Contributions of fluoride to the sensitivity of oral isolates to some anti-microbial agents

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Abstract

Objective: Plaque formation on teeth surfaces, gingivitis and caries has been associated with the presence and activities of oral flora; and several agents have been demonstrated to exhibit anti-plaque potentials. This Study is designed to evaluate the inhibitory potentials of some agents on fluoride-exposed and non-exposed oral isolates capable of causing dental plaque and infections.
Method: Agar dilution method was used to determine the Minimum Inhibitory Concentration (MIC) of triclosan, ampicillin and chlorhexidine against fluoride-exposed and non-exposed *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Bacillus subtilis, and Candida albicans.*
Results: All the agents showed remarkable antimicrobial activities against fluoride-exposed oral isolates. Our study also showed a 75% decrease ($p<0.05$) in the MICs of all the agents used against the test organisms after 4 weeks of exposure to 0.11 w/w% fluoride. In contrast, the MIC of the non-exposed isolates were significantly higher($p<0.05$) and did not change throughout this period. Triclosan and chlorhexidine showed, in addition to their antibacterial activities, outstanding antifungal action resulting in more than 93% increase in the susceptibility of *Candida albicans.* These observations may be attributable to the synergistic effect of fluoride on the antimicrobial activity of these agents.
Conclusion: It can be inferred from this study that regular daily use of fluoride containing dentifrice having antimicrobial agent such as triclosan or in conjunction with mouthwash containing chlorhexidine will aid in the prevention of plaque formation.

Key words: Fluoride, oral isolates, antimicrobial, Minimum Inhibitory Concentration.

Introduction

Many oral flora are pathogenic and have been associated with diseases such as dental caries and periodontal infections [1, 2]. In order to control these diseases, fluoride is usually included in dental products or may be added to drinking water supply[3, 4]. Fluoride is able to primarily interfere with the demand demineralization balance between enamel and the oral fluid[5]. In addition, anti-microbial agents on the other hand interfere with microbial colonization, growth and metabolism of dental plaque[6].
Fluoride has been implicated as a very potent anti-plaque agent, and a very economical way of administering it for the purpose of dental health is through the use of dentifrice paste. A daily use of this paste is bound to increase the fluoride level in the oral cavity which might influence the sensitivity of oral microbes to anti-microbial agents. The aim of this study is therefore to investigate the effect of fluoride on some oral isolates with a view to studying their sensitivities to some antimicrobial agents.

Materials and Methods

Antimicrobial Agents
Triclosan (Chemical and Allied Products Limited CAPL, Lagos), Ampicillin (Doyin Pharmaceutical Industries Limited, Lagos), Chlorhexidine (Chemical and Allied Products Limited CAPL, Lagos)

Microorganisms
All the test organisms were obtained as oral isolates. They included: *Staphylococcus aureus, Staphylococcus epidermidis, Candida albicans, Streptococcus pyogenes and Bacillus subtilis.*

Media
Nutrient Agar (Oxoid), Tryptone Soya Agar (Oxoid)

Isolation of Microorganisms
The isolated microorganisms were obtained from 40 selected volunteers who did not use fluoride toothpaste for oral hygiene. Their prevalence rates were Bacillus.subtilis, 60%, *Staphylococcus aureus,* 15%, *Staphylococcus epidermidis,* 20%, *Streptococcus pyogenes,* 25% and *Candida. albicans* 10%. All the isolates were identified using the method of Cowan & Steel, 1966 [6] and were maintained in tryptone soya agar slopes at 4 - 5°C pending use.

Preparation of Isolates for Use in Sensitivity Tests
Each isolate was divided into two sets. One set was grown in tryptone soya agar containing 0.85% w/v of sodium monofluorophosphate equivalent to 0.11% fluoride. The other grown without fluoride served as control. Fluoride concentration of 0.15% is allowed in dentifrice products[4]. The two sets were subsequently subcultured every three days for four weeks.

Determination of Antimicrobial Activity of Triclosan, Chlorhexidine and Ampicillin

Agar dilution method was also used in this study. Stock concentration containing 100 g/ml each of triclosan; ampicillin or chlorhexidine was prepared in sterile water. Working concentrations of 10-0.03 g/ml were further prepared in 20ml molten agar plate and mixed thoroughly. The mixture was allowed to set on the bench and then dried in an oven for 2 hours at 40°C to allow for easy absorption of the inoculum. The plates were then divided into five sectors corresponding to the 5 test organisms used.

For each test organism, 5 drops of overnight dilution containing $1x10^6$ cells per ml were placed on the appropriately labelled sector and time allowed for absorption. The plates were then incubated at 37°C for 24hours and observed for presence or absence of growth. The minimum inhibitory concentration (MIC) of the agent was taken as the least concentration of the drug allowing no growth for a particular isolate. The above procedure was repeated at 0, 7, 14, 21, 28 days respectively for the fluoride-exposed and non-fluoride-exposed isolates.

Results

The in-vitro effectiveness of the various anti-microbial agents on fluoride-exposed and non-exposed oral microbial flora is shown in Figures 1-4. The results showed that, the anti-microbial agents were more effective in inhibiting growths of fluoride-exposed isolates. With the exception of *Candida albicans,* all the organisms showed a progressively increasing susceptibility to ampicillin as exposure time to fluoride increased .Consequently, the MIC of ampicillin against the *Staphylococcus* species and *Bacillus subtilis* respectively decreased from 2.50 to 0.08 g/ml after 4-weeks of exposure to fluoride. *Streptococcus pyogenes* however, showed less susceptibility, giving a higher MIC of 1.25 g/ml within the same period. In contrast, the non-exposed isolates showed correspondingly lower susceptibility to test antimicrobial agent (Table 1). Also, when the percent decrease in MIC was evaluated, the exposed isolates decreased to a value of 96.8% each for *Staphylococcus* and *Bacillus subtilis* whilst *Streptococcus pyogenes* fell to 75.0% after 4 weeks of exposure (Figure 1).

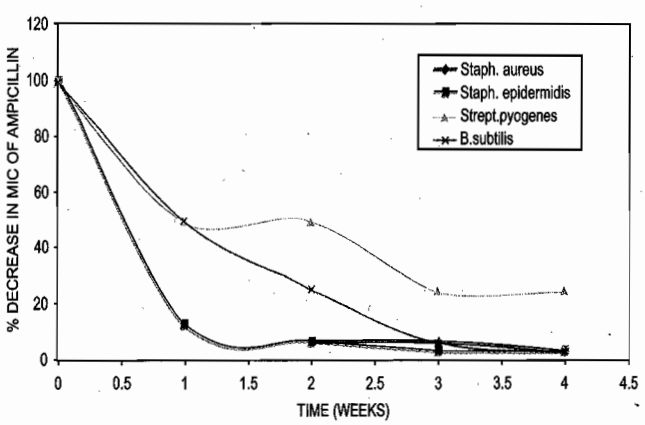

Figure 1. Effect of Fluoride-exposed Cells on MIC of Ampicillin

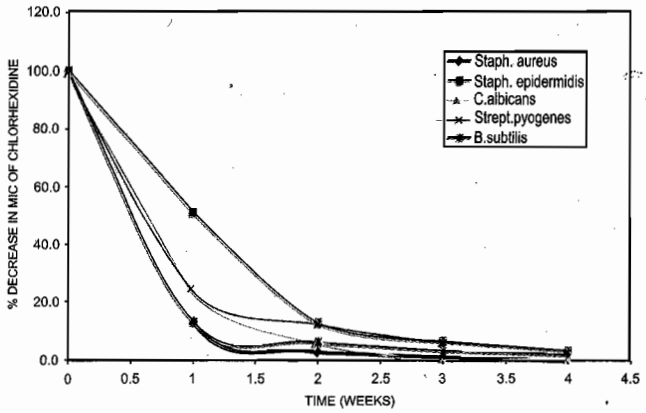

Figure 2. Effect of Fluoride-exposed Cells on MIC of Chlorhexidine

Characteristically, ampicillin, chlorhexidine and triclosan were all effective against the bacterial isolates but surprisingly, the rate of inhibition of growths of the organisms was particularly faster within the first week for ampicillin and chlorhexidine (Figures 1 & 2). For triclosan however, this period extended to two weeks (Figure 3) implying a slower rate of kill by the latter. Unfortunately, ampicillin was comparatively less effective on *Streptococcus Pyogenes* compared to other bacterial isolates (Figure 4). Triclosan and chlorhexidine in addition showed appreciable antifungal activity against *Candida albicans* (Tables 2 & 3). This study has equally revealed that

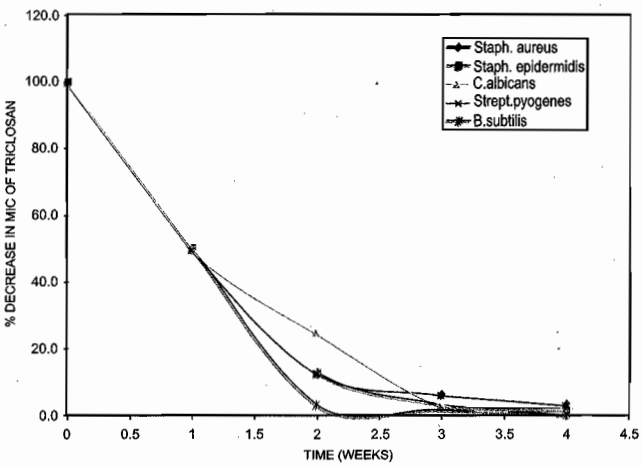

Figure 3. Effect of Fluoride-exposed Cells on MIC of Triclosan

chlorhexidine is very effective when compared to triclosan or ampicillin. This is because the minimum inhibitory concentration of chlorhexidine against all the isolates were lowest compared with the corresponding values of ampicillin or triclosan (Tables 1-3). With triclosan, the results gave an MIC range of 5.0-0.04 g/ml especially in the presence of fluoride (Table 3).

Discussion

Many workers have shown that some antimicrobial agents are effective in inhibiting the growth of oral microbes [6, 8, 9]; while others have established the importance of fluoride as a potent anti-plaque agent [10, 11]. Hence, assessing the

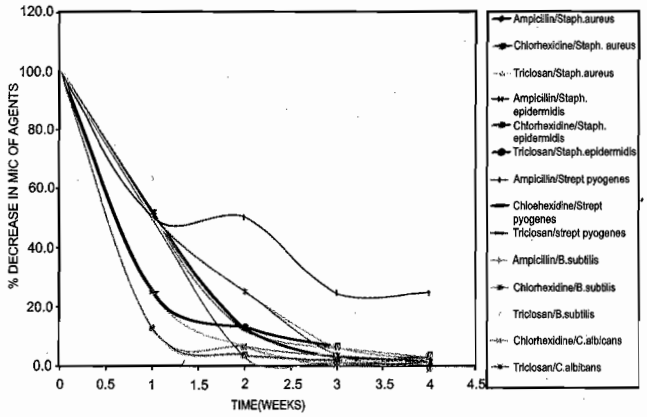

Figure 4. Susceptibility of Isolates to Ampicillin, Chlorhexidine and Triclosan

contributions of fluoride in enhancing the susceptibility of oral isolates to antimicrobial agents will further consolidate the strategies normally adopted to safeguard oral health. This study has indeed confirmed that fluoride-exposed isolates are more susceptible to such agents than non-exposed oral microbes .The significance of this observation is farfetched and reassuring, particularly in the use of fluoride to prevent plaque and dental caries [12, 13].

For each antimicrobial agent studied, the varying MIC values obtained against the test organisms may be attributed to the nature of the species [14], the mechanism of action of the agent, and the duration of exposure to fluoride. Consequently, the longer the duration of exposure to fluoride by the isolates, the higher the level of susceptibility observed, implying that fluorides thus interfere with the inhibition of oral isolates [15]. On the other hand, the lower activity of ampicillin particularly against *Streptococcus pyogenes* is indicative of some form of resistance, suggesting the use of more potent drugs for streptococcal infections [16]. Chlorhexidine, a bisbiguanide is

Table 1 : Minimum inhibitory concentrations of ampicillin on fluoride exposed (A) and non-exposed isolates (B)

Exposure Time (weeks)	Minimum Inhibitory Concentration (g/ml) against								
	Staphylococcus aureus		*Staphylococcus epidermidis*		*Candida albicans*	*Streptococcus pyogenes*		*Bacillus subtilis*	
	A	B	A	B	-	A	B	A	B
0	2.50	2.50	1.25	1.25	-	5.00	5.00	2.50	2.50
1	0.32	2.50	0.32	1.25	-	2.50	5.00	1.25	2.50
2	0.16	2.50	0.16	1.25	-	2.50	5.00	0.64	2.50
3	0.16	2.50	0.08	1.25	-	1.25	5.00	0.16	2.50
4	0.08	2.50	0.08	1.25	-	1.25	5.00	0.08	2.50

established to be very bactericidal against Gram positive and Gram negative microorganisms and has been reported to be very effective against plaque [17,18]. Similarly, triclosan monophosphate being a non-ionic broad spectrum antimicrobial agent that is broken down in -vivo to the active agent [19], has been reported to be effective at low concentration in the range of 0.01 3 g/ml [20]. Furthermore,

Table 2 : Minimum inhibitory concentrations of Chlorhexidine on fluoride exposed (A) and non-exposed isolates (B)

Exposure Time (weeks)	Minimum Inhibitory Concentration (g/ml) against									
	Staphylococcus aureus		*Staphylococcus epidermidis*		*Candida albicans*		*Streptococcus pyogenes*		*Bacillus subtilis*	
	A	B	A	B	A	B	A	B	A	B
0	2.50	2.50	1.25	1.25	10.0	10.0	5.00	5.00	2.50	2.50
1	0.32	2.50	0.64	1.25	2.50	10.0	1.25	5.00	0.32	2.50
2	0.08	2.50	0.16	1.25	1.25	10.0	0.64	5.00	0.16	2.50
3	0.04	2.50	0.08	1.25	1.25	10.0	0.32	5.00	0.08	2.50
4	0.02	2.50	0.04	1.25	0.64	10.0	0.16	5.00	0.04	2.50

Table 3 : Minimum inhibitory concentrations of Triclosan on fluoride exposed (A) and non-exposed isolates (B)

Exposure Time (weeks)	Minimum Inhibitory Concentration (ug/ml) against									
	Staphylococcus aureus		*Staphylococcus epidermidis*		*Candida albicans*		*Streptococcus pyogenes*		*Bacillus subtilis*	
	A	B	A	B	A	B	A	B	A	B
0	2.50	2.50	2.50	2.50	5.00	5.00	5.00	5.00	2.50	5.00
1	1.25	2.50	1.25	2.50	2.50	5.00	2.50	5.00	2.50	5.00
2	0.32	2.50	0.32	2.50	1.25	5.00	0.64	5.00	0.16	5.00
3	0.16	2.50	0.08	2.50	0.16	5.00	0.32	5.00	0.08	5.00
4	0.08	2.50	0.04	2.50	0.08	5.00	0.16	5.00	0.04	5.00

the broad spectrum activities of chlorhexidine and triclosan will be particularly advantageous in the control of mixed infections in the mouth [21, 22].

Generally, the clinical effectiveness of any antimicrobial agent depends on the ability to kill microbes with minimum toxicity to host cells [23], and any microorganism is considered sensitive if it is inhibited by considerably less than the average blood level attained with ordinary dosage. The low MIC values observed against the fluoride-exposed isolates can attest to this fact, even though the test microorganisms were fluoride-tolerant, and were capable of growing in the presence of low concentrations of fluoride ions. For this reason, the influence of fluoride on the susceptibility of the microbial cells to agents used in this study as shown by their low MICs should necessitate the enforcement of inclusion of adequate amount of fluoride in dentifrices.

Conclusion

This study has confirmed the relevance of certain antimicrobial agents such as ampicillin, chlorhexidine, and triclosan as potential plaque inhibitory agents. Notwithstanding the variability in the susceptibility of the oral isolates to these agents, the primary role of fluoride in suppressing the proliferation of oral flora thereby preventing plaque formation is clearly obvious. Therefore; inclusion of these agents in dentifrices will significantly improve the anti-plaque properties of these products.

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