ABSTRACT

Background: The use of antimicrobial agents was advocated for a number of years using different compounds that delivered through mouth rinses to control intra- and extra-oral disease in immunocompromised patients. The purpose of this research is to find out and to compare between the antibacterial properties of 0.2% chlorhexidine digluconate (CHX)  on oral β-hemolytic streptococci and Staphylococcus aureus isolated from patient with renal failure.

Materials and methods: β-hemolytic streptococci and Staphylococcus aureus were isolated stimulated saliva samples collected from patients receiving steroids therapy. These bacteria were purified and diagnosed according to morphological characteristic, biochemical and antibiotic susceptibility tests.

Results: Agar diffusion technique demonstrated that chlorexidine digluconate inhibited the growth of both types of isolates, but the antibacterial effect against Staphylococcus aureus was less than that against β-hemolytic streptococci.

Conclusion: The use of CHX 0.2% as a mouth wash to remove those pathogens from the oral cavity to inhibit their infections in immunocompromised patients is highly indicated.

Key words: Staphylococcus aureus, β-hemolytic streptococci, chlorhexidine, immunocompromised. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):166-169).

INTRODUCTION

The oral cavity is the most complex and the most accessible microbial ecosystem of the human body where the teeth, gingivae (gums), tongue, throat and buccal mucosa (cheeks) all provide different surfaces for microbial colonization (1).

The organisms present in the oral cavity are a mixture of commensals and pathogens, as many commensal bacteria can, under certain conditions be associated with human disease like in subjects whose immune systems are not working optimally, i.e. immunocompromised, are especially susceptible to infections by microbes that are commensal in healthy individuals. For these reasons, commensals are nowadays often referred to as opportunistic pathogens (2).

Staphylococci are found in the saliva of approximately 30% individuals, but they are considered transients rather than components of the resident oral microbiota and can play a significant role in oral and respiratory tract infections of a compromised host (3). The staphylococci are gram-positive spherical cells, usually arranged in grapelike irregular clusters. They grow readily on many types of media and are active metabolically, fermenting carbohydrates and producing pigments that vary from white to deep yellow. Some are members of the normal flora of the skin and mucous membranes of humans; others cause suppuration, abscess formation, a variety of pyogenic infections, and even fatal septicemia (4).

The pathogenic staphylococci often hemolyze blood, coagulate plasma, and produce a variety of extracellular enzymes and toxins. Clumping factor, fibronectin-binding protein, and collagen-binding protein bind specifically to fibrinogen, fibronectin, and collagen, respectively, and are instrumental in adhesion to tissues and foreign bodies covered with the appropriate matrix protein. Protein A binds to the Fc portion of immunoglobulins (IgG). It is assumed that “false” binding of immunoglobulins by protein A prevents “correct” binding of opsonizing antibodies, thus hindering phagocytosis. Staphylococci rapidly develop resistance to many antimicrobial agents and present difficult therapeutic problems (5).

Staphylococcus aureus (S. aureus) infection can result from direct contamination of a wound, eg, postoperative staphylococcal wound infection or infection following trauma like chronic osteomyelitis subsequent to an open fracture, meningitis following skull fracture. If S. aureus disseminates and bacteremia ensues, endocarditis, acute hematogenous osteomyelitis, meningitis, or pulmonary infection can result. The clinical presentations resemble those seen with other bloodstream infections. Secondary localization within an organ or system is accompanied by the symptoms and signs of organ dysfunction and intense focal suppuration (6). Contact spread of staphylococcal infections has assumed added importance in hospitals, where a large proportion of the staff and patients carry antibiotic-resistant staphylococci in the nose or on the skin; in hospitals, the areas at highest risk for severe staphylococcal infections are the newborn
nursery, intensive care units, operating rooms, and cancer chemotherapy wards (7). Massive introduction of "epidemic" pathogenic \textit{S. aureus} into these areas may lead to serious clinical disease. Personnel with active \textit{S. aureus} lesions and carriers may have to be excluded from these areas. In such individuals, the application of topical antiseptics may diminish shedding of dangerous organisms (8).

Streptococci are Gram-positive, non-motile, catalase-negative, facultatively anaerobic cocci that occur in chains or pairs. They are classified based on their hemolytic capacity (α-, β-, γ-hemolysis) and the antigenicity of a carbohydrate occurring in their cell walls (Lancefield antigen) (9). β-hemolytic group \textit{A} streptococci (\textit{Streptococcus pyogenes}) cause infections of the upper respiratory tract and invasive infections of the skin and subcutaneous connective tissue. Depending on the status of the immune defenses and the genetic disposition, this may lead to scarlet fever and severe infections such as necrotizing fasciitis, sepsis, or septic shock. Sequelae such as acute rheumatic fever and glomerulonephritis have an autoimmune pathogenesis (10).

Streptococcal diseases can be classified as either acute, invasive infections or sequelae to them. Invasive infections occur as the pathogens enter through traumas or microtraumas in the skin or mucosa and cause invasive local or generalized infections. The rare cases of severe septic infection and necrotizing fasciitis occur in persons with a high-risk MHC II allotype (11).

Although humans can be asymptomatic nasopharyngeal or perineal carriers of \textit{Streptococcus pyogenes}, the organism should be considered significant if it is detected by culture or other means. The ultimate source of group \textit{A} streptococci is a person harboring these organisms. The individual may have a clinical or subclinical infection or may be a carrier distributing streptococci directly to other persons \textit{via} droplets from the respiratory tract or skin. The nasal discharges of a person harboring \textit{Streptococcus pyogenes} are the most dangerous source for spread of these organisms (12). Resistance against streptococcal diseases is \textit{M} type-specific. Thus, a host who has recovered from infection by one group \textit{A} streptococcal \textit{M} type is relatively immune to reinfection by the same type but fully susceptible to infection by another \textit{M} type. Anti-\textit{M} type-specific antibodies can be demonstrated in a test that exploits the fact that streptococci are rapidly killed after phagocytosis. \textit{M} protein interferes with phagocytosis, but in the presence of type-specific antibody to \textit{M} protein, streptococci are killed by human leukocytes (13). Antibody to streptolysin \textit{O} develops following infection; it blocks hemolysis by streptolysin \textit{O} but does not indicate immunity. High titers (>250 units) indicate recent or repeated infections and are found more often in rheumatic individuals than in those with uncomplicated streptococcal infections (14).

Compromised hosts are people with one or more defects in their body's natural defenses against microbial invaders. Consequently immunocompromised people can become infected with any pathogen able to infect immunocompetent individuals they are much more liable to suffer from severe and life-threatening infections (15). Modern medicine has effective methods for treating many types of cancers, is improving organ transplantation techniques and has developed technology that enables people with otherwise fatal diseases to lead prolonged and productive lives but a consequence of these achievements, however, is an increasing number of compromised people prone to infection (3).

Compromise can take a variety of forms, falling into two main groups:

\begin{itemize}
  \item Defects, accidental or intentional, in the body's innate defense mechanisms
  \item Deficiencies in the adaptive immune response.
\end{itemize}

These disorders of the immune system can be further sub-classified as primary or secondary:

\begin{itemize}
  \item Primary immunodeficiency is inherited or occurs by exposure in utero to environmental factors or by other unknown mechanisms. It is rare, and varies in severity depending upon the type of defect.
  \item Secondary or acquired immunodeficiency is due to an underlying disease state or occurs as a result of treatment for a disease (16).
\end{itemize}

Immunodeficiency results in:

\begin{itemize}
  \item drastic effects on the structure of the lymphoid organs.
  \item gross reductions in the synthesis of complement components
  \item sluggish chemotactic responses of phagocytes
  \item lowered concentrations of secretory and mucosal IgA
  \item reduced affinity of IgG
  \item in particular, a serious deficit in T-cell number leading to inadequate cell-mediated responses.
\end{itemize}

The use of antimicrobial agents to control plaque and oral disease has been advocated for a number of years. Different compounds have been delivered through mouth rinses or tooth pastes or by topical application. Some chemical agents have proven to be helpful against plaque accumulation.
and thereby to some extent also against caries (17). Chlorhexidine (CHX) is a broad-spectrum antimicrobial agent whose effects are more potent on gram-positive microorganisms than on gram-negative microorganisms, and effective against aerobes, anaerobes and against organism associated with diseases of the oral cavity (18).

Chlorhexidine disrupts cell membrane and cell wall permeability of many Gram- positive and Gram-negative bacteria and interferes with the adherence of plaque-forming bacteria, thus reducing the rate of plaque accumulation; chlorhexidine can inhibit the adenosine triphosphatase (ATPase) which is an important enzyme that is linked to cytoplasmic membrane and thus can inhibit the process of returning potassium ions into cells in exchange for sodium and hydrogen ions, also inhibits metabolic enzymes such as phosphoenolpyruvate phosphotransferase (19).

MATERIALS AND METHODS

Stimulated saliva samples were collected under standard conditions to obtain 20 microbial samples from patients receiving steroid therapy aged 21-23 years were selected to participate in this study. Ten-fold serial dilutions were prepared using sterile normal saline. Two dilutions were selected and inoculated on blood Agar (B.A.) plates which are incubated anaerobically by using gas packs supplied in an anaerobic jar to isolate group A streptococci; and mannitol salt agar plates which are incubated aerobically for 24 hrs at 37°C for the isolation of S. aureus. Colonial morphology, cell morphology, biochemical activities and antibiotic susceptibility tests were manipulated to diagnose the isolated bacterial species.

A single colony from each plate was transferred to 10 ml sterile BHI-B and then incubated for 24 hrs aerobically at 37°C to activate the inoculums. Agar diffusion technique was applied to study the antimicrobial effects of CHX against the isolates spreaded on Muller Hinton Agar (MHA); wells of equal sizes and depths were prepared in the agar using Kork porer. Each well was filled with 50µl of 0.2% CHX. Inhibition zones diameters were measured using a scientific ruler; resistance of the isolates to CHX was indicated when there were no zones of inhibition.

RESULTS

On mannitol salt agar plates, smooth circular golden yellow colonies appeared indicating (Fig. 1-A) from which colonies were subjected to catalase production test (+ ve) and tube coagulase production test (+ ve).

On blood agar plates, group A streptococci colonies appeared as small, circular colonies surrounded by clear zones of hemolysis (Fig. 1-B); bacitracin susceptibility test performed (bacitracin sensitive) to identify group A streptococci (Streptococcus pyogenes)

All the isolates were gram positive (Figure 2). The motility of all types of microbial cells was examined under microscope by direct smear and without staining; the isolates were non-motile.

Diameters of inhibition zones for CHX were found to be indicator for the bacterial isolates sensitivity. Figure 3 illustrates the mean diameters of the inhibition zones in relation to CHX. Student’s t-test showed highly significant differences among diameters of inhibition zones produced by CHX in the inoculated MHA plates.
The comparison between the antibacterial effect of CHX in relation to *S. aureus* and *Streptococcus pyogenes* showed highly significant difference using T-test analysis (Table 1).

**Table 1: Student’s t-test for the comparison between the Effect of CHX on *S. aureus* and *Streptococcus pyogenes* (in vitro)**

<table>
<thead>
<tr>
<th></th>
<th>S.D.</th>
<th>t-test</th>
<th>P-value</th>
<th>Sig</th>
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<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>0.891</td>
<td>-40.867</td>
<td>P&lt;0.01</td>
<td>HS</td>
</tr>
<tr>
<td><em>S. pyogenes</em></td>
<td></td>
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HS: highly significant difference at level P<0.05.

From the results shown above, it is quite obvious that CHX had exerted antimicrobial action against *S. aureus* and *Streptococcus pyogenes* but was less effective against *S. aureus* than *Streptococcus pyogenes* which could be due to the hereditary contents or attraction ability or the permeability of the cell wall of the microorganisms.

**REFERENCES**