

ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *STREPTOCOCCUS MUTANS* CAUSING DENTAL CARIES AMONG STUDENTS OF JAZAN UNIVERSITY, KINGDOM OF SAUDI ARABIA

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ABSTRACT

Dental caries is an age old disease in which the tooth enamel is demineralized by the secretion of acid in microenvironment by bacteria. The most common among the bacteria causing dental caries is Streptococcus mutans. The synthesis of polysaccharide (Dextran) forms the plaque on smooth surface of gums in which gets adhere other bacteria viz Lactobacillus acidophilus, Actinomyces viscosus etc producing acid and bring about dissolution of tooth enamel. Many age old therapies are still being used for controlling tooth decay viz. twigs of Miswak in Saudi Arabia and twigs of Neem in India. However the increasing use of sugar and poor oral hygiene has increased the prevalence of dental caries throughout the globe. The treatment therefore becomes necessary to stop the proliferation of caries causing bacteria either by conventional methods or by use of Antibiotics. Since there is increasing trend of bacteria becoming resistant to various antibiotics, its therefore useful to study the effect of same antibiotics in different regions to know the pattern of its susceptibility. In the present study, the emphasis was given on the antibiotic susceptibility pattern of Streptococcus mutans which was isolated from among the students of College of Applied medical sciences, Jazan University, Kingdom of Saudi Arabia. The antibiotics used in the study are tetracycline, cefaclor, chloramphenicol, ofloxacin, ceftazidime, gentamycin, ceftaxime sodium, clindamycin, cefprozil, tobramycin, bacitracin, cefuroxime, ceftizoxime, sulphamethoxazole and lomefloxacin. The susceptibility of Streptococcus mutans was tested by disc diffusion method of kirby-Bauer technique. In the present study Streptococcus mutans was found to be highly sensitive to tetracycline, cefprozil,

cefuroxime sodium, ceftaxime sodium and ceftizoxime similar to the findings of Wayne and David.

Introduction:

Dental caries is an ecological disease in which the diet, the host and the microbial flora interact over a period of time in such a way as to encourage demineralization of the tooth enamel with resultant caries formation(8). Dental caries is still one of the most common diseases in the world today. Until recently almost everyone had experienced tooth decay in their lifetime. However, today many people are caries free and there has been a 40-60 % reduction in the incidence of tooth decay around the Western world.

Teeth are composed of a thin layer (1-2mm) of dental enamel which forms the hard protective coating over the tooth. This consists mainly of calcium, phosphate and other ions in a structure known as "hydroxyapatite". Dental enamel is porous and is susceptible to acid dissolution during the process of demineralization.

Dental caries will not occur if the oral cavity is free of bacteria. These bacteria are organized into a material known as dental plaque(1) which is yellowish coloured film on the surface of the teeth. Of the many types of bacteria in the mouth, the most caries active appear to be *Streptococcus mutans*, *Lactobacillus spp.*, *Veillonella spp.* and *Actinomyces spp.*

Streptococcus mutans is a gram positive, facultative anaerobic bacteria described by JK Clark(2) in 1924 after he isolated it from carious lesions. It metabolizes carbohydrates specially sucrose and produces extracellular polysaccharide "glucan" by which the bacteria stick to smooth surfaces and forms plaque.

The effects of *Streptococcus mutans* can be counteracted through proper oral hygiene and use of fluoride. Fluoride has been found to be the most effective agent against caries because it acts through topical mechanisms inhibiting the demineralization of enamel and tooth structure, enhancement of re-mineralization and inhibition of bacterial enzymes such as enolases, phosphatases, pyrophosphatases etc. However the large section of population still suffers from

caries even having good oral hygiene. Thus the use of antibiotics therefore plays an important role in controlling the caries disease. In the present study the *Streptococcus mutans* isolated were tested against various antibiotics available in Jazan Region.

Review of Literature

Infections of the teeth have plagued humans constantly, despite a quest for better oral hygiene. As early as 200 BC, a bronze wire root canal filling was found in the skull of a Nabataean warrior. Infections usually arise from pulpitis and associated necrotic dental pulp that initially begins on the tooth's surface as dental caries(9).

Resistance to cefuroxime, penicillin, tetracycline, and mercury is reported for 839 *Streptococcus mutans* isolates from 209 human study subjects by Jorma Leistevuo (5).

Involvement of *Streptococcus mutans* in dental caries and bacterial endocarditis has prompted interest in the susceptibility of this organism to antimicrobial agents.

David J. Hyman (10) isolated forty-one strains of *Streptococcus mutans* (34 from blood specimens from patients with endocarditis and 7 from stock cultures) which were tested for susceptibility to penicillin, ampicillin, methicillin, erythromycin, cephalothin, vancomycin, chloramphenicol, tetracycline, gentamicin, streptomycin, and kanamycin. Minimal inhibitory and bactericidal concentrations were determined by a broth microdilution procedure. Most of the strains were very susceptible to ampicillin, penicillin, and erythromycin, with most strains having minimal inhibitory concentrations of 0.08 µg/ml or less. Most of the strains were also susceptible to cephalothin, methicillin, chloramphenicol, tetracycline, and vancomycin. Gentamicin was the most effective aminoglycoside. The antimicrobial susceptibility patterns are similar to those of other viridans streptococci. *Streptococcus mutans* strains have proven to be difficult for some microbiologists to identify. But when organisms suggesting *S. mutans* are isolated from patients with endocarditis, they should be at least identified as non-enterococcal streptococci so that appropriate therapy can be initiated.

Because physicians frequently encounter patients with dental complaints, familiarity with the initial management of these conditions is important. Issues that may arise include the appropriate use of antibiotics and the indications for immediate dental referral. Antibiotics may be unnecessarily prescribed in up to 30% of cases, which may increase costs and risk of side effects (4). When needed, penicillin and its derivatives, erythromycin, clindamycin, and metronidazole are appropriate adjuncts to definitive dental treatment (11).

William Buwembo (13) evaluated the antibiotic susceptibilities of cariogenic mutans streptococci isolated from participants attending a dental and an HIV clinic in Kampala-Uganda. About 14.3% and 23.8 % of the isolates were resistant to cotrimoxazole and amoxicillin, respectively. Resistance to ceftriaxone, vancomycin, Chloramphenical, erythromycin and tetracycline was found in 46.4, 27.3, 14.3, 11.9 and 54.8% of the isolates

The main objective of the research project is to study and evaluate the antimicrobial susceptibility of various antibiotics against the streptococcus mutans isolates responsible for dental caries. The *Streptococcus mutans* is to be isolated by Kohler and Brathall (6) method from the saliva of students of Jazan University and the antibiotic susceptibility is determine by disc diffusion method of Kirby-Bauer technique (7).

Materials and Methods:

Sample collection and Culture:

Twenty students of College of applied medical sciences, Jazan university were selected for sampling. The sampling of saliva for the isolation of *Streptococcus mutans* was done as per the method suggested by Kohler and Brathall. According to this method each student was given a chewing gum and was asked to chew it at least for five minutes to generate more saliva. Each student was then asked to scrap the gums and tooth to collect saliva by a sterilized wooden spatula. The spatula was than pressed against the surface of of mitis salivarius agar base (Difco

Laboratories, Detroit, Mich.), 1% potassium tellurite (Merck OY, Espoo, Finland), 15% sucrose, and 0.1 U of bacitracin (Sigma Chemical Co., St. Louis, Mo.) per ml.

Plates were incubated for 3 days at 35°C in a 5% CO₂ atmosphere to facilitate identification. Judging by colony appearance, dark, raised, mucoid *S. mutans*-like colonies were identified as *mutans streptococci*.

Antibiotic susceptibility by Kirby-Bauer technique:

Antibiotic susceptibility testing was done by disc-diffusion method as suggested by Kirby-Bauer technique using Mueller-Hinton agar. The antibiotics used were tetracycline, cefaclor, chloramphenicol, ofloxacin, ceftazidime, gentamycin, ceftaxime sodium, clindamycin, cefprozil, tobramycin, bacitracin, cefuroxime, ceftizoxime, sulphamethoxazole and lomefloxacin.

Preparation of Inoculum:

The selected colonies of *Streptococcus mutans* are inoculated in 5ml sterile nutrient broth and incubated at 37⁰c for 2-8 hours till moderate turbidity is developed. The inoculum turbidity was compared by mixing 0.5ml of 1.75% barium chloride and 99.5ml of 0.36N sulphuric acid, as recommended by W.H.O. Wherever necessary the inoculum was diluted or incubated further to attain comparative turbidity. The *Streptococcus mutans* isolates were then tested for their antibiotic resistance / susceptibility pattern by Kirby-Bauer technique using the above antibiotics. The plates were incubated at 37⁰c for 18-20 hours under anaerobic condition. The test is done in triplicate for each antibiotic and zone of inhibition was recorded.

Result:

The zone of inhibition of each antibiotic is read using a zone scale reader. Readings of the antibiotic susceptibility test were recorded and average of triplicate was noted (Table 1). Total fifteen antibiotics were used viz. tetracycline, cefaclor, chloramphenicol, ofloxacin, ceftazidime, gentamycin, ceftaxime sodium, clindamycin, cefprozil, tobramycin, bacitracin, cefuroxime sodium, ceftizoxime, sulphamethoxazole and lomefloxacin.

Discussion:

The third generation antibiotics were used which are the recent development in antimicrobial activity. Out of total fifteen antibiotics, all *Streptococcus mutans* isolates were found to be resistant to bacitracin, tobramycin and gentamycin (Table 2). All 20 isolates were found to be highly sensitive to tetracycline, 19 to cefuroxime sodium, 18 each to cefprozil and ceftaxime sodium and 17 to ceftizoxime. The number of isolates which are moderately sensitive to antibiotics are 17 each for cefaclor and sulphamethoxazole, 16 for chloramphenicol, 2 each for ceftizoxime and lomefloxacin, 1 each for ceftaxime sodium, cefprozil, ofloxacin, ceftazidime and ceftizoxime. All 20 isolates were less sensitive to Clindamycin. Others which are less sensitive are 19 each for ofloxacin and Ceftazidime, 16 for lomefloxacin, 3 for Chloramphenicol, 2 for Sulphamethoxazole and 1 each for Chloramphenicol, cefprozil and ceftizoxime. Beside 3 isolates were found to be resistant to cefaclor, 2 for lomefloxacin and 1 each for Chloramphenicol, Cefprozil and sulphamethoxazole (Graph 1 and 2). Our study correlates with the study of Wayne A (12) in which all isolates were found to be sensitive to tetracycline also the study of David B. Uzuegbu in which the *Streptococcus mutans* isolates were sensitive to tetracycline, ceftaxime sodium and ceftizoxime (3).

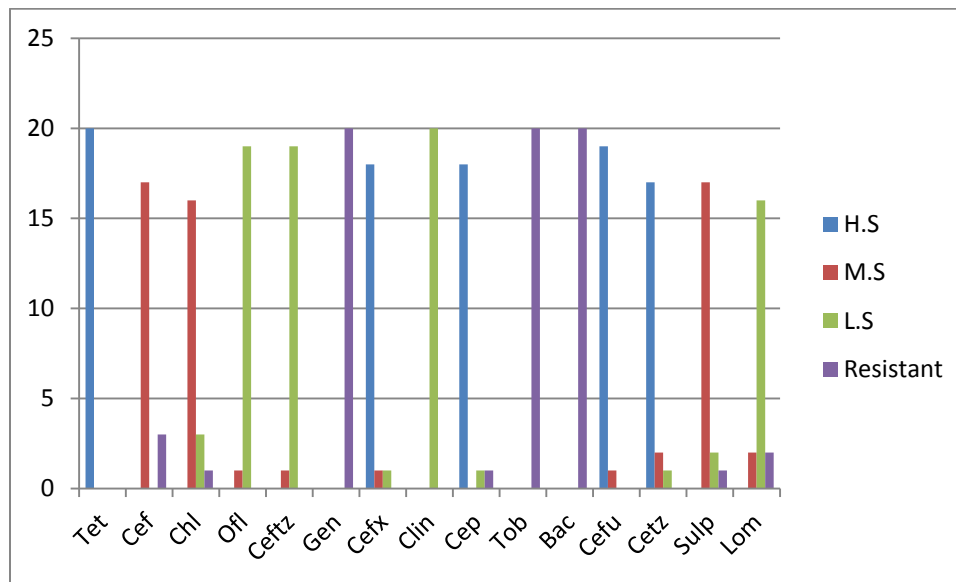
Table:1 Percentage susceptibility of *Streptococcus mutans* against various antibiotics.

Antibiotics	Number of Isolates				% Sensitive	% Resistant
	H.S	M.S	L.S	Resistant		
Tetracycline	20	-	-	-	100	nil
Cefaclor	-	17	-	03	85	15
Chloramphenicol	-	16	03	01	95	05
Ofloxacin	-	01	19	-	100	-

Ceftazidime	-	01	19	-	100	-
Gentamycin	-	-	-	20	-	100
Ceftaxime sodium	18	01	01	-	100	-
Clindamycin	-	-	20	-	100	-
Cefprozil	18	-	01	01	95	05
Tobramycin	-	-	-	20	-	100
Bacitracin	-	-	-	20	-	100
Cefuroxime Sodium	19	01	-	-	100	-
Ceftizoxime	17	02	01	-	100	-
Sulphamethoxazole	-	17	02	01	95	05
Lomefloxacin	-	02	16	02	90	10

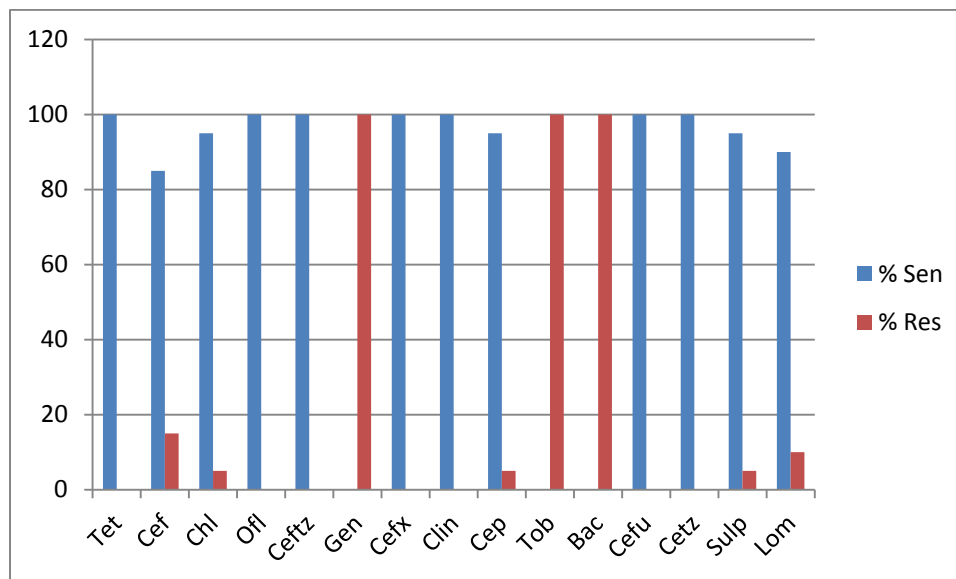
H.S (Highly sensitive), M.S (Moderately sensitive), L.S (Less sensitive)

Graph 1: Isolates showing sensitivity and resistance to various antibiotics



H.S (Highly sensitive), M.S (Moderately sensitive), L.S (Less sensitive)

Graph 2: % susceptibility pattern of Isolates against various antibiotics



Conclusion:

The Streptococcus mutans isolates in our present study were found to be highly sensitive to tetracycline, cefprozil, cefuroxime sodium, ceftaxime sodium and ceftizoxime similar to the findings of Wayne and David. It is absolutely necessary to treat the patient with caries with suitable antibiotics, since if not, may lead to dangerous endocarditis. Beside antibiotics the caries can be prevented by proper brushing of tooth, control intake of sugary diet and proper oral hygiene.

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