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ANTIBIOTIC SENSITIVITY PATTERN OF STREPTOCOCCUS AGAINST COMMERCIALLY AVAILABLE DRUGS & COMPARISON WITH EXTRACT OF *PUNICA GRANATUM.*

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ABSTRACT

Streptococcus is the leading cause of dental diseases worldwide and is considered to be the most cariogenic of all of the oral bacteria. The total 150 dental plaque sample were collected from Uttranchal dental college and hospital dehradun and different dental clinics of dehradun. Out of 320 isolates recovered prevalence of *Streptococcus sp.* was 50%. 10 antibiotics were used against the recovered isolates. 80% of the recovered isolates revealed the sensitive activity against amoxicillin (25 mcg), ampicillin (25 mcg), chloramphenicol (30 mcg), erythromycin (30 mcg), clindamycin (30 mcg), tetracycline (30 mcg), penicillin (100 mcg) and resistant activity against metronidazole (30 mcg), ciprofloxacin (25 mcg), and gentamycin (25 mcg). The antibacterial activity of *Punica granatum* against the recovered isolates was studied by using agar well diffusion method. Out of four different solvents used in the present study 90 % of the recovered isolates showed the maximum zone of inhibition against the methanolic extract of *Punica granatum* i.e. 25 mm.

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KEYWORDS

Punica granatum, Dental plaque, Dental caries, Antibacterial activity.

INTRODUCTION

The two most common types of dental disease, dental caries and periodontal disease, are plaque-related infections. A bio-film known as dental plaque, which provides ground for the formation and inhabitancy of pathogenic bacteria, is formidable in its contribution to various factors that lead to tooth decay, gingivitis, and periodontitis [1]. Dental caries is one of the most important problems in public health because of its ubiquitousness in civilized population. The prevalence of dental caries in industrialized countries like India is on the rise. As the treatment is very costly and requires a lot of manpower, the prevention at the primary level is the solution of the choice [2-3]. The disease is marked by a localized progressive demineralization of the hard tissues of the crown and root surfaces of the tooth. The demineralization is caused by acids produced by bacteria, particularly, *Streptococcus mutans* that ferment dietary carbohydrates [4]. *S. mutans* is gram-positive cocci, non-motile facultative anaerobic microorganism which can metabolize carbohydrates and is considered to be the principle etiological agent of dental caries [5-6]. The demineralization occurs within a bacteria-laden gelatinous material called dental plaque that adheres to the tooth surfaces and become colonized by bacteria [7]. Secondary infections are caused by *Lactobacillus* species, and yeasts such as *Candida albicans* [8-9]. In the present clinical scenario globally, there is a great interest in the use of antimicrobial agents for prevention and treatment of dental diseases due to the spread of antibiotic resistance [10]. The widespread concern about the increasing problem of antibiotic resistance has emphasized the need for rationalization of antibiotic use in the treatment of dental caries [11]. Inappropriate antibiotic prescribing and use have been identified as major factors in the emergence of antibiotic resistance in *S. mutans*. Consequently, modification and surveillance of prescribing attitudes have become crucial.

In the recent years, a shift from narrow-spectrum antibiotic prescriptions which included penicillin to broad-spectrum aminopenicillins which include amoxicillin by dental professionals has been reported and the increase of bacterial isolates resistant to the former antibiotics is blamed for such a shift in prescription practices [12]. The paper aims to evaluate the efficacy of current commercially available drugs in India against *Streptococcus sp.* which is primarily responsible for causing dental caries and for also comparing with the extracts of *Punica granatum.* Seeds of the pomegranate are used in medicinal purpose. It is rather an extraordinary fruit with a complete medicinal power contained within its seeds [13].

MATERIALS AND METHODS

In the present investigation, a total of 150 dental plaque samples were collected from Uttranchal dental college and hospital dehradun and different dental clinics of dehradun. Total recovered isolates were 320 out of which 160 Streptococcus sp. were isolated. So the prevalence of Streptococcus was 50%. The samples were collected ascetically in sterile 50 ml Oakridge tubes and inoculated in nutrient broth for 24 hrs at 37^0C. Inoculated samples were streaked on Brain heart infusion agar and other selective media. The isolates obtained were identified on the basis of colony morphology and biochemical reactions. *Streptococcus* (MTCC 497) was used as control strain. The inoculums of

Streptococcus sp. were adjusted according to 0.5 McFarland standard which was prepared by adding 0.05ml of barium chloride ($BaCl_2$) (1.17% w/v $BaCl_2.2H_2O$) to 9.95ml of 0.18M H_2SO_4 (1.0%w/v) with constant stirring. The inoculums of test strains was adjusted to 1.5 x 10^8 CFU/ml equal to that of the 0.5McFarland standard by adding sterile distilled water.

The antimicrobial sensitivity of the test strains to 10 antibacterial drugs was determined by Kirby-Bauer disc diffusion method [14-17]. 20 ml of Muller Hinton agar melted and cooled at 45^0C was poured into sterile petri plates and allowed to solidify completely. A lawn of test pathogen was prepared by evenly spreading 100µl inoculums (1.5 x 10^8 CFU/ml) with the help of a sterilized spreader onto the entire surface of agar plate. The plates were allowed to dry before applying antibiotic disc. The discs were firmly applied to the surface of agar plates within 15 minutes of inoculation. For the preparation of punica extract the seeds of ripened fruit were dried under shade and stored into fine powder using electric blender. 50g of dried powder sample was taken and extracted by soxhlet apparatus using distilled water, methanol, petroleum ether and ethanol separately. The solvents were removed under reduced pressure in a rotary evaporator until they become completely dry. 500µl of the extracts (500 mg/ml) was introduced into the wells. All the agar plates were incubated at 37^0C. If antimicrobial activity was present on the plates, it was indicated by an inhibition zone. The diameter of the inhibition zones were measured in millimeter at 24 hours using a scale. The experiments were conducted in triplicate for each test antibiotic/test pathogen. The diameter of inhibition zones was measured. An organism was interpreted as highly susceptible if the diameter of inhibition zone was more than 19 mm, intermediate if diameter was 15-18 mm and resistant if diameter was less than 13 mm.

RESULTS AND DISCUSSION

The antibacterial activity of 10 commercial drugs was assayed by Kirby-Bauer disc diffusion method and data on the diameter of inhibition zones produced by *Streptococcus* was compared with the data obtained with *Punica granatum* extract. 80% of the recovered isolates exhibited the sensitive activity against amoxicillin, ampicillin, chloramphenicol, erythromycin, penicillin, clindamycin, tetracycline, and resistant activity against metronidazole, ciprofloxacin and gentamycin. The antibacterial activity of *Punica granatum* against the recovered isolates was studied by using agar well diffusion method. Four different solvents viz., distilled water, methanol, petroleum ether and ethanol were used to obtain the extract of *Punica granatum*. Out of these solvents, 90 % of recovered isolates showed the maximum zone of inhibition against the methanolic extract of *P. granatum* i.e. 25mm, 24mm 23mm and 22mm. Among 10 test drugs, the diameter of inhibition zones observed in 6 drugs particularly amoxicillin (34mm), ampicillin (32mm), penicillin (31mm), chloramphenicol (30mm), erythromycin (28mm), clindamycin (26mm), tetracycline (25mm), Metronidazole, ciprofloxacin and gentamycin displayed no inhibition zones against the growth of *Streptococcus sp*., hence considered to be resistant against these drugs. Among the antibacterial drugs tested amoxicillin, penicillin and ampicillin showed maximum zone of inhibition against *Streptococcus*. *Streptococcus sp.* have been found to be most susceptible against amoxicillin as revealed by the data, the maximum zone of inhibition was found in amoxicillin (34mm). The observations from the present study substantiate the frequent use of broad spectrum amoxicillin in dental practice as also worked out by Al-Harooni and Skoog [12]. This antibiotic is routinely prescribed as prophylaxis to the patients prior to massive dental procedures. It has been reported that the introduction of penicillin in the prophylactic

treatment has reduced the infection, but the long-term use of penicillin could be compromised by the emergence of resistant strains [6]. Erythromycin and clindamycin have been recommended as alternative options for patients who are allergic to penicillin and are also widely used for antibiotic prophylaxis of endocarditis associated with dental procedures [18-19]. These two antibiotics have not developed resistance against the strains of *Streptococcus* as revealed by the zone of inhibition in the present study. Tetracycline produces side effects mainly on the digestive system which include mild stomach pain or upset, nausea, vomiting and diarrhea but it is effective in inhibiting the growth of *Streptococcus* and hence should be recommended for use. However as a precautionary measurement tetracyclines should not be recommended for children or pregnant women because they can discolour developing teeth and alter bone growth [20]. Gentamycin, an aminoglycoside, may lead to side effects which include damage to the ears and kidneys. Metronidazole has been most frequently prescribed by the dental professionals. However, the emergence of resistance to this drug may be slower than if it were used alone, because in order to target both aerobic and anaerobic organisms, metronidazole is used empirically in combination with one or more antibiotics, although the resistance to the drug may be associated with mobile genetic elements, aiding spread [10].

It may be envisaged from the present study that due to lack of appropriate knowledge of prescribing antibiotics for the treatment of dental caries on part of dental professionals, the microbial flora responsible for causing dental caries has developed resistance to the commercially available drugs. It may also be recommended that amoxicillin and penicillin G are the most effective antibacterial drugs for the treatment of dental caries. Further investigation and education are required to attempt to slow resistance development and lessen the future impact on antibiotic prescribing in dentistry. Due to a rapid increase in the rate of infections, antibiotic resistance in microorganisms and due to side effects of synthetic antibiotics, medicinal plants are gaining popularity over these drugs [21]. Although medicinal plants produce slow recovery, the therapeutic use of medicinal plant is becoming popular because of their lesser side effects and low resistance in microorganisms [22]. *Punica granatum* fights dental plaque, the yellowish buildup of microorganisms on the teeth that can lead to cavities and gum disease and pomegranate extract kills microorganisms isolated from the dental plaque of healthy adults. Pomegranate seeds are a source of many nutrients and can also be used for medicinal purposes. So we can effectively use mouthwash containing pomegranate for one minute which can reduce the amount of microorganisms from dental plaque [23,24].

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