

Research article

# Multi-Drug Resistance of Bacterial Isolates among Dental Caries Patients

Yadav K<sup>1</sup>, Prakash S<sup>2</sup>, Yadav NP<sup>3</sup>, Sah RS<sup>4</sup>

Department of Microbiology, Janaki Medical College  
Ramdaiya, Janakpur, Nepal

<sup>1</sup>Lecturer, Krishna Medical technical research centre

<sup>2</sup>Assistant Prof, Department of Biochemistry, Janaki Medical College, Janakpur, Nepal

<sup>3</sup>Assistant Prof, Department of Microbiology, Janaki Medical College, Janakpur, Nepal

<sup>4</sup>Department of Dentistry, Janaki Medical College, Janakpur, Nepal

## ABSTRACT

**Background and Objectives:** Dental caries is a well known major oral health problem in most developing countries which has multifactorial etiology caused by many facultatively anaerobes. *S. mutans* is the main pathogen associated with this disease. Recently Multi-Drug Resistant (MDR) species of *S. mutans* were identified from the dental caries patients against many commercial antibiotics. MDR is a natural phenomenon, posing a serious worldwide threat to public health. Several therapeutic agents are available to treat or prevent tooth decay, but still global burden of the disease with MDR are emerging. Therefore, the present study was designed for assessing the antibiotic sensitivity pattern of commercially available antibiotics.

**Material and Methods:** This cross-sectional study was carried out by following Standard protocols of Bergey's Manual of Systematic Bacteriology to isolate and identify the organism and further followed by antibiotic susceptibility test of bacterial isolates by disc diffusion method.

**Results:** *Streptococcus mutans* (40%) was the most predominant to cause dental caries followed by *S. aureus* with 28.92. Gram positive isolates were found to be frequently resistant towards penicillin and tetracycline whereas Gram negative isolates were found to be Cotrimoxazole resistant.

**Conclusion:** A high frequency of penicillin resistance in oral isolates and its co-resistance to erythromycin, tetracycline, gentamycin and ampicillin among the patients was observed. The various awareness programmes should be facilitating the appropriate use of antibiotic to re-establish dominance over diseases must be implemented.

**Key words:** Dental carries, antibiotic resistant, MDR, *S mutans*, Penicillin

## INTRODUCTION

The occurrence of microbial infections has augmented significantly during the last few decades. Irrational use of antimicrobial drugs in treating infections has led to the

emergence of resistance among the various strains of microorganisms. Multi-Drug resistance (MDR) is defined as insensitivity or resistance of a microorganism to the administered antimicrobial medicines which

are structurally distinct and have different molecular targets despite earlier sensitivity to it [1,2]. Nowadays, multiple drug resistance has been developed in human pathogenic microorganisms due to the haphazard utilization of commercial antibacterial drugs frequently used in the treatment of infectious diseases [3]. Resistance of numerous bacterial pathogens to many antibiotics continues to increase worldwide. Frequencies, pattern, and distributions of resistant bacteria diverge extensively with geographic regions and often reflect the usage patterns of antibiotics [4]. Factor that increase in resource-poor and resource-rich nation include total antibiotic consumption as well as under use through lack of access, inadequate dosing, poor adherence, and substandard antimicrobial treatment [3].

Moreover, inappropriate antibiotic prescribing and its use have been identified as major factors in the appearance of antibiotic resistance. Nowadays, 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 [5]. Fluoroquinolones are quinolone antimicrobials which are active against many  $\beta$ -lactam resistant bacteria. Amoxicillin/clavulanic acid, combination of a  $\beta$ -lactam antibiotic (amoxicillin trihydrate) and a  $\beta$ -lactamase inhibitor (potassium clavulanate) has broad antimicrobial spectrum and effective against amoxicillin resistant bacteria that produce  $\beta$ -lactamase [6]. Such antimicrobial agent may prove valuable for managing dental infections. Production of  $\beta$ -lactamase is, however,

unusual for most of streptococci, where resistance is happening by slight alteration in penicillin binding proteins [7]. Bacterial resistance to antibiotics such as penicillin and other  $\beta$ -lactam is a health issue in numerous parts of the world.

In Nepal, the oral health system is currently in transition phase. The higher prevalence of dental caries is influenced by the lack of dental awareness and practice [8] as compared to developed countries [9] due to geo-socio-political, economic factors and inadequate health care assets. Internationally, dental caries is considered as a foremost public health problem due to its high prevalence and significant social impact in important life activities [10]. It has been associated with low self-esteem, adverse pregnancy outcomes, increased risk of myocardial infarction, cardiovascular, respiratory, erectile, diabetes complications [11], cavernous sinus thrombosis and Ludwig angina which can be life-threatening [12].

The microbial community of caries is diverse and contains many facultatively and obligately anaerobic bacteria belonging to the genera *Actinomyces*, *Bifidobacterium*, *Eubacterium*, *Lactobacillus*, *Parvimonas* and *Rothia* [8]. It can also be caused by other bacteria, including members of the mitis, anginosus and salivarius groups of streptococci, *Propionibacterium*, *Enterococcus faecalis*, *Scardovi*, *Prevotella*, *Selenomonas*, *Dialister*, *Fusobacterium*, *Pseudoramibacter*, *Veillonella*, *Atopobium*, *Granulicatella*, *Leptotrichia* and *Thiomonas*. *Streptococcus mutans* is considered to be the principle etiological agent of dental caries [10] and has the ability to cause bacteria endocarditis and other severe complications [13].

The widespread uses of available antibiotics together with the length of time led to major problems of resistant organisms contributing to morbidity and mortality [14]. People infected with drug-resistant organisms are more likely to have longer and more expensive hospital stays, and may have a higher mortality rate. The antibacterial susceptibility patterns of bacterial isolates are important for determining appropriate empirical therapy for infections in critically ill patients. Therefore, the present study was carried out to determine drug resistance trends among isolates of dental caries which will help health care authorities for further planning, implementation and evaluation in public dental health service by building the effective management program for it.

## **MATERIALS AND METHODS**

The present research work was a cross-sectional study which was conducted in the Microbiology laboratory of Janaki Medical College Teaching Hospital (JMCTH), Janakpur, Nepal from April to November, 2014. All patients were examined clinically in the dental OPD by Dental Surgeon in the Department of Dentistry.

*Sample collection and processing:* A sterile cotton swab was taken and dipped in 1% glucose solution. The swab was then squeezed on the wall of clean, dry, sterile test tube and pressed gently on the portion of teeth cavity. The swab was lightly rotated 2-3 times in the cavity and again dipped into the same tube containing glucose solution and the tube was labelled with name, age and sex. Collected specimens were immediately incubated at 37°C for 3-4 hours. All culture media were prepared as instructed by the manufacturer company (Hi-media). A loopful of inoculum from glucose broth was streaked

on Blood Agar (BA) and Mac Conkey Agar (MA) plates. Plates were then incubated at 37°C for 24 hours and observed for significant growth of isolates which were sub-cultured on Nutrient Agar (NA) and BA plates and incubated at 37°C for 24 hours. Colony morphology was noted and Gram staining was performed for the identification of bacteria after incubation. Gram positive organisms were tested by catalase tests, oxidase test and their specific biochemical tests. Catalase, optochin sensitivity, indole, urease bile solubility and specific carbohydrate fermentation tests were done for the identification of *Streptococcus* spp. Similarly, coagulase, urease and mannitol fermentation tests were performed for the identification of *Staphylococcus* spp. The identification of various Gram negative isolates were done by using standard microbiological techniques described in Bergey's manual of Systematic Bacteriology, 2<sup>nd</sup> edition.

*Antibiotic susceptibility testing:* Antibiotic susceptibility test of the isolated organisms were done by using modified Kirby Bauer disc diffusion method. Bacterial inoculum was prepared by suspending the freshly grown bacteria in 2 ml of sterile nutrient broth and Brain Heart Infusion broth (BHIB) with yeast extract for non-fastidious and fastidious organisms respectively and incubated at 37°C for 3-4 hours. The turbidity of tube was matched with 0.5 Mc Farland turbidity standard. The inoculum was streaked on entire Muller-Hinton agar (MHA) plate. For fastidious organisms, it was streaked onto MHA plate with 5% blood. Antibiotic discs were placed and incubated at 37°C for 24 hour. Diameter of zone of inhibition was measured and zone diameter criterion was used to interpret the level of susceptibility to

each antibiotic Clinical and Laboratory Standards Institute (CLSI) [15].

*Data analysis*

The data were analyzed SPSS 16.0 version statistical software and Microsoft Excel 2007. The p-value <0.05 was considered statistically significant.

**RESULTS**

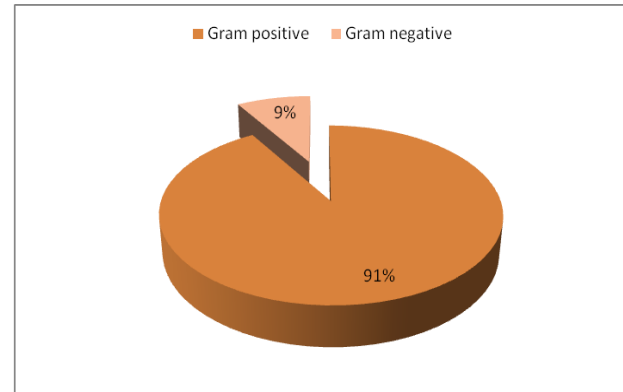
A total number of 330 patients were suspected as dental caries by dental surgeon attending dental OPD of JMCTH. Among them, 145 were male and 185 were female patients. The highest numbers of dental caries patients were found to be less than 20 years old of 56.06% followed between 21-40 years with 34.54%. The results are shown in table 1.

**Table 1: Age wise distribution of dental caries patients**

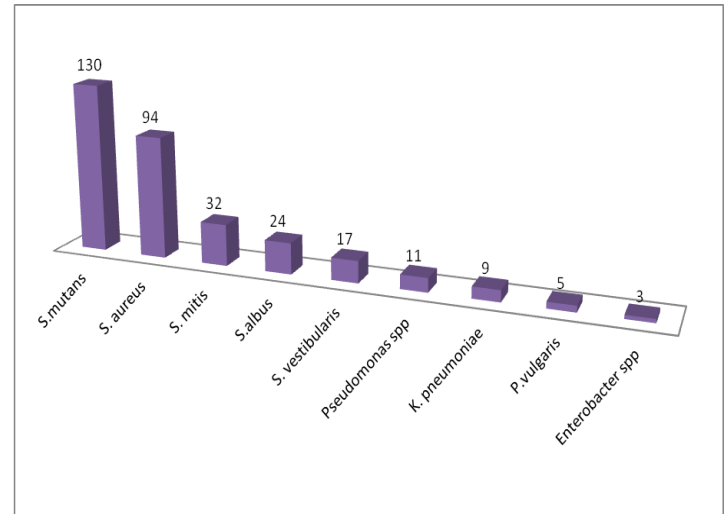
Age group ( yrs)	Male (%)	Female (%)	Total (%)
<20	82 (56.55)	103 (55.67)	185 (56.06)
21-40	47 (32.41)	67 (36.21)	114 (34.54)
>40	16 (11.03)	15 (8.10)	31 (9.39)
<b>Total</b>	<b>145 (100)</b>	<b>185 (100)</b>	<b>330 (100)</b>

*Bacteriological profile of dental caries patients:* A total of 325 bacterial isolates were identified in which 297 (91%) isolates were Gram positive and 28 (9%) isolates were Gram negative (figure 1). Of all Gram positive bacterial isolates, 130 (40%) were *S.mutans*, 94 (28.92%) were *S. aureus*, 32 (9.84%) were *S. mitis*, 24 (7.38%) were *S. albus*, 17 (5.23%) were *S. vestibularis*. Similarly, among Gram negative bacterial isolates, 11 (3.38%) were *Pseudomonas* spp., 9 (2.76%) were *K. pneumoniae*, 5 (1.52%) were *P. vulgaris* and 3 (1%) were *Enterobacter* spp. The results are shown in figure 2.

**Figure 1: Gram staining reaction**



**Figure 2: Bacteriological profile of dental caries patients**



*Antibiotic resistance pattern of bacterial isolates:* *S. mutans* was highly resistant to penicillin. More than sixty-six percent (66.15%) isolates of *S. mutans* were resistant to Penicillin; 60.76% were to tetracycline and 20% were resistant to cotrimoxazole. *S. aureus* was found to be highly resistant towards penicillin (91.48%) followed by tetracycline (86.17%) and ampicillin (61.70%). *S. mitis* was resistant to tetracycline (78.12%) followed by ciprofloxacin (65.62%). *Pseudomonas* spp were highly resistant to tetracycline followed by cotrimoxazole (90.90%). The results are shown in table 2.

**Table 2: Antibiotic resistance pattern of bacterial isolates**

Isolates	Amp	Ceft	Cip	Cot	Ery	Gen	Tet	Pen	Imp
<i>S.mutans</i>	26.92%	41.56%	22.30%	20%	0%	0%	60.76%	66.15%	-
<i>S. aureus</i>	61.70%	20.21%	21.24%	50%	46.80%	58.51%	86.17%	91.48%	-
<i>S. mitis</i>	40.62%	34.37%	65.62%	21.84%	43.75%	28.12%	78.12%	46.87%	-
<i>S.albus</i>	7.40%	7.83%	1.54%	7.83%	2.63%	1.73%	2.43%	6.43%	-
<i>S. vestibularis</i>	5.38%	4.31%	5.72%	1.21%	5.72%	2.43%	4.12%	5.05%	-
<i>Pseudomonas spp</i>	72.72%	0%	0%	90.90%	-	18.18%	100%	-	9.09%
<i>K. pneumoniae</i>	22.22%	11.11%	22.22%	55.55%	-	11.11%	33.33%	-	33.33%
<i>P.vulgaris</i>	10.71%	0%	0%	7.14%	-	3.57%	17.85%	-	0%
<i>Enterobacter spp</i>	2.12%	0%	1.01%	1.23%	-	2.63%	3.57%	-	0%

Amp- Ampicillin; Ceft- Ceftriaxone; Cip- Ciprofloxacin; Cot- Cotrimoxazole;  
Ery- Erythromycin; Gen- Gentamicin; Tet- Tetracycline; Pen- Penicillin

## DISCUSSION

Dental caries is the most studied oral disease worldwide and the majority of studies are concentrated in school children, with not enough research on the situation of the disease in young adults and old ages. The present study showed the frequency of dental caries was found to be higher in age group of less than 20 years old followed by adulthood. This may be attributed to the higher consumption of sugary foods such as chocolate, candy, jellies, soft drinks, etc..

The present study identified nine different types of bacterial isolates of Gram positive bacteria (*S.mutans*, *S. aureus*, *S. mitis*, *S.albus*, *S. vestibularis*) and Gram negative bacteria (*Pseudomonas spp*, *K. pneumoniae*, *P.vulgaris* and *Enterobacter spp*) isolated from dental caries patients. All isolates were subjected to the antibiotic susceptibility tests.

Antibiotic is routinely prescribed for 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 results in emergence of resistant strains [16]. 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 [17]. It may also be recommended that amoxicillin and penicillin G are the most effective antibacterial drugs for the treatment of dental caries [18].

A substantial resistance was observed to a number of commonly used antibiotics in the present study. *S. mutans* showed higher resistance toward penicillin (66.15%) and tetracycline (60.76%). Maripandi et al. (2011) also reported 22.98% *Streptococcus spp.* was

resistant to penicillin and the same percentage was sensitive to tetracycline which is vast difference with the present study [19]. But, in a study conducted by Dwivedi et al. (2011), the resistance percentage against penicillin and tetracycline was 48% and 66% respectively [20]. Similarly, Rozkiewicz et al. (2006) reported that 52% of *S. mutans* was resistant to tetracycline [21] which is almost in accordance with this study.

*S. aureus* was found to be highly resistant towards penicillin (91.48%) and tetracycline (86.17%). *S. mitis* was found to be highly resistant towards tetracycline (78.12%) followed by ciprofloxacin (65.62%) and penicillin (46.87%) and 28.12% to gentamycin. Rozkiewicz et al. (2006) also found *S. mitis* resistant to ciprofloxacin (55.2%) and gentamicin (25.9%) [21] which is almost alike with this study.

All the *Pseudomonas* spp were found to be resistant to tetracycline followed by cotrimoxazole of 90.90% and ampicillin of 72.72%. *K. pneumoniae* was found to be resistant towards cotrimoxazole with 55.55%. The study conducted by Omolaja et al. (2013) reported *P. aeruginosa* and *K. pneumoniae* showed highest level of resistance to chloramphenicol and tetracycline.

Out of 325 culture positive samples, 267 isolates (82.15%) were found to be MDR. In gram negative isolates, 21.42% (6/28) were found MDR whereas in gram positive isolates, 46.80% (139/297) were found MDR but was statistically insignificant ( $p=0.72$ ). MDR is defined as resistance of two groups of the antimicrobial agents. The high frequency of multiple antibiotics resistance might be a reflection of inappropriate use of

antimicrobials, lack of laboratory diagnostic tests, unavailability of guideline for the selection of antibiotics.

Bacterial resistance to  $\beta$ -lactam antibiotics is primarily due to the production of  $\beta$ -lactamase enzyme which attacks  $\beta$ -lactam ring of the antibiotics rendering them inactive [22]. In some developing countries antibiotics are available without prescription and this potentially facilitates overuse. Use of closely related drugs for other condition also acts a role in the broadening resistance [23]. Inappropriate practices like misuse and abuse of antibiotics and unskilled practitioners can also lead to emergence of resistance in bacteria. Expired antibiotics, self medication, counterfeit drugs, inadequate hospital control measures promote the development of resistance in clinical isolates [24]. The worldwide increase in both community and hospital-acquired antimicrobial-resistant bacteria is threatening the capability to efficiently treat patients, emphasizing the requirement for continued surveillance, more appropriate antimicrobial prescription, prudent infection control and new treatment alternatives [14] [24]. The use of molecular biology techniques would also enhance the molecular identification of resistance genes [24].

## CONCLUSION

Dental caries, a chronic disease is unique among human and is one of the most common important global oral health problem which hinders the achievement and maintenance of oral health in all age groups in the world today [25] The present study concluded the incidence of dental caries was found to be higher in age group of less than 20 years old. *Streptococcus mutans*, *Streptococcus mitis*, *Staphylococcus aureus*, *Staphylococcus albus*

and *Pseudomonas aeruginosa* were directly associated with the establishment of dental infections. *S. mutans* and *S. aureus* has become an important theme of concern in dental caries because of its higher predominance of isolation and emergence of drug resistance. Abuse of antibiotics has led to the emergence of Multi Drug Resistant bacteria which are difficult to control as these bacteria are resistant to most of the antibiotics. The emergence of multi drug resistance of bacterial isolates was observed which suggests the need for a regular scrutiny program at time interval regularly to control and manage the antibiotic resistance bacteria.

#### ACKNOWLEDGEMENT

Authors wish to acknowledge the support of all the participants of the study and are profusely thankful to Dr. Ram Snehi Sah, Dental Surgeon, and Mr. N.P Yadav, Assistant professor in Microbiology, Janaki Medical College Teaching Hospital, Janakpurdham for their trustworthy support carry out this research.

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