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A Systematic Evaluation of the Effect of Sodium Hypochlorite on Pathogenic Aerobic Bacteria and Its Possible Use as a Chemical Disinfectant for Microbiological Culture Plates

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A B S T R A C T

Sodium hypochlorite has been used as a proven disinfecting agent for more than 150 years. Of late, the ill effects of sodium hypochlorite on the human health and environment have come to light through studies of various agencies and governments are looking forward for proper recommendations. In the light of this, the current study gains lots of importance since there are only a few systematic studies available. The current study tries to determine the minimum effective dose and time required to disinfect common pathogenic aerobic bacteria on exposure to Sodium hypochlorite. The results have shown that 0.1 % of Sodium hypochlorite is sufficient to disinfect common pathogenic bacteria with a minimum exposure time of 5 minutes. It also shows that 0.5 % Sodium hypochlorite with a minimum exposure time of 5 minutes could be used to decontaminate culture plates used in Microbiology laboratories. Chemical disinfection with Sodium hypochlorite is safe, cost effective and can be used in all setups in a shorter period of time without the use of electricity.

Introduction

Sodium hypochlorite as a chemical disinfectant is well known. However, there are many studies that point to its ill effects on human health and on environment.

The Chlorine Institute Inc. (2017); In: Chlorine: Effects on health and environment. 3rd ed. pp. 1 – 8; Doris Horvath (1992) In: Report on the STOA Scholar seminar (Dekant, 2008). After establishing the effect of 1% Sodium hypochlorite on blood containing vacutainers (Sneha Kukanur *et al.*,), it was generally felt that there was a need to study the optimum dose and time of exposure to Sodium hypochlorite on various clinical samples. In the light of this, the current study was planned to find out the effects of Sodium hypochlorite on common pathogenic aerobic bacteria encountered in a clinical Microbiology laboratory.

The current study was designed to study the effects of various concentrations and duration of exposure to Sodium hypochlorite on the culture plates growing the pathogenic organisms.

The main aim and objectives of this study includes, to evaluate the disinfectant action of different concentrations and duration of exposure to Sodium hypochlorite.

To evaluate action of Sodium hypochlorite as chemical disinfectant for bacteriological culture plates growing known pathogens.

Materials and Methods

The study was carried out over a period of two months (from 2nd June 2016 to 30th July 2016). A total of 129 culture positive samples were included in the study. A total of twelve pathogenic organisms that were isolated were included in the study. Culture plates which were still under process and had no growth of bacteria were not included in the study.

Since effectiveness of 1 % sodium hypochlorite was established earlier in blood samples contained in discarded vacutainers (Sneha Kukanur, *et al.,*), Sodium hypochlorite concentrations of 0.1 %, 0.5 %, 0.75 % and 1.0 % and exposure time of 5 minutes and 10 minutes were used across the samples.

Similarly based on the previous experience, the minimum time of exposure studied was 5 minutes and the maximum time of exposure was 10 minutes.

Titration of dose and time required for complete disinfection of commonly encountered bacteria

Four sterile test tubes per isolate (to be tested) were placed in a test tube rack.

Known organisms from the pure solid cultures were picked up and suspended in 0.5 ml normal saline placed in these test tubes and adjusted to 0.5 Macfarland turbidity standards (Scott Sutton).

Four different concentrations (0.2 %, 1.0 %, 1.50 % and 2.0 %) of Sodium hypochlorite were prepared separately in four different containers (Fig. 1a).

Equal quantities (0.5 ml) of different concentrations of sodium hypochlorite prepared were poured into each test tube with bacterial suspension in saline to get a final concentration of 0.1 %. 0.5 %, 0.75 % and 1.0 % (Fig. 1b).

At the end of 5 minutes, one loop full of the suspension was placed on a plate of blood agar and another on Mac Conkey agar. Similarly, a second set of same dilutions were prepared after exposure for 10 minutes as shown in Figure 1C. The plates were then incubated for 24 hrs. at 37°C and looked for the kill effect of different concentrations of Sodium hypochlorite.

Decontamination procedure for Microbiology positive culture plates

Culture plates from where the clinical isolates were grown on Muller Hinton agar were studied for the disinfectant action of Sodium hypochlorite at 0.5 % and 1 % with exposure time of 5 minutes and 10 minutes. Muller Hinton agar was selected since the pure cultures are made on a single plate and secondly, the lawn cultures have more bacterial load than either blood, chocolate or Mac Conkey agars normally used in the Microbiology laboratory where streak cultures are made for isolation the organisms. This study also gave us the effect of Sodium hypochlorite on bacteria in a log phase and gave important evidence regarding the penetration of the chemical into the agar gel.

Results and Discussion

A total of 129 pathogenic bacterial isolates were included in the study. Distributions of

different bacteria isolated are depicted in table 1. Among the isolates included in the study, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella* spp. were the main organisms (61.0 %).

Results showed that 0.1 % of Sodium hypochlorite with minimum exposure time of 5 minutes were sufficient to disinfect all the isolates studied (Table 2).

Similarly, the culture plates with different isolates studied were disinfected with 0.5 % and 1 % Sodium hypochlorite with an exposure period of 5 minutes and 10 minutes (Table 3).

Sodium hypochlorite is being used as a disinfectant agent for more than 150 years. It is a cost effective chemical disinfectant. Daniel Mekonnen *et al.,* (2015) and Adolfo Paolin *et al.,* (2016) have done some studies on the effect of Sodium hypochlorite using different bacterial isolates and exposed to different intervals of time. As compared to the studies referred above, our study results in comparison have shown the effectiveness at lower concentrations and at shorter exposure time. Recent literature reports the ill effects of Sodium hypochlorite on human health and environment, prompting a second look regarding the use of chlorine containing disinfectants. There are insufficient scientific studies to evaluate the effects of Sodium hypochlorite in relation to its concentration and duration of exposure.

Current practice of disinfection of the Microbiological culture plate is autoclaving and disposing autoclaved bags as infective waste as per the Biomedical Waste management recommendation (Government of India BMW rule, 2018). Hence there is a need to look into the safety issues of using Sodium hypochlorite as a disinfectant in the disposal of biomedical waste, especially of the bacteriological waste from the Microbiology laboratories. The present study has looked into all these needs and hence the importance of this study (Fig. 2).

Table.1 Depicting the number of isolates studied

Sl. No	Organism	No. of samples	Percentage
1	*Citrobacter* spp	1	0.78
2	*Staphylococcus aureus*	14	10.85
3	MRSA	10	7.75
4	CONS	4	3.10
5	MR CONS	5	3.88
6	*Enterococcus* spp	7	5.43
7	*Escherichia coli*	31	24.03
8	*Klebsiella oxytoca*	3	2.33
9	*Klebsiella pneumoniae*	13	10.08
10	NF GNB	11	8.53
11	*Proteus mirabilis*	2	1.55
12	*Proteus vulgaris*	5	3.88
13	*Pseudomonas aeruginosa*	21	16.28
14	*Pseudomonas* spp (Non-pigmented)	2	1.55
	Total isolates studied	129	100.00

Table.2 Results of the exposure of different concentrations of bacteria to Sodium hypochlorite

		0.1 %		0.5 %		0.75 %		1.0 %	
Organisms	**No. of samples**	**0.1% (5 mins)**	**0.1% (10 mins)**	**0.5% (5 mins)**	**0.5% (10 mins)**	**0.75% (5 mins)**	**0.75% (10 mins)**	**1% (5 mins)**	**1% (10 mins)**
***Citrobacter*spp**	1	NG	NG	NG	NG	NG	NG	NG	NG
Staphylococcus aureus	14	NG	NG	NG	NG	NG	NG	NG	NG
MRSA	10	NG	NG	NG	NG	NG	NG	NG	NG
CONS	4	NG	NG	NG	NG	NG	NG	NG	NG
MR CONS	5	NG	NG	NG	NG	NG	NG	NG	NG
***Enterococcus* spp**	7	NG	NG	NG	NG	NG	NG	NG	NG
Escherichia coli	31	NG	NG	NG	NG	NG	NG	NG	NG
Klebsiella oxytoca	3	NG	NG	NG	NG	NG	NG	NG	NG
Klebsiella pneumoniae	13	NG	NG	NG	NG	NG	NG	NG	NG
NF GNB	11	NG	NG	NG	NG	NG	NG	NG	NG
Proteus mirabilis	2	NG	NG	NG	NG	NG	NG	NG	NG
Proteus vulgaris	5	NG	NG	NG	NG	NG	NG	NG	NG
Pseudomonas aeruginosa	21	NG	NG	NG	NG	NG	NG	NG	NG
***Pseudomonas* spp (Non pigmented)**	2	NG	NG	NG	NG	NG	NG	NG	NG

* NG = No Growth

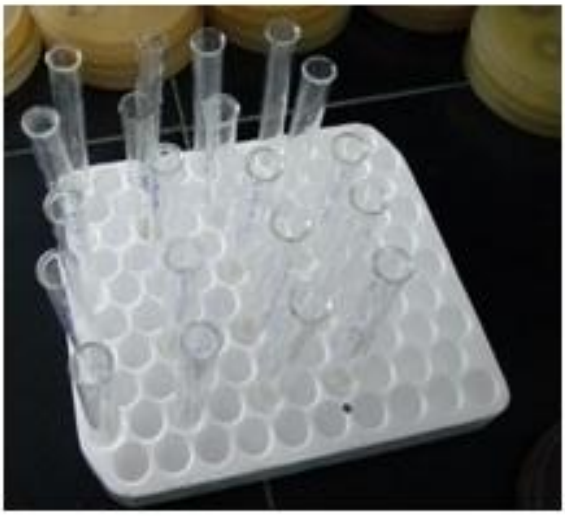
Table.3 Results of different concentrations of Sodium hypochlorite on Bacterial culture plates

Organisms	**No. of cases**	**0.5% Sod Hypo (plate)**		**1% Sod Hypo (plate)**	
		5 min	**10 min**	**5 min**	**10 min**
***Citrobacter*spp**	1	NG	NG	NG	NG
Staphylococcus aureus	14	NG	NG	NG	NG
MRSA	10	NG	NG	NG	NG
CONS	4	NG	NG	NG	NG
MR CONS	5	NG	NG	NG	NG
***Enterococcus* spp**	7	NG	NG	NG	NG
Escherichia coli	31	NG	NG	NG	NG
Klebsiella oxytoca	3	NG	NG	NG	NG
Klebsiella pneumoniae	13	NG	NG	NG	NG
NF GNB	11	NG	NG	NG	NG
Proteus mirabilis	2	NG	NG	NG	NG
Proteus vulgaris	5	NG	NG	NG	NG
Pseudomonas aeruginosa	21	NG	NG	NG	NG
***Pseudomonas* spp (Non-pigmented)**	2	NG	NG	NG	NG

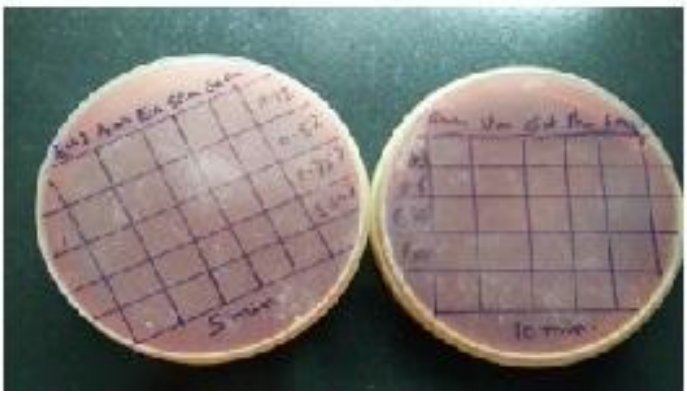
* NG = No Growth

Fig.1 Determination of effective dose of Sodium hypochlorite on known Clinical bacterial isolates

I a: Preparation of the dilutions of Sodium hypochlorite

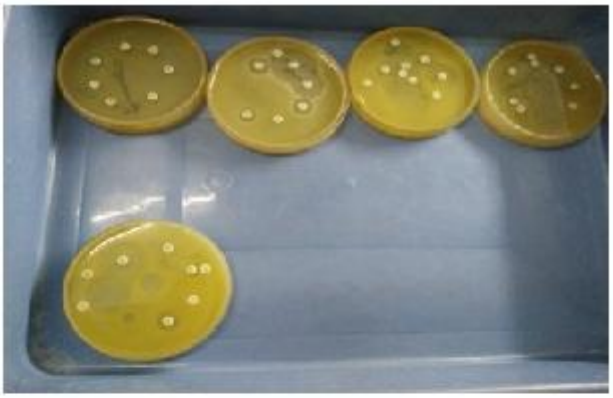


I b: in each tube, loopful of known organisms were suspended in 0.5 ml normal saline to which 0.5 ml of 0.2, 1.0, 1.5 & 2.0 % Sodium hypochlorite solution was added to get a final concentration of 0.1, 0.5, 0.75 &1.0 %

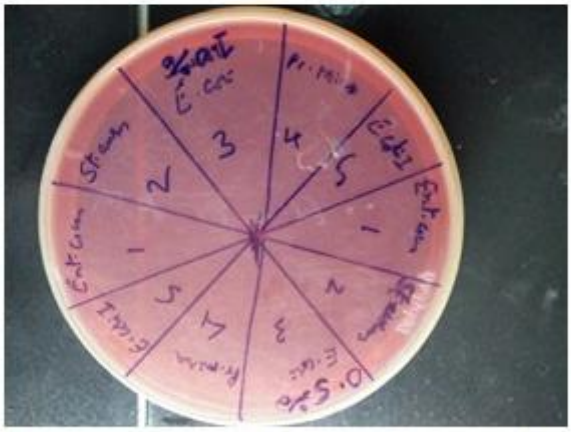


I c: Two Mac conkey agar plates showing inoculation of organisms exposed for 5 and 10 min. to different conc. of Sodium hypochlorite

Fig.2 Determination of disinfection action of Sodium hypochlorite on culture plates using 0.5 % or 1.0 % dilution and exposure time of 5 or 10 minutes



II a: Culture plate of known organisms exposed to 0.5 % Sodium hypochlorite for 5 or 10 min.



II b: Plates exposed to either 0.5 % or 1 % and exposed to 5 or 10 minutes are cultured on to Mac conkey and blood agar plates

The current recommendation of WHO regarding Sodium Hypochlorite as a disinfectant is the use of 1 % with an exposure time of 20 minutes. The same is followed by almost all the biomedical waste management rule in most of the countries, including India.

The current study, clearly illustrates the minimum concentration of Sodium hypochlorite required for the disinfection of commonly encountered bacteria in a Clinical Microbiology laboratory is at a dose of 0.1 % and the minimum exposure time is 5 minutes.

The current study also makes an attempt to study an alternate method of effective chemical decontamination and disinfection of Microbiological culture plates using Sodium hypochlorite. Chemical disinfection using Sodium hypochlorite is simple, affordable and easy to operate compared to autoclaving, which requires the use of electricity and large quantity of water.

A limitation of the current study is the non-inclusion of the effects on Mycobacterium tuberculosis. A separate study would be planned to prove the concentration and duration required to kill Mycobacterium tuberculosis which is the toughest organism to disinfect.

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