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Antibacterial efficacy of *Syzygium aromaticum* extracts on multi-drug resistant *Streptococcus mutans* isolated from dental plaque samples

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Abstract

Over the last few decades there has been a remarkable increase in the prevalence rate of dental caries.*Streptococcus mutans* has been implicated as major cariogenic bacteria because they produce high levels of lactic acid and extracellular polysaccharide. In the present study, 38 % of *S. mutans* was recovered from the dental plaque samples collected from patients. Antibiotic sensitivity tests revealed the emergence of Multi-Drug Resistance (MDR) with all the isolates being completely resistant to penicillin, amoxycillin and ampicillin. Also, a decrease in sensitivity to Bacitracin was observed. The isolates were sensitive to the antibiotics erythromycin, clindamycin, ciprofloxacin and azithromycin. Alternatively, *Syzygium aromaticum* (clove), a traditional household spice with medicinal importance was attempted for its efficacy against the MDR *S. mutans* isolates. It was observed that the *Syzygium aromaticum* extract had a preponderant efficacy at a concentration of 1600 µg/ml with the maximum zone of inhibition. It was concluded that *Syzygium aromaticum* extracts could be an important alternate therapeutic agent in the management of drug resistant *S. mutans*.

Key words: Multi drug resistance, *S. mutans*, *Syzygium aromaticum* Dental caries.

Introduction

Streptococcus mutans is the leading cause of dental caries (tooth decay) worldwide and is considered to be the most cariogenic of all of the oral *Streptococci*. In addition to being both acidogenic and aciduric, many of the *S. mutans* group are able to produce large quantities of intracellular and extracellular polysaccharide, and this is believed to be an important factor in their cariogenicity. Since the discovery of *S. mutans* as an etiological agent of dental caries, much attention was focused on this bacterium, as a target for the prevention of disease through the use of antimicrobial agents and vaccine preparation. Conventional drugs usually provide effective antibiotic therapy for bacterial infections. However, there is an increasing problem of antibiotic resistance and a continuing need for new solutions. In recent years, the isolates of *S. mutans* have begun to show considerable resistance to commonly used antibiotics, for treatment. Alternatively worldwide, hundreds of plants are used in traditional medicine as treatment for bacterial infections. Many plant extracts are being studied for their efficacy against the drug resistant isolates. Various plants such as *Humulus lupulus Piper betle* (Nalina et al *2007), Elettaria cardamomum* (Purshotam Kaushik et al. 2010), *Ficus carica* (Mi-Ran Jeong et al. 2009)*, Allium sativum* are reported to have preponderant antibacterial activity. However, studies on these herbal extracts in the management of dental caries are obscure. Such studies could provide a platform for the development of a novel formulation to combat the drug resistant *S. mutans*. Hence the present study was aimed to determine the pattern of drug resistance in *S. mutans* isolated from Bangalore region and to screen the efficacy of Syzygium *aromaticum* extracts against the drug resistant *S. mutans*.

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Materials and Methods

Isolation and Identification

Dental plaque samples were collected from patients visiting M. S. Ramaiah Dental College, Bangalore, India. A total of 100 dental plaque samples were obtained from the patient's molar teeth using a sterile tooth pick and was immediately transferred to the transport medium and was subjected to isolation and identification studies as described elsewhere (Igarashi et al. 2001). All the isolates were taken for their identification study using routine lab diagnostic tools such as hemolysis on blood agar plates, gram staining, and Phenol red test to identify them as *S. mutans*. A standard *S. mutans* strain MTCC 497 received from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India was also included as control. All the isolates along with the standard were then sub-cultured and maintained in Todd Hewitt medium.

Antibiotic sensitivity Tests

All the isolates identified as *S. mutans* were tested for their sensitivity pattern with the routinely used antibiotics by Kirby-Bauer disk diffusion technique (Kirby et al. 1966) on Mueller-Hinton agar (MHA) plates. The antibiotics tested were amoxycillin (10 µg), ampicillin (10 µg), azithromycin (30 µg), bacitracin (10 µg), ciprofloxacin (05 µg), clindamycin(02 µg), erythromycin (15 µg), penicillin (10 units), and streptomycin (10 µg). The tests were conducted in triplicates to determine the resistance pattern among the isolates.

Preparation of herbal extracts

Syzygium aromaticum (Clove) extract was prepared according to the method described earlier (Zubaidah et al. 2006). The fresh clove bulbs (10g) obtained from the local market of Bangalore was homogenized using a blender/grinder. The powder was then suspended in acetone and methanol. The suspended solutions were mixed well by means of a vortex mixer and stored overnight to allow the medicinal components of the clove powder to enter the solvent. The solutions were diluted to final concentrations of 800 mg/ml and 1600 mg/ml.

Antimicrobial Screening

The antibacterial activity of extract at various concentrations (20µl, 40µl and 80µl) was screened by well diffusion technique on sterile Muller Hinton Agar plates (Kirby et al. 1966). Antibacterial activity in terms of zones of inhibition (mm) was recorded after 24 hours of incubation. The antagonistic action of the extracts was tested against the test organism in triplicates.

Results and discussion

Among the 100 plaque samples collected, 38 % were confirmed to be harbouring *S. mutans* by routine lab diagnostic tools. All the 38 GAS isolates were identified as gram positive *cocci* in chains. The colonies grown on Mitis Salivarius Bacitracin agar plates tested positive for greenish hemolysis on blood agar plates, and positive for Sorbitol & Mannitol fermentation studies confirming the identification of *S. mutans*. The present study revealed a high rate (38/%) of *Streptococcus mutans* incidence in Bangalore urban population.

In a similar study conducted to compare the prevalence and pattern of caries in children of urban Bangalore and non-urban Chickaballapur within Karnataka state, India, the results showed caries prevalence of 66.3% in Bangalore city whereas in Chickballapur, the prevalence was 58.4% (Virjee et al. 1987). Whereas in a recent study, it was concluded that caries prevalence in preschool children of urban Bangalore was 27.5% (Prashanth et al. 2012) and showed a decreased trend of caries prevalence in urban Bangalore from 1987 to 2005. But the present study significantly showed that 38 % dental plaque samples harbouring *S. mutans,* the predominant oral microflora causing dental caries. In another study conducted among the school children of Urban Delhi, the prevalence of dental caries was found to be 52.3%. Although it can be seen that the prevalence of caries in urban Bangalore population is comparatively lower than Delhi, but it is alarming that the rate has significantly increased in Bangalore population too. Thus the current study emphasizes the need for regular surveillance programs to screen for *S. mutans* and its role in causing dental caries.

In order to understand the antibiotic susceptibility pattern among the clinical isolates of *S. mutans*, a battery of routinely used antibiotics were tested for its sensitivity. The isolates showed complete resistance to penicillin (zone sizes between 7 to 11mm) and ampicillin (zone sizes between 10 to 17mm). While 30% of the isolates showed intermediate sensitivity to Amoxycillin, 70% showed complete resistance (zone sizes between 7 to 17mm). The isolates showed decreasing sensitivity towards Bacitracin (zone sizes between 10 to 14 mm) (Fig.1). Ciprofloxacin, erythromycin, clindamycin and azithromycin proved to be the most effective antibiotics against *S. mutans* (zone sizes ≥ 36mm). The standard MTCC 497 was sensitive to all the antibiotics tested.

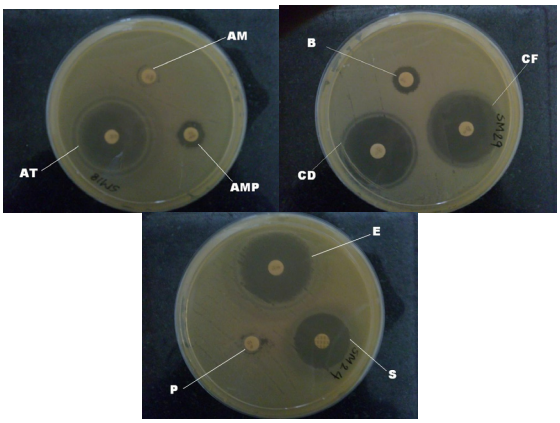

Figure 1: The isolates of *Streptococcus mutans* exhibiting multi drug resistance to the antibiotics Penicillin (P), Amoxycillin (AM) and Ampicillin (AMP) and decreasing sensitivity to Bacitracin (B). The isolates were sensitive to other antibiotics like Ciprofloxacin(CF), Clindamycin(CD), Erythromycin(E), Azithromycin (AT) and Streptomycin(S).

Viridans group S*treptococci* resistant to antibiotics have increasingly been reported over the past decade, while studies on antibiotic resistance of *Streptococcus mutans* group are few. *Streptococcus mutans,* being gram-positive cocci, is expected to be certainly susceptible to cell wall synthesis inhibiting antibiotics like penicillin, cephalosporin and protein synthesis inhibiting antibiotics like chloramphenicol and tetracycline. But recent studies indicate the emergence of penicillin resistance in *Streptococcus mutans*, which are generally considered uniformly susceptible to penicillin. In a recent study from the isolates obtained from a hospital, the *Streptococcus mutans* was found to be uniformly resistant to the β – lactam antibiotic, penicillin (Jesse Joel et al. 2011). In another recent study *S. mutans* isolates were multi drug resistant with resistance to amoxicillin, ceftriaxone, chloramphenicol, erythromycin, and tetracycline. Thus the present study which revealed the emergence of multi drug resistance in *Streptococcus mutans* in the urban Bangalore region has actually not only made general prophylaxis a very cumbersome affair but also has further intricate the antibiotic resistance pattern of this region.

Traditional antibacterial approaches are falling out of favor due to increased incidence of resistant organisms and virulence-targeted therapies are gaining increased interest. Natural products discovered from medicinal plants have provided numerous clinically used medicines and there is an abundance of evidence that naturally occurring compounds, especially from foods that we already consume, may have therapeutic potential in managing caries. Therefore, in view of contriving an alternate therapeutic agent against drug resistant isolates, *Syzygium aromaticum* extract was prepared and tested for its antibacterial efficacy. The antibiotic

sensitivity test was conducted using the clove extracts prepared in different solvents and at various concentrations on all the clinical isolates. The inhibition zone size were noted and tabulated. The maximum zone of inhibition in clove extracted with acetone was found to be 23 mm, whereas the maximum zone size with methanolic clove extract was found to be 22 mm (Fig. 2).

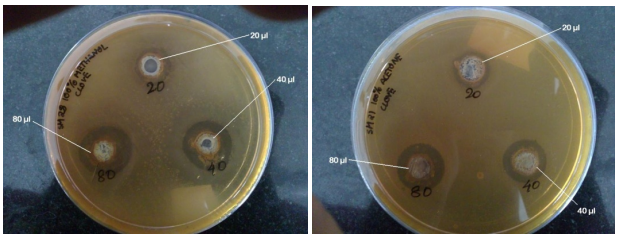

Figure 2: Antimicrobial effect of Methanol Clove Extract on the multi drug resistant isolates of *S. mutans*.

It was found that the acetone clove extract of concentration 1600µg/ml showed antimicrobial activity equivalent to that of streptomycin when tested against standard and clinical isolates. Methanol clove extract of concentration 1600µg/ml also showed antimicrobial activity equivalent to that of streptomycin when tested against standard and clinical isolates. Thus, at the higher concentration the extract was as effective as Streptomycin (Fig. 3). The extracts in fact were more effective than most commonly prescribed antibiotics such as amoxycillin, ampicillin, bacitracin and penicillin. This showed that the natural extract could be used as an alternative to the conventional antibiotics, to which the microorganisms develop resistance.

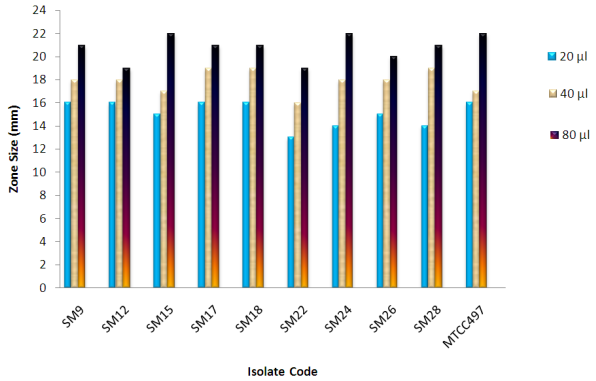

Figure 3: Antimicrobial effect of Methanol Clove extractat various concentrations on the multi drug resistant isolates of *S. mutans*.

In a similar study (Fani et al. 2007) have showed that garlic has an inhibitory activity against MDR *S.mutans*. But they have also found that intravenous administration of garlic extract causes minor side effects like nausea, diarrhea and vomotting. In another study the antimicrobial activities of the crude ethanolic extracts of *A. nilotica, C. zeylanicum and S. aromaticum* were screened against multidrug resistant (MDR) strains of *Escherichia coli, Klebsiella pneumoniae* and *Candida albicans* and were found to be effective. These results were in agreement with the present study, where *S. aromaticum* extracts were found to have anti microbial activity against the MDR *S. mutans* isolates.

Conclusion

The present study revealed the emergence of Multi-Drug Resistance in at least this region. Based upon the present study it is concluded that *S. aromaticum* extracts could be a possible alternate source to obtain new and effective herbal medicines to treat infections caused by multi-drug resistant strains of *S. mutans*. However, it is necessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties before being supplemented as a therapeutic agent. Therefore it is concluded that *S. aromaticum* extracts which exhibited excellent anti-bacterial activity cannot supplant the orthodox therapeutic agents for the dental caries, but can be used as an adjuvant to manage the drug resistance.

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