**International Journal of Biology Research**
**ISSN: 2455-6548, Impact Factor: (RJIF 5.22)**
**www.biotechjournals.com**
**Volume 1; Issue 1; March 2016; Page No. 53-55**

# Comparative study on isolation of r-plasmids from ampicillin resistant isolates of caries and non-caries patients

**S Karikalan, A Mohankumar**

Division of Microbial Technology, PG and Research Dept. of Zoology, Chikkanna Govt. Arts College, Tirupur 641 602, Tamil Nadu, India.

**Abstract**
The oral bacterial isolates have been multi-drug resistant in tradition for several decades and specific antibiotic was resistant to caries pathogens. The aim of the present study was to prove the Ampicillin antibiotic completely resistant to oral isolate and a comparative study about the R- plasmid isolated from caries causing, Ampicillin resistant bacteria *Streptococcus mutans.* The samples were collected from caries and non-caries patients. The extracted tooth and the saliva samples were collected from them. The effectivity of caries was determined by the caries susceptibility test. *S.mutans* the predominant cariogens were isolated and R-plasmids were isolated from oral pathogen and compare with caries and without caries isolates. Antibiogram of *S.mutans*, Ampicillin showed 100% resistant. The main objective of this study was assessed the isolation of R- plasmids from dental caries causing, Ampicillin resistant isolate of *S. mutans* from the caries and non-caries patients.

**Keywords:** Antibacterial, Dental caries, *Streptococcus mutans,* R-plasmid.

## 1. Introduction
Tooth decay is the leading cause of tooth loss and while this disease will lead to odontogenic infections (Ryan *et al.,* 2004) [11]. This infectious disease caused by an important oral viridans streptococci and Clark was first described this *Streptococcus mutans* in 1924 (Clark, 1924) [2]. *S. mutans* is a Gram positive, non-motile, non-spore forming, Catalase-negative, facultative anaerobic cocci bacterium commonly found in the human oral cavity, is a significant contributor to tooth decay (Loesche and Patterson, 1996) [9] and has the ability to cause bacteria endocarditis. It is a member of the viridans streptococci (Patterson, 1996) [9] and occurs in chains. Several researchers reported that *S.mutans* has virulent character like resistant to leading antibiotics. The *S.mutans* isolate has been multi-drug resistant in tradition for several decades and specific antibiotic was resistant to caries pathogens. Normal oral flora of this pathogenic group species proved their resistant power against some helpful antibiotics like cell wall breaking penicillin group for recent decades and especially those were isolated from human saliva samples. Particularly some studies were reported on this type of oral pathogenic microbes. Resistant plasmid (R-Plasmid) is a conjugative feature in bacterial cells that support resistance to antibiotics, metal ions, ultraviolet radiation, and bacteriophage. Another group was resistance (R) plasmids which carry that encode resistance to antibiotics or poisons (Kennath dodar, 2008).
In this study compare the two isolates of caries and without caries possible to high resistance R-plasmids were found in Ampicillin resistant isolates and isolated the plasmids from dental caries pathogen *S.mutans.*

## 2. Materials and Methods
### 2.1. Sample Collection
Extracted tooth sample and saliva were collected from dental caries and non caries patient in critical condition from a dental clinic in Tirupur, Tamil Nadu, and India. Only two samples were collected during a period of this investigation. Decayed tooth from the patient was collected with a sterile forceps and emptied into Mitis Salivarius Bacitracin agar, broth-based system (Hi-media, Mumbai) and the extracted samples were placed into the peptone water (Transported media) and it was used as per requirement.
Selective media of this species was of Mitis-Salivarius Bacitracin Agar (MSB) and the collected samples were plated and incubated at 37 °C for 24h. Cultures showing hemolysis were included in this study. The stock cultures of the isolates were then prepared in Brain-Heart Infusion (BHI) Broth (Hi-Media Laboratories, Mumbai) incubated at 37 ºC for 24h and stored at 4 ºC. To revive the microorganisms, a single pellet of the cultures was resuspended in Brain-Heart Infusion (BHI) Broth (Hi-Media Laboratories, Mumbai), followed by incubation at 37 ºC for 24h.

### 2.2. Bacterial identification
The isolates were identified on the basis of their morphological, physiological and biochemical characteristics. The tests were performed on Biochemical tests included, Gram staining, Motility, Catalase test, Voges-proskauer, Hemolysis, Oxidase, Carbohydrate fermentation tests included Mannitol and Sorbitol. The colonies were sub-cultured repeatedly for obtaining pure cultures on Mitis Salivarius Bacitracin Agar slants and stored at 4 °C for further investigations (Shklair and Keene, 1974) [13].

### 2.3. Antibiotic susceptibility test
The disc diffusion test by Kirby-Bauer was employed to check the antibiotic susceptibility of the isolates (Kirby *et al.,* 1966) [8]. Antibiotics used were Penicillin-G, Ampicillin,

Tetracycline, and Amoxicillin. Only selective isolates were used to this assay.

### 2.4. Plasmid DNA extraction

Extra chromosomal DNA of *S.mutans* was isolated by $ZnCl_2$ method (Holmes and Quigley, 1981) [6].

## 3. Results and Discussion

All the samples were collected from 20 - 60 year old male and female patients (Caries and Non-caries patients for isolation process (Table 1). Suspected colonies of *S.mutans* (Fig 1) were subjected to various biochemical tests (Table 2).

In the tests comprising the antibiotic susceptibility (Table 3) the isolates obtained was found and conspicuously resistant (Fig 2) to Tetracycline (30µcg) and Ampicillin (5µcg) Penicillin-G (10µcg) and Amoxicillin (30µcg).

Among the tested isolates of *S. mutans* in extracted tooth samples of different individuals was found to be 47.24%, and was higher among individuals with caries (57.04%) as compared to individuals without carries (34.82%). Most of the (100%) *S.mutans* isolates were resistant to Ampicillin and (90%) were Penicillin G, (85%) were Amoxicillin and lowest of 50% to Tetracycline. All the 10 isolates of *S. mutans* were found to be multi drug resistant and were resistant to a minimum of 4 antibiotics and maximum of 100% of isolates were resistant to Ampicillin antibiotic (Fig 3).

Similar results obtained on *S.mutans* resistant against Penicillin, Amoxicillin and Ampicillin antibiotics (Fani *et al.*, 2007) [5]. And Dhamodhar *et al.*, (2012) [4]. A contradictory report documented on Tetracycline antibiotic sensitive against on *S.mutans* (Devi *et al.*, 2011) [3].

Ampicillin resistant isolates were selected to isolate the R-plasmids from both caries and one isolate from without caries. The Ampicillin resistant R-plasmids of caries patients isolate and absence of band observed in non-caries isolate (Fig 4).

**Table 1:** Extracted tooth and Saliva sample collection

| No of Patients | Gender | Age | (Dental caries) Extracted tooth | Without caries (Saliva) |
|---|---|---|---|---|
| 30 | Female | 20-60 | Positive | Positive |
| 20 | Male | 21-52 | Positive | Positive |

**Table 2:** Identification of *Streptococcus mutans*

| S. No | Test | Results |
|---|---|---|
| 1. | Gram stain | Positive |
| 2. | Motility | Negative |
| 3. | Colour | Blue |
| 4. | Catalase | Negative |
| 5. | Oxidase | Negative |
| 6. | VP test | Positive |
| 7. | Mannitol | Positive |
| 8. | Sorbitol | Positive |
| 9. | Hemolysis | α |

**Table 3:** Antibiotic Susceptibility of *Streptococcus mutans*

| S. No | Antibiotic Used (Disc) | Concentration of the disc (µcg) | Zone of Inhibition | Results | |
|---|---|---|---|---|---|
| | | | | With caries | Without caries |
| 1. | Ampicillin | 5µcg | 9mm | R | R |
| 2. | Amoxicillin | 30µcg | 9mm | R | R |
| 3. | Pencillin-G | 10µcg | Nil | R | R |
| 4. | Tetracycline | 30µcg | 18mm | R | R |

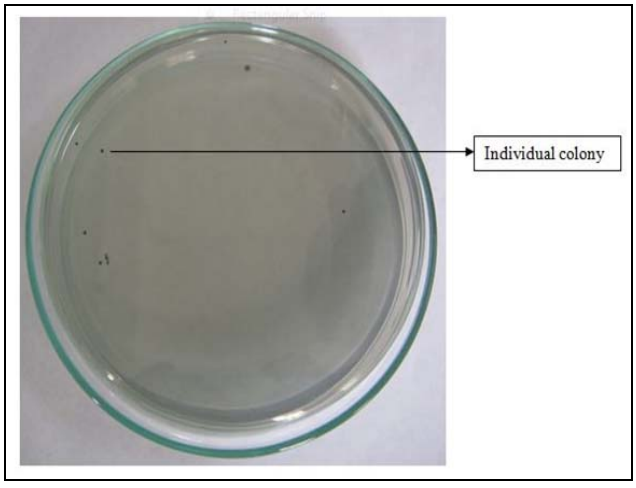


**Fig 1:** Colonies of *S.mutans* on the mitis-salivarius agar

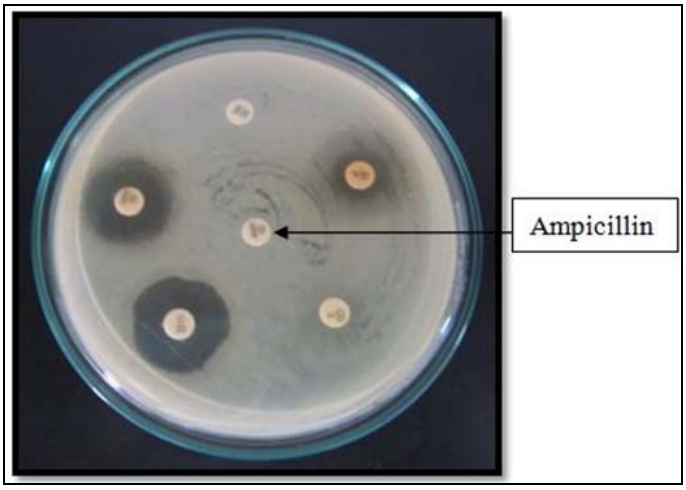


**Fig 2:** Showed the Ampicillin resistant of *S.mutans*

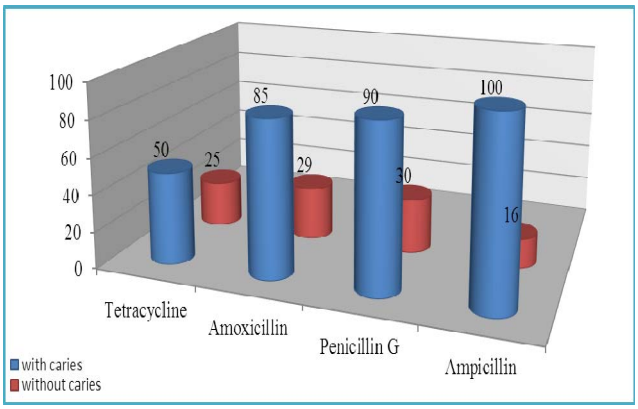


**Fig 3:** Graph showed 4 antibiotics were high resistant to concern isolates

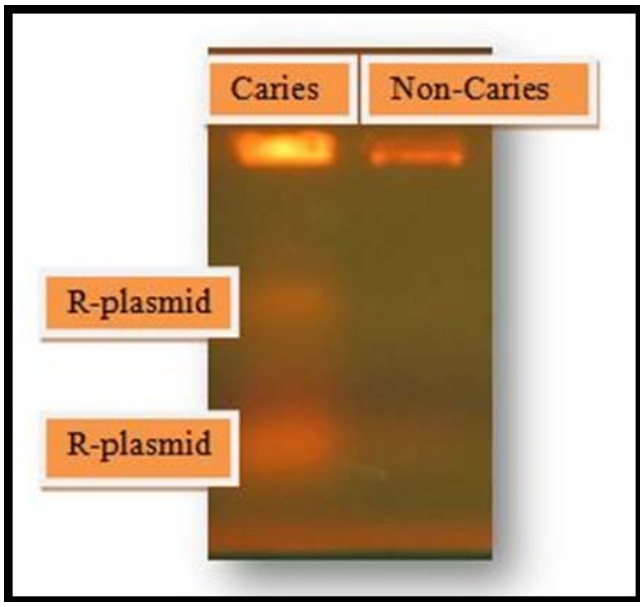


**Fig 4:** Showed the Ampicillin resistant R-plasmids in caries isolate and not shown in Non-caries isolate of *S.mutans*

**Plasmid isolation:** The presence of plasmids in the dental caries and non-caries bacterial isolates was determined using a modification of boiling preparation method.

**Gel electrophoresis:** Plasmid DNA was separated by electrophoresis on a 0.7% agarose gel (w/v) at 50 volts overnight. The gel was stained with Ethidium-bromide, visualized under UV transillumination and photographed.
Two important works were performed, only caries patient exhibited the Ampicillin resistant plasmids and not in saliva sample of non-caries patient. This study proved that non caries sample isolate not shown the R-plasmid.

## 4. Conclusion

In this comparative study on *S.mutans* isolates between the caries and non-caries patient, when checked for Antibiotic Susceptibility it was found to be Ampicillin showed Multiple Antibiotic Resistance (MAR) to all the other antibiotics. Compare than saliva sample isolate only R-plasmids recovered from dental caries isolate. Based on the results obtained, this study deal with identified the particular antibiotic resistant against Ampicillin and it containing R-plasmids for treatment of Dental Caries but R-plasmids were not observed in the non-caries patients. This investigation showed and only compared the presence or absence of R-plasmids in caries and non-caries patients. It used to helpful and identify the pathogenic (R-plasmids) bacterial strains found in the non caries patients. An opportunity for proper checkup may be useful to prevent the dental caries among the non-caries patients.

## 5. References


1. Ash MM, Ward ML, Arbor AK, Ash. Oral Pathology. An introduction to general and oral pathology for hygienist. Philadelphia: Lea and Febiger Publisher, 1986, 144-233.
2. Clark JK. On the bacterial factor in the etiology of dental caries. Br. J Exp. Pathol. 1924; 5:141-147.
3. Devi A, Singh V, Bhatt AB. Antibiotic Sensitivity Pattern of Streptococcus against Commercially Available Drugs and Comparison with Extract of Punica Granatum., International Journal of Pharma and Bio Sciences. 2011; 2(2):504-508.
4. Dhamodhar P, Sreenivasa Murthy, Channarayappa, Karthik R, Neha G, Shanthakumar SS, George JV. Antibacterial efficacy of Syzygium aromaticum extracts on multi-drug resistant Streptococcus mutans isolated from dental plaque samples. J Biochem Tech. 2012; 3(5):155-157.
5. Fani MM, Kohanteb J, Dayaghi M. Inhibitory activity of garlic (Allium sativum) extract on multidrug-resistant Streptococcus mutans. J Indian Soc. Pedod. Prev Dent. 2007; 25:164-168.
6. Holmes DS, Quigley M. A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem 1981; 114:193-197.
7. http://www.textbook of bacteriology.net/author Kenneth todar (accessed on 30/06/08).
8. Kirby WM, Bauer AW, Sherris JC**,** Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966; 45(4):493-496.
9. Loesche WJ. Microbiology of dental decay and periodontal disease. In: Baron ST Albrecht, Castro G *et al*., editors. Baron's Medical Microbiology. Galveston, Texas: University of Texas Medical Branch, 1996, 1169-1170.
10. Patterson MJ. Viridians Streptococci can cause endocarditis. In: Baron S, Albrecht T, Castro G, *et al*., editors. Baron's Medical Microbiology. Galveston, Texas: University of Texas Medical Branch, 1996, 199-212.
11. Ryan KJ, Ray CG, Sherris JC. Sherri's medical microbiology. An introduction to infectious diseases, 4th edn. New York: McGraw-Hill, 2004.
12. Seymour RA, Lowry R, Whitworth JM, Martin MV. Infective endocarditis, dentistry and antibiotic prophylaxis; time for a rethink? Br. Dent. J. 2000; 189(11):610-616.
13. Shklair IL, Keene HJ. A biochemical scheme for the separation of type five varieties of Streptococcus mutans. Arch. Oral Biol 1974; 19(11):1079-81.
14. Wagman RJ. The teeth and gums In: The New Complete Medical and Health Encyclopedia Chicago: Ferguson Publishing Company 2000; 3:719-740.