IN VITRO EVALUATION OF THE ANTIMICROBIAL POTENCY OF SOME MOUTHWASHES IN ONDO STATE, NIGERIA

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ABSTRACT

The search for potent antimicrobial agent is unending. The use of mouthwashes to improve oral hygiene is being favoured for its presumed antimicrobial activity. This study therefore evaluated the antibacterial activity of common mouthwashes against relevant clinical isolates. Five mouthwash samples (coded; LIS; COL; TOT; FLU; BRE) bought from major super markets in Ondo State, Nigeria, were examined for antimicrobial activity using agar well diffusion method. The kinetic of activity and MIC determination of the mouthwashes were done by standard methods. All the mouthwashes were registered with the appropriate regulating agent. Three of the mouthwashes were alcohol based. The best activity was against Streptococcus mutans by LIS (19mm). TOT recorded poor activity against all isolates. COL and FLU demonstrated antifungal activity against Candida albicans. LIS has the lowest MIC of 1:8 against Streptococcus mutans. BRE and FLU were able to kill all the Staphylococcus aureus cells by the 12th hour. The mouthwashes exhibited varying degree of antimicrobial activity. Alcohol based mouthwashes demonstrated better antimicrobial activity. It is therefore confirmed in vitro, the beneficial activity of using mouthwashes to improve oral hygiene.

KEYWORDS: Mouthwashes; Antimicrobial; Ondo State; Minimum Inhibitory Concentration; Killing rate.

INTRODUCTION

Development of dental diseases is often as a result of colonization of teeth by cariogenic bacteria which produce acids as by-products of carbohydrate metabolism.[1] Dental caries and periodontal diseases are significant health problems of human that affect 60-90% of children.
and 10-15% of adults in industrialized countries. However, their occurrence has been reported to be irrespective of socio-economic class of individuals affected.\cite{2,3} It is important to equally mention that a key factor in both caries and periodontal disease as observed by Malic et al.,\cite{4} is the oral microflora and the biofilms produced by these organisms which are at the centre of disease pathogenesis. In controlling biofilm, mechanical debridement and use of adjunctive antimicrobials are important and will equally help in preventing plaque-mediated diseases. The two commonly implicated microbes in oral diseases are Streptococcus mutans and Candida albicans. Individuals with heavy colonization of cariogenic bacteria are considered to be at high risk for dental caries making their eradication an important step in dental treatment.\cite{5} To prevent oral diseases is much easier and cheaper than its treatment. Mouthwashes are primarily used as an aid to breath freshness and cleaner to the mouth, however, some few products in circulation claim to have some antiseptic properties.\cite{6} A broader definition by Shree et al.,\cite{7} describe mouthwash as non-sterile aqueous solution used mostly for its deodorant, refreshing or antiseptic effects and also used in rinsing and reducing oral bacteria, remove food particles, temporarily reduce bad breath and provide a pleasant taste. The active ingredients in mouthwashes may be an antibacterial (to reduce bacteria flora around lesions) antihistamine (for local anesthetic effect) antifungal (to stop any fungal growth) and a steroid (to reduce inflammation). It has been reported that Chlorhexidine gluconate is currently the most effective among the antimicrobials often incorporated into mouthwashes to reduce plaques and gingivitis.\cite{8} Bagis et al.,\cite{9} and Hooper et al.,\cite{10} reported that mouthwashes that contain chlorhexidine are associated with mucositis, altered taste, burning sensation, staining of the dental tissues which has resulted into the chemical analysis of their major components. Some mouthwashes contain alcohol which acts as a solvent for other ingredients. Though alcohols at concentrations of 10-12% serves as a preservative and antiseptic, however its addition in mouthwashes is considered a health hazard by many consumers and researchers.\cite{11} Little has been done in the area of antimicrobial assessment of mouthwashes especially in Nigeria. This study was therefore designed to determine the antimicrobial properties of 5 commonly available mouthwashes against some clinical (oral) isolates. The study will also provide opportunity for Dentist in prescribing mouthwashes that are most appropriate and at minimal concentration with fewer side effects.
MATERIALS AND METHODS

Sources of mouthwashes used in the study

Five mouthwashes (alcohol and non-alcohol based) were purchased in popular supermarkets in Akure and Ikare towns in Ondo State of Nigeria. The mouthwashes were coded for this study. The physical properties of the samples were documented.

Sources of microorganisms used in the study

The test organisms were clinical isolates from dental caries and periodontal infections. They include Escherichia coli, Staphylococcus aureus, Streptococcus mutans, and Candida albicans. All the organisms were reactivated through sub-culturing on freshly prepared Blood agar and Nutrient agar prior to use for the study. The organisms were re-identified using standard biochemical and staining methods described by Aneja[12] and Benson.[13]

Preparation of inoculums

A modified method of McFarland[14] was used. Uniform suspensions of overnight pure cultures of the test organisms were made in peptone water. The turbidity of the suspensions was adjusted until they matched 0.5 McFarland turbidity standard which was prepared by adding 0.05ml of BaCl₂ (1% w/v) to 9.95ml of H₂SO₄ (1% V/V). This produced a suspension of approximately 1.0 x10⁶ cells/ml. The optical densities of the 0.5 McFarland turbidity standard and the organism suspensions were compared using Unico 2100 Spectrophotometer at 520nm wavelength and adjustments were made by either further diluting the organism suspension or adding more suspension from the stock.

Screening for antimicrobial activity

Agar well diffusion method was used. Pure isolates were transferred using sterile loop into a tube containing normal saline (0.85% NaCl) and density of each microbial suspension was adjusted equal to that of 10⁶ cfu/ml (0.5 McFarland standard) and used as inoculum. Wells of 6mm in diameter were bored into the seeded Mueller-Hinton agar plate where 100μl volume of different mouthwashes was dispensed. The plates were allowed to stand for ten minutes for diffusion of the mouthwashes to take place and incubated at 37⁰C for 24h. The antimicrobial activity, indicated by an inhibition zone surrounding the well containing the mouthwashes was recorded. The mean values of the diameter of inhibition zones were found and taken as inhibition zone for each mouthwash at neat concentration.
Determination of minimum inhibitory concentration (MIC) of the mouthwashes

Agar dilution method as described by Musa et al., [15] was used. The mouthwashes were serially diluted from neat (1:0) to a 1:8 concentration with molten Muller-Hinton agar and poured aseptically to sterile Petri dishes and allowed to set. Sterile paper discs (Whatman No 1, 5mm in diameter) were firmly placed on the agar surface. Twenty microlitres (20µl) of the standardized bacteria cultures were then placed on the paper disc and incubated at 37°C for 24hrs. The lowest concentration that inhibited growth was taken as the MIC.

Kinetic of bactericidal activity of the mouthwashes

Modified method of Olonitola et al., [16] was used. The MIC of the mouthwashes were prepared with sterile normal saline and incubated with 20µl of 1.5 x 10^8 cfu/ml of S. aureus that had earlier exhibited sensitivity to the mouthwashes. At different time intervals (0, 2, 4, 6, 8, 10 and 12h), 1ml of the reaction mixtures were withdrawn and decimal dilutions were prepared with sterile normal saline. From the dilution, 0.02ml was placed on 5 different spots of a freshly prepared plate count agar which was incubated at 37°C. The average of the five plate counts were recorded to the nearest whole number.

RESULT

Table 1: Physical assessment of the mouthwash samples

<table>
<thead>
<tr>
<th>Mouthwash Types</th>
<th>Expiry Dates</th>
<th>Regd. Number</th>
<th>Colour</th>
<th>Alcohol based</th>
<th>Non-Alcohol based</th>
</tr>
</thead>
<tbody>
<tr>
<td>Listerine (LIS)</td>
<td>Nov., 2015</td>
<td>3574660603835</td>
<td>Yellow</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Colgate (COL)</td>
<td>Sept., 2015</td>
<td>7891024130544</td>
<td>Green</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Brett (BRE)</td>
<td>Feb., 2015</td>
<td>04-0367</td>
<td>Yellow</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Fluorodine (FLU)</td>
<td>Dec., 2015</td>
<td>5060072080480</td>
<td>Blue</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Total Care (TOT)</td>
<td>Oct., 2015</td>
<td>5020535002565</td>
<td>Red</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 2: Susceptibility pattern of the mouthwash samples

<table>
<thead>
<tr>
<th>Organism/Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouthwashes</td>
</tr>
<tr>
<td>LIS</td>
</tr>
<tr>
<td>COL</td>
</tr>
<tr>
<td>TOT</td>
</tr>
<tr>
<td>FLU</td>
</tr>
<tr>
<td>BRE</td>
</tr>
</tbody>
</table>
Table 3: The lowest mouthwash concentrations that inhibited the visible growth of tested organisms after overnight incubation (MIC).

<table>
<thead>
<tr>
<th>Organisms</th>
<th>LIS</th>
<th>COL</th>
<th>TOT</th>
<th>FLU</th>
<th>BRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>1:4</td>
<td>1:4</td>
<td>1:0</td>
<td>1:4</td>
<td>1:4</td>
</tr>
<tr>
<td>S. aureus</td>
<td>1:4</td>
<td>1:4</td>
<td>1:0</td>
<td>1:4</td>
<td>1:2</td>
</tr>
<tr>
<td>Str. mutans</td>
<td>1:8</td>
<td>1:4</td>
<td>NI</td>
<td>1:4</td>
<td>1:4</td>
</tr>
<tr>
<td>C. albicans</td>
<td>NI</td>
<td>1:0</td>
<td>NI</td>
<td>1:0</td>
<td>1:0</td>
</tr>
</tbody>
</table>

Key: NI - No inhibition

Figure 1a. Rate of microbial killing by LIS on Staphylococcus aureus

Figure 1b. Rate of microbial killing by COL on Staphylococcus aureus
Figure 1c. Rate of microbial killing by BRE on Staphylococcus aureus

Figure 1d. Rate of microbial killing by FLU on Staphylococcus aureus

Figure 1e. Rate of microbial killing by TOT on Staphylococcus aureus
DISCUSSION
The results of antimicrobial potentials of the five mouthwashes varied widely. Of the mouthwashes tested at full strength, FLU was the most active, closely followed by COL and BRE. Incidentally, the 3 most active mouthwashes were alcohol based. Apart from TOT, all other mouthwashes gave better activity against Staphylococcus aureus and E. coli thereby making their activity broad spectrum as against the finding of Anyanwu,\cite{17} who reported that alcohol containing mouthwashes were more active against Gram-ve bacteria while non-alcohol based were more active agent Gram+ve. However bacteria in their biofilm state have been reported to show less susceptibility to mouthwashes and thus pose a serious challenge to dental hygiene. Therefore keeping oral hygiene, which is expected to help in avoiding the biofilms formation is recommended to ensure maintaining susceptibility of oral bacteria to mouthwash\cite{18}

No activities were discovered against Candida albicans by LIS and TOT while others (COL, FLU and BRE) showed very little activity although Almekhlafi et al.,\cite{19} reported that mouthwashes containing Yemeni myrrh as a single active constituent had good antimicrobial activity against S. aureus and C. albican. Parker\cite{20}, explained that the difference in activity may be due to the active product concentration and its interaction with other constituents in addition to differences in their formulations.

However, since alcohol is known as a drying agent, releasing more malodorous volatile sulphur compounds, alcohol based mouthwashes can therefore induce halitoses among individual users or worsened halitosis in those who already have it.\cite{21} Aneja et al.,\cite{22} reported that the mean average inhibition zone of one mouthwash may not be directly compared with that of another mouthwash because they may be constituted of different active ingredients that may diffuse at different rates. Systemic pathologies that are linked to transient bacteraemia that is always generated during dental manipulations and the need to prevent oral infections, such as caries, candidiasis and especially periodontitis have increased the use of mouthwashes globally. By this standard, a good mouthwash should have good antibacterial activity.\cite{23,24} Malic et al.,\cite{4} suggested incorporation of natural compounds to enhance their antimicrobial effects against common oral pathogens and to reduce reported problems of several-over-the-counter mouthwashes, (like enamel staining, burning sensation, alterations in taste) and presence of an alcohol component. Chlorhexidine has been reported to be the most potent antimicrobial agent in most mouthwashes.\cite{25,26} Essential oil-based
mouthwashes too with or without alcohol have been reported to have good antibacterial activity when used undiluted \cite{23}. The MIC of most of the mouthwashes was at 1:4 and this is in agreement with the study of Anyawu et al. \cite{17} This indicated the level of dilutions that can be made of the mouthwashes without losing their potency. The rate of killing demonstrated by the mouthwashes showed that at the 12th hour of incubation, COL and FLU were able to bring viable bacteria count to zero, giving dental care giver a good guide as to prescription intervals especially where S. aureus is the incriminated pathogen. Oluremi,\cite{27} agreed that anticaries activity is optimally achieved by using appropriate mouthwash combination and at the right dilution as this prevents both the initiation and progress of dental caries.

CONCLUSION
The varying antimicrobial activities demonstrated by the mouthwashes are indication that not all mouthwashes are suitable for the prevention and control of dental caries as against the belief that all mouthwashes are potent antimicrobials.

REFERENCES


