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Antibacterial Activity of Chlorine Disinfectant against Potential Biofilms Forming Bacteria

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Abstract: ***Chlorination of bacteria by active chlorine compounds with the aim of killing them occurs both in a variety of disinfection processes. The research was carried out to determine the antibacterial activity of chlorine disinfectant against potential biofilms forming bacteria. The samples were collected from Oluyole Tap, Mark Mercy Tap and Mark Mercy Sink. Standard analytical method was used to determine the parameters. The result showed that the bacterial contaminant was Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus cereus. The result also showed that Pseudomonasaeruginosa from Mark Mercy Tap had the highest zone of inhibition 34.0mm at 25% concentration of chlorine disinfectant. At 75%, concentration Pseudomonasaeruginosa had 20.0mm zone of inhibition, at 50% concentration 13.0mm zone of inhibition and at 100% concentration 11.0mm zone of inhibition. In Staphylococcusaureus from Oluyole Tap, the highest inhibition concentration is 28.0mm zone of inhibition at 50% concentration followed by 24.0mm zone of inhibition at 100% concentration and 22.0mm zone of inhibition at 75% concentration. While Bacilluscereus form Mark Mercy sink had the highest inhibition concentration of 22.0mm zone of inhibition at 75% concentration, followed by 18.0mm zone of inhibition at 50% concentration and 14.0mm zone of inhibition at 100% concentration while there is no inhibition at 25%. Antimicrobial activity for Staphylococcusaureus, Pseudomonasaeruginosa and Bacilluscereus was done by 50% of chlorine which was found to be effective in all organisms. It was concluded that the disinfectant solution are effective for 27 to 37 days if stored in screw cap bottles, they have the potential to be used in the dental setting as a surface disinfectant and a sterilant for semi-critical heat sensitive instruments.***

Keywords: Antibacterial, Biofilms, Chlorine, bacteria, concentration.

1. Introduction

Chlorine is a chemical element and a strong oxidant agent among the element with highest electron affinity (Mohammed *et al.*, 2014). Elemental chlorine is commercially produced from brine by electrolysis, predominantly in the chlor-alkali process. It is used in the manufacture of a wide range of consumer products, about two-thirds of them organic chemicals such as polyvinyl chloride (PVC), many intermediates for the production of plastics and other end products which do not contain the elements. As a common disinfectant, elemental chlorine and chlorine-generating compounds are used more directly in swimming pools to keep them sanitary (Taneja *et al.*, 2011). Chlorinated compounds are often used in dental clinics and laboratory environment due to their broad spectrum of antimicrobial activity, low toxicity, low cost and efficacy in biofilms (Merritt *et al.*, 2010). Antiseptics and disinfectants are used extensively in hospitals and other health care settings for a variety of tropical and hard surface applications. In particular, they are an essential part of infections control practices and aid in the prevention of nosocomial infections (Rutala, 2015; Larson, 2006). Bacterial biofilms are responsible for industrial biofouling, microbial regrowth in distribution systems, persistent infection (Fux *et al.*, 2005) and many other expensive and life-threatening problems. Therefore, the control of biofilms is now understood to be crucial. However, there are still few effective control strategies, and they are poorly understood in many contexts. Many antimicrobial agents that are effective against planktonic cells turn out to be ineffective against the same bacteria growing in a biofilm state (Stewar*et al.*, 2010). Biofilm cells exhibit susceptibilities to certain antimicrobial concentration control strategies (Walsh, 2013). Chlorination of bacteria by active chlorine compounds with the aim of killing them occurs both in a variety of disinfection processes it will be of significance in the health sector as it will reduce the effect and spread of bacteria on man. It will provide insightful information on individuals in order to educate them on the properties of chorine disinfectants.

2. Material and method

This study was carried out at two different laboratories (mark mercy hospital via the laboratory and Oluyole laboratory) from two sites (sink pipe and tap water pipe). The chlorine disinfectant was purchased from a local market in Ibadan. The samples used for this study was taken with the aid of swab stick from the two locations in the laboratories. It was then streaked in the nutrient agarin petri dish inoculation on media. Also, the samples were inoculated in Mannitol salt agar medium, MacConkey agar medium and blood agar. These was incubated for 24hours at room temperature. The microorganism was identified using gram staining method and biochemical characterization (using indole, oxidase, catalase and gelatinase test) (Scott, 2011).

Gram staining

A smear of each of the bacterial isolates was made on clean grease free slide and heat-fixed using flame. Crystal violet stains (0.3% w/v) was added and allowed to stand for one minute. The stain was washed off with distilled water iodine (0.4% w/v), a mordant was added and allowed to stand for one minute before being rinsed off with distilled water. Ethanol (95% w/v), a decolouriser was added and allowed to stand for 30 seconds before being rinsed off with distilled water and then counter stained with the secondary stain, safranin (0.4% w/v) and allowed to stand for one minute. This was washed off with distilled water and dried. The stained smear was observed under the microscope using oil immersion lens magnification (×100). Taneja *et al.*, 2011

Indole Test

The test organisms were inoculated into a broth that contained tryptophan and incubated at 37°C for 48hours. Then 2ml of the broth suspension was transferred to another test-tube under aseptic conditions. About 0.5ml of Kovacs reagent was added to the broth. The mixture was shaken properly to ensure a thorough mixing and then observed for colour reaction. A positive result was indicated by a pink coloured ring round the interface between the broth suspension and alcohol reagent which rose to the surface (Stewart *et al.*, 2010).

Antimicrobial activity

Sensitivity disc diffusion method was employed for checking the antimicrobial activity of the disinfectant samples. All the isolates were cultivated for 24hours for sensitivity test. The Mueller Hinton Agar was prepared and autoclaved at 121ºC for 15 minutes. The plates were swabbed with respective organisms and marked according to the organism. Sterile disc was placed and different concentration levels (25%, 50%, 75% and 100%) of the chlorine disinfectants was poured into each disc. After the disc is placed, the plate was incubated at 37ºc for 24hours. A zone of inhibition indicate that the disinfectant was effective, measured and recorded (Stewar *et al.*, 2010).

Statistical analysis

Data was subjected to analysis using SPSS2017 version. Results were presented as mean± standard deviations followed by one-way analysis of variance (ANOVA) was used for comparison of the mean.

3. Results and Discussion

Table 1: Biochemical Characterization of Bacterial Isolates

Isolate codes	Grams reaction	Shape	Motility	catalase	Oxidase	Endospore formation	Capsule	Growth in KCN	Nitrate reduction	Gelatin Hydrolysis	Citrate	Methyl red	Voge Proskaeur	Coagulase	Indole	Glucose	Fructose	Galactose	Lactose	Sucrose	Maltose	Xylose	Ribose	Raffinose	Arabinose	Probable bacteria
TO	-	R	+	+	-	-	-	-	+	+	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	Pseudomonas aeruginosa
TM	+	cc	-	+	-	-	-	-	+	-	+	-	-	+	-	+	+	+	+	+	+	-	+	-	+	Staphylococus aureus
SM	+	R	+	+	-	+	-	+	+	-	+	-	-	-	-	+	+	-	-	-	+	-	+	-	-	Bacillus cereus

Key: R= Rod, CC=cocci in clusters,
+ =positive test, - =negative test,
TO= Tap Oluyole, TM =Tap Mark mercy,
SM =Sink Mark merc

Table 2: Susceptibility of Bacterial Isolates

	Zone of inhibition (mm)		
Concentration of chlorine (%)	Staphylococcus aureus	Pseudomonas aeruginosa	Bacillus cereus
25	22.0a	34.0b	7.1a
50	28.0a	13.0c	18.0b
75	23.0a	20.0d	22.0c
100	24.0a	11.0c	14.0d

The biochemical test on the isolates from the two sites (water tap pipe and sink pipe) confirmed the presence of the bacterial contaminants as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus cereus* (as shown in table 1) as obtained and identified from the two laboratories, this is in agreement with the findings of Mohammed*et al.*,2015who discovered the presence of *B.cereus* in a washing base sink due to day to day activities in the laboratory and some species of bacteria were found in the tap due to the constant running of water. The study also collaborated with the finding of Walsh et al., 1997 who stated that living biological contaminants can be transmitted through the air and can be found on wet surface known as biofilms. Also from table 2, at different concentration levels (25%, 50%, 75%, 100%) of chlorine disinfectant, there was no significant difference ($p>0.05$) in the inhibitory effect of chlorine disinfectant at the different concentration levels on *S.aureus* with the value (22.0a, 28.0a, 23.0a, 24.0a). for *B.cereus*, there was a significant difference in the inhibition values of chlorine disinfectant at the different concentration levels. There was no significant difference ($p>0.05$) in the inhibitory effect of chlorine disinfectant at the concentration levels (50%×100%) on *P.aeruginosa* with the values of 13.0c and 11.0c. this agreed with the work of Thompson, (1999) who determine that disinfectant should be used to kill microorganisms but should not be applied on living tissues and commonly on an inanimate object such as floors and instrument. Also, from the Fux *et al.*, 2005 findings which also collaborated with this study that chlorine serve as a great control on potential biofilms forming bacterial and it is clearer that biofilms are complex mixture of different species rather than the model single species biostructures studied by the majority laboratories. Hence, the results showed that 50% of chlorine disinfectant will eliminate most of the contaminants including mycobacteria within 30seconds which suggested that lower concentration could be used for housekeeping and laboratories surfaces. Therefore, pre-cleaning of heavily contaminated instrument with the disinfectant is advantageous and proper care on laboratory area is also to be noted.

4. Conclusion and Recommendation

This study has revealed that chlorine is more advantageous because it is non-censive which make it suitable for its use as microbiocidal and sporicidal. It also serves as sterilant for semi-critical heat sensitive instruments. It is advisable that 50% of chlorine should be used.

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