80 and normal saline. Conidial suspension was diluted with normal saline to have turbidity of 0.1 OD having 5x10^6 CFU/ml. 50 µl of conidial suspension was exposed to 500 µl each of undiluted and five and ten times diluted SOW for 1 minute.

Subsequently, 5 µl suspensions were inoculated on culture media. Bacterial and fungal suspensions were inoculated on nutrient agar and Sabouraud's dextrose agar (SDA) respectively. For bacteria, nutrient agar plates were incubated overnight at 37^{o}C. For fungi, SDA plates were incubated till there was growth on control (without exposure to SOW).

*Ethical approval***:** Not required as the present work is an in vitro study, and was executed on laboratory preserved strains of bacteria and fungus.

Results

After overnight incubation, 6 log reduction of bacteria and *Candida* spp. was observed after exposure to undiluted, 5 and 10 times diluted freshly generated SOW. In case of filamentous fungi, growth was inhibited only after exposure to undiluted SOW (Figure 1, Table 1).

Table 1: Showing the effect of different dilutions of superoxodised water over growth of different microbes.

Microorganism	Freshly generated SOW		
	Undiluted	5 times diluted	10 times diluted
Staphylococcus aureus	+	+	+
Enterococcus faecalis	+	+	+
Escherichia coli	+	+	+
Klebsiella pneumoniae	+	+	+
Acinetobacter baumannii	+	+	+
Candida albicans	+	+	+
Candida krusei	+	+	+
Candida parapsilosis	+	+	+
Candida tropicalis	+	+	+
Aspergillus spp.	+	-	-
Fusarium spp.	+	-	-
Curvularia spp.	+	-	-
Bipolaris sp.	+	-	-

Note: Growth inhibition (+); No Growth inhibition (–)

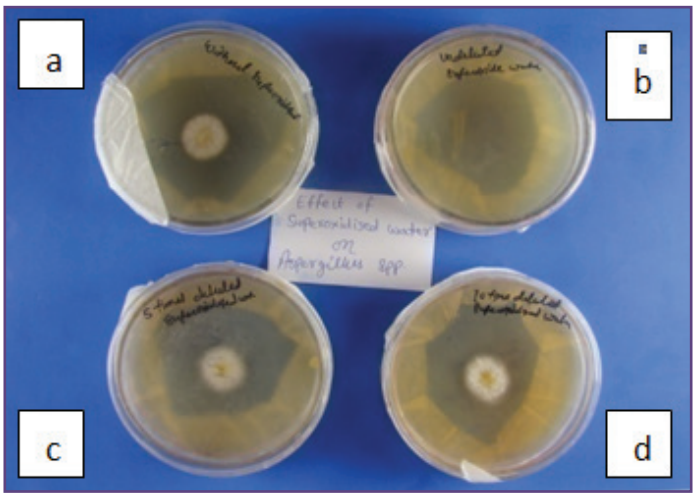

Fig. 1: Effect of different dilutions of superoxidized water over the growth of Aspergillus spp. (a) Showing growth on SDA without SOW, (b) No growth on SDA with undiluted SOW, (c) Showing growth on SDA with 5 times diluted SOW, (d) Showing growth on SDA with 10 times diluted SOW.

Discussion

Nosocomial infections have a major impact on morbidity and mortality on the patients suffering with chronic illness, immunocompromised status and taking broad spectrum antibiotics. These infections are usually exogenous as they are transmitted by hospital ecosystem including hospital health care personnel, food, water and air. Ecosystem is contaminated from spillage of human secretions and excretion which requires prompt cleaning with disinfectant. [7]Secondary bacterial infections are common among inpatients but fungi may also cause life threatening infection during hospital stay especially in ICU patients. With the emergence of disinfectant resistant microbes, there is need for newer highly effective disinfectant. [8,9,10]

SOW is new, high level disinfectant with no effect on human tissues at neutral pH. [11]It is prepared by passing the normal saline over titanium coated electrode at 9 amp. The raw material for SOW generation, normal saline and electricity are cheap and easily available but the generating equipment is costly. End product, hypochlorous acid is not harmful to the environment. Although it is highly efficient against the microbes but its efficacy is reduced in the presence of organic material such as blood and pus which should be removed before SOW application. [12]

In this present study, SOW was effective against all the microbes including filamentous fungi. SOW is also effective against microbes (Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae) that are frequently encountered as multi drug resistant strains. [13].Previous studies have also shown the higher efficacy of SOW against various bacteria and yeast like fungi (*Candida*). [14,15,16] In our study, efficacy of diluted SOW was also noted against *Candida krusei* and *Candida glabrata* having intrinsic antifungal drug resistant property. Use of SOW as disinfectant can prevent the outbreaks of the drug resistant microbes including *Candida* as fungal infection are the fourth most common cause of nosocomialsepticemia. [17].Molds especially *Aspergillus* species and *Fusarium* species are most frequently isolates whose incidence has increased in present era of immunocompromised state. [18] These filamentous fungi were inhibited only by undiluted freshly generated SOW, that necessitates the use of undiluted SOW.

Freshly generated 10 times diluted SOW showed disinfectant property against various bacteria and yeast but filamentous fungi were inhibited only by undiluted SOW. There was no inhibition of filamentous fungi in diluted SOW. Efficacy of SOW in diluted form (1/2) have been shown by Gunaydin M et al; against the clinical isolates but not at higher dilution. They also reported the efficacy of 1/2 diluted SOW against *Candida* sp. but in contrast our study showed the potent efficacy of SOW against Candida spp. even in 10 times dilution. Against filamentous fungi, similar results were observed.[19]Being high level disinfectant, SOW should be used in undiluted form. Available disinfectants are effective in diluted states so that there is usual tendency of dilute disinfectant before their use. Use of diluted SOW especially in ICU setting, tertiary care centre may result in molds outbreak. Thus freshly prepared SOW should be used in undiluted state.

Conclusion

The present study indicates the use of undiluted SOW in hospital settings as disinfectant, to prevent the occurrence of nosocomial infections specially caused by filamentous fungi. However, we have observed the efficacy of superoxidised water in different dilutions against various microbes. Further studies are warranted to corroborate these results in clinical scenario for conclusive validation.

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