Study of Molecular Mechanism of Antibiotic susceptibility against Polymicrobial Oral infection

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Abstract

Introduction- Antibiotic to treat dental caries infection are routinely prescribed. Bacteria have increased resistance to the currently recommended antibiotics. The purpose of this investigation was to perform antibiotic susceptibility on a panel pathogenic strain of bacteria isolated step by step from dental caries infection.

Materials and Methods-Bacteria were isolated from caries site of patients and cultivated identified at the species level. Each of 150 species of bacteria was tested for antibiotics susceptibility to a five antibiotics using Etest. Antibiotic used were Amoxicillin, Cloxocillin, Erythromycin, Tetracycline, Penicillin-V.

Results- The percentages of Resistance in treatment for each antibiotic in this study were penicillin V: 72/150 (48%), Tetracycline: 99/150 (66%), amoxicillin: 135/150 (90%), Cloxocillin: 117/150 (78%), and Erythromycin: 90/150 (60%) (Table 1). If combination antibiotic therapy had been used to treat the bacteria isolated from dental caries, the percentage of Resistance for the combination of penicillin V/Amoxicillin would have been 39/150 (26%), and the combination of amoxicillin/ Erythromycin would have been 45/150 (30%).

Conclusion- This study demonstrated the Molecular Mechanism of antibiotic resistance and susceptibility pattern, of bacteria causing dental caries. The comprehensive results data obtained will allow in investigating spatial distribution of pathogenic antibiotic resistant bacteria in patients of dental caries. In turn this will allow the development of novel diagnostic and treatment methods.

Introduction

Dental caries, also known as **tooth decay** or **cavity**, is a disease where bacterial processes damage hard tooth structure (enamel, dentin, and cementum). (1) Tissues progressively break down, producing dental caries (cavities, holes in the teeth). Two groups of bacteria are responsible for initiating caries: *Streptococcus mutans* and *Lactobacillus casie*. If left untreated, the disease can lead to pain, tooth loss, infection, and, in severe cases, death. (2) Today, caries remains one of the most common diseases throughout the world.

The presentation of caries is highly variable; however, the risk factors and stages of development are similar. Initially, it may appear as a small chalky area that may eventually develop into a large cavitation. Sometimes caries may be directly visible, however other methods of detection such as radiographs are used for less visible areas of teeth and to judge the extent of destruction. (3)

Tooth decay is caused by specific types of acid-producing bacteria that cause damage in the presence of fermentable carbohydrates such as sucrose, fructose, and glucose. (4)(5)(6). The mineral content of teeth is sensitive to increases in acidity from the production of lactic acid. Specifically, a tooth (which is primarily mineral in content) is in a constant state of back-and-forth demineralization and remineralization between the tooth and surrounding saliva. When the pH at the surface of the tooth drops below 5.5, demineralization proceeds faster than remineralization (meaning that there is a net loss of mineral structure on the tooth's surface).

Antibiotics (antimicrobials) are often prescribed for the adjunctive treatment of dental caries. The choice of antibiotic is usually based on previously published susceptibility testing and previous clinical success. There is concern that bacteria have increased resistance to the currently prescribed antibiotics. Antibiotic susceptibility testing is determination of antibiotics resistance pattern of bacteria. It will be ideal if susceptibility testing could always undertake before the prescription of antibiotics.

Previously the most common methods of susceptibility testing were the disk diffusion test, agar dilution, and both micro- and macro-broth dilution. Disadvantages of these systems include: nonquantitative interpretation, inconsistent application for slow growing

bacteria and anaerobes, limited use for direct testing of clinical material, and very time consuming (14). The Etest (AB Biodisk, Culver City, CA) is based on the diffusion of a continuous, exponential concentration gradient of the antimicrobial from a plastic strip containing the antibiotic. The Etest overcomes several of the above disadvantages while producing an accurate, reproducible reference minimum inhibitory concentration (MIC), which has been used for susceptibility testing of dental caries isolates (7, 8). Each plastic strip is 5- X 50-mm with the dried antibiotic in a concentration gradient on one side and an MIC interpretive scale on the other side (Fig. 1). The MIC scale corresponds to 15 two-fold dilutions. After incubation of the Etest strip on agar media with a lawn of bacteria, an ellipse of inhibition is formed around the strip. The point where the ellipse intersects the strip is where the MIC is read from the interpretive scale. The Etest is technically simple to use. In the future, Etest strips may be developed for use with fungi, investigational antimicrobials, antimicrobial combinations, and as a surveillance tool around the world.

The purpose of this study was to determine the detailed mechanism of antibiotic susceptibility of 150 strains of bacteria recently isolated from the 50 patients sample using the Etest. The antibiotics tested were Amoxicillin, Cloxacillin Erythromycin, Tetracycline and Penicillin.

Materials and Methods

A total 50 clinical samples were taken from patients at outpatient department of oral surgery & department of oral medicine and radiology and other OPDs of R.K.D.F. Dental college and Peoples Dental Academy, Bhopal, M.P. India. Out of 50 patients, 18 were female, 23 were male 09 were children.

The sample were collected from different legions in oral cavity the patient were showing Dental caries, Dental decay Calculus Plaque throat swabs. Samples transported to the laboratory under aseptic conditions. Total 150 strains of bacteria were cultivated from 50 samples. All culturing of bacterial strains was performed at 37° C on Nutrient Agar Media, Blood Agar media, and streaked on Mitis, Salavarious Agar and de Man, Rogosa Sharpe Agar (MRS agar) (Himedia laboratories, India) to obtained confluent growth.

Five different Etest strips with the antimicrobials. Amoxicillin (Am), Cloxocillin (Cx), Erythromycin (E), Tetracycline (T), Penicillin-V (Pv) (Himedia laboratories, India), were placed on the plates, which were incubated in the candle jar chamber to produce a confluent growth of bacteria. After incubation, an ellipse of inhibition around each antibiotic strip was read to determine the MIC calibrated in µl/ml. (Figure- 1) The National Committee for Clinical Laboratory Standards (NCCLS) has established MIC interpretive standards for resistance of facultative anaerobes: penicillin G (penicillin V) ≤2 µl/ml, Tetracycline ≤16 µl/ml, Cloxocillin, resistance at ≤32 µg/ml, Erythromycin, ≤8µl/ml, and amoxicillin ≤2µg/ml.

Result

S*treptococcus mutans* (ATCC 25175) as a reference strain conformed to the values for quality control. One fifty strains of bacteria isolated from dental caries were facultative anaerobes, Susceptibility pattern and intermediate MIC end points are both considered amenable to antibiotic therapy by the NCCLS. The percentages of Resistance in treatment for each antibiotic in this study were penicillin V: 72/150 (48%), Tetracycline: 99/150 (66%), amoxicillin: 135/150 (90%), Cloxocillin: 117/150 (78%), and Erythromycin: 90/150 (60%) If combination antibiotic therapy had been used to treat the bacteria isolated from dental caries, the percentage of Resistance for the combination of penicillin V/Amoxicillin would have been 39/150 (26%), and the combination of amoxicillin/ Erythromycin would have been 45/150 (30%).(Table 1) all the isolates were less susceptibility pattern shows by antibiotics but in the combination of antibiotics increasing the susceptibility against bacterial isolates of dental caries . (Figure-2)

Discussion

Antibiotic prescription should be adjunctive to proper clinical treatment. Selection of an antibiotic regimen should be based on knowledge of the efficacy of an antibiotic for the bacteria. It should be remembered that dental caries infections are ecosystems of bacteria in which by products of one species of bacteria may be nutrients for other species of bacteria (9).

Antibiotic resistance studies have generally relied on the isolation of bacteria on antibiotic free plates and subsequent testing of susceptibility to a range of antibiotics. The majority of the antibiotic-resistant bacteria isolated were members of the normal oral microflora; however, some pathogenic and opportunistic pathogenic bacteria were also isolated. Antibiotic-resistant bacteria were isolated that are important agents of pneumonia, otitis media, sinusitis, meningitis, deep seated abscesses, endocarditis and dental diseases including caries and periodontal diseases. The carriage of these pathogenic and opportunistic pathogens is of clinical importance because infection caused by such antibiotic-resistant bacteria could result in treatment failure and lead to a chronic infection. All subjects were having antibiotics resistant bacteria, demonstrating the potential of the oral cavity to act as a reservoir of antibiotic-resistant organisms. A few of the subjects had very high percentages of their oral microflora resistant to specific antibiotics. The high percentages of their oral microflora resistant to specific antibiotics suggests that there is a subset population that could act as a reservoir of antibiotic-resistant bacteria (10).

Resistances for penicillin have three basic mechanism, Production of Beta lactamase, inability of binding protein to penicillin, barriers to cell wall penetration. In previous study determined that 23% isolates were resistance to penicillin (11) but we find the 48% isolates were resistance for penicillin.

Molecular Mechanism of drug resistance

Most of the resistance is due to one of the verity of plasmid or transposon encoded β-lactamases such as TEM-1 (the most common, TEM-2) SHV-1, PSEs, and OXAs. The enzymes coded by these genes typically active against ampicillin and other penicillin and to a lesser extend of third generation of antibiotics.

Antibacterial drug sensitivity results have shown that cell wall was not able to withstand osmotic pressure and bacteria literally explode, not only used drugs also inhibit the DNA guyrase, topoisomerase IV enzyme reducing the resistance property of test bacteria.

Amoxicillin is used in dentistry for the treatment of dental infection; factors that promote an increase in amoxicillin resistance in dental infection are of concern to both dental and medical practitioners. The use of amoxicillin significantly increased both the total

number of amoxicillin resistance bacteria and its proportion (12). About 64% isolates resistance for amoxicillin were found in this study.

Tetracycline is prescribed by dentists frequently; tetracycline resistance is encoded by *tet* gene which are found in oral bacterial species. Tetracycline resistance mechanism include the synthesis of efflux protein, production of ribosome protection protein and enzyme modification of the antibiotic (13) (14). About 78% isolates were found to resistance for tetracycline.

Erythromycin interferes with aminoacyl translocation, preventing the transfer of the tRNA bound at the A site of the rRNA complex to the p site of the rRNA complex. Erythromycin displays bactericidal activity, it is frequently prescribed for use in oral infection in childern, 48% isolates were found to resistance erythromycin. (Figure-3)

Cloxocillin is a semisynthetic antibiotic in the same class as penicillin, cloxacillin is used against *Staphylococci* that produce beta lactamase. Due to its large R chain which does not allow the beta lactamases to bind. 40% isolates were found to resistance for Cloxocillin.

Combination of two antibiotics is also tested for resistance patterns, were the penicillin-amoxicillin and amoxicillin-erythromycin show decrease rate of resistance pattern in all isolates.(table-1)

In conclusion, this study demonstrated the molecular picture of antibiotic resistance and susceptibility pattern, for dental caries causing bacteria. The comprehensive results data obtained will allow to investigating spatial distribution of pathogenic antibiotic resistant bacteria in patients of dental caries. In turn this will allow the development of novel diagnostic and treatment methods. Some more studies in this direction should be needed to find out the susceptible gene pattern which can be possible drug target against dental caries.

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Table–1 showing efficacy of antibiotics against clinical isolates of dental caries.

Name of oral pathogens	**No of Isolates**	**Name of the Antibiotics*** (Number of drug resistant isolates)						
		AMX	**TE**	**P**	**COX**	**E**	**P/AMX**	**E/AMX**
Gram Positive cocci								
Streptococcus mutans	61	57	40	32	44	37	17	22
Streptococcus sobrinus	16	14	10	7	12	9	4	2
Stretococcus oralis	14	12	9	9	7	8	5	6
Streptococcus sanguinis	16	14	13	5	9	11	3	1
Gram Positive Bacilli								
Lactobacillus acidophilus	18	16	12	7	14	10	6	8
Lactobacillus rhamnosus	12	10	10	7	9	7	2	3
Lactobacillus fermentum	13	12	4	5	10	8	2	3
Total	150	135/150 (90%),	99/150 (66%),	72/150 (48%)	117/150 (78%),	90/150 (60%)	39/150 (26%),	45/150 (30%)

*****AMX-Amoxicillin, TE- Tetracycline, P- Penicillin, COX- Cloxacillin, E- Erythromycin, P/AMX-penicillin /Amoxicillin, E/AMX- amoxicillin/ Erythromycin.

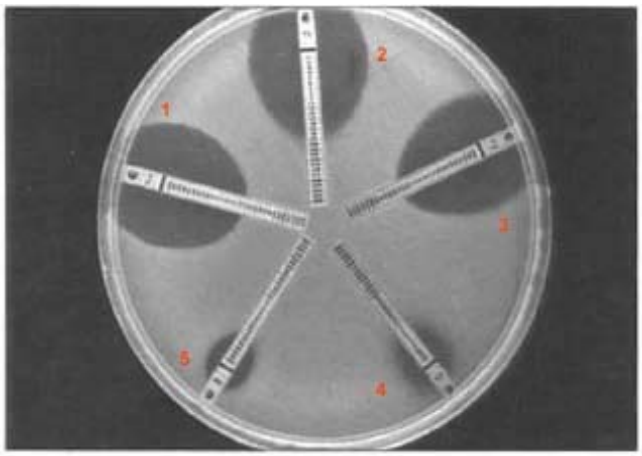
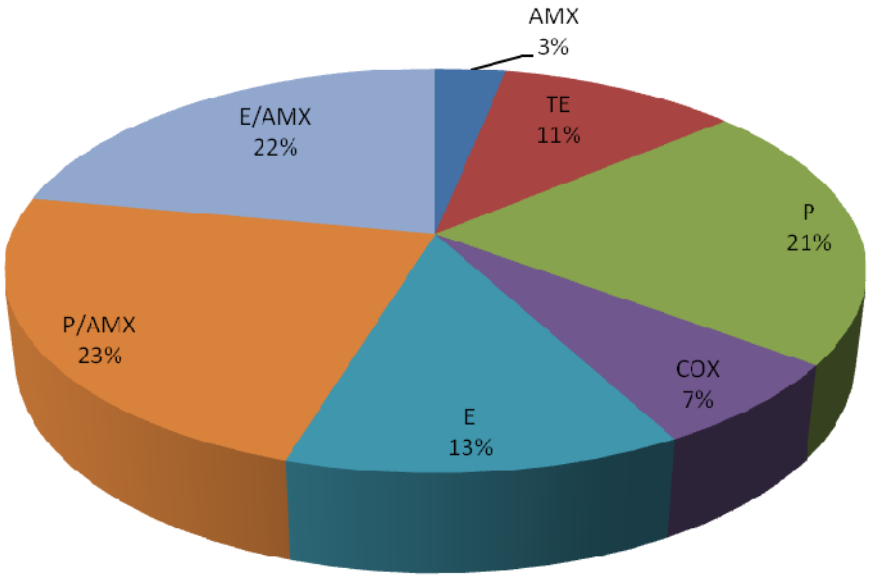


Figure-1 E test Strip for Resistance pattern of Five antibiotics used in dental caries isolates.

1- Amoxicillin, 2- Tetracyciline, 3-Penicllline, 4- Erythromycine, 5- **cloxacillin**

Figure-2 Showing Suseptibility pattern of Antibiotics in phathogenic bacterial isolates from Dental Caries



*AMX-Amoxicillin, TE- Tetracycline, P- Penicillin, COX- Cloxacillin, E- Erythromycin, P/AMX- penicillin /Amoxicillin, E/AMX- amoxicillin/ Erythromycin

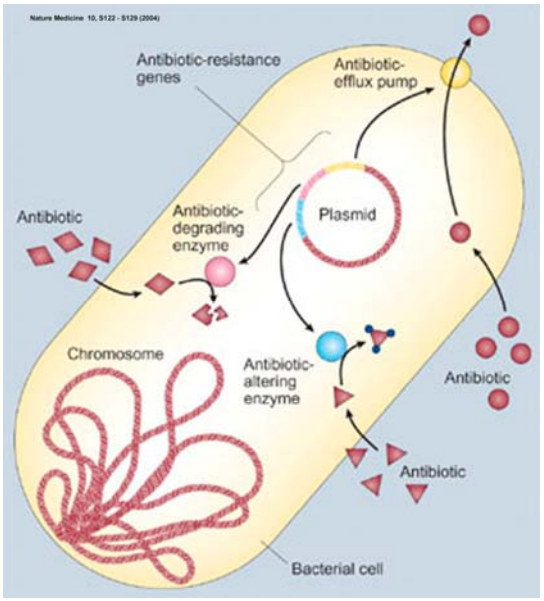


Figure-3 Showing Molecular Mechanism of Drug Resistance in bacteria