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Antibiogram profiles against polymicrobial pathogens among dental caries patients at Janaki Medical College teaching hospital, Nepal

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Abstract

Objectives: The microbial community of dental caries with many of the facultatively and obligately-anaerobic bacteria is diverse and frequencies, pattern, and distributions of resistant bacteria vary significantly with geographic regions and often reflect the usage patterns of antibiotics. Therefore, the present study aims to determine polymicrobial pathogens causing dental caries and to determine the antibiotic sensitivity pattern of bacterial isolates.

Materials and Methods: This cross-sectional study was carried 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: The incidence rate of dental caries was found to be 62.5% among study population. *Streptococcus mutans* was most predominant to cause dental caries. All bacteria isolates were found to be most sensitive towards ciprofloxacin followed by other tested antibiotics.

Conclusion: Female had more dental caries than male in present study. Ciprofloxacin was the most effective drugs against dental pathogens and β -lactam with β -lactam inhibitors were recommended for bacterial resistance.

Keywords: Dental caries, *S. mutans*, β -lactam inhibitors, Ciprofloxacin, β -lactamase.

1. Introduction

Dental caries, known as tooth decay or tooth cavity is considered as a major public health problem globally due to its high prevalence and significant social impact. World Health Organization reports 60-90% of school children worldwide have experienced caries, with the disease being most prevalent in Asian and Latin American countries [1]. At present, the distribution and severity of dental caries vary in different parts of the world and within the same country or region. Nepal is a developing country with little awareness and practice regarding oral health amongst Nepalese population and has a high morbidity of dental caries in all age groups of both genders [2]. The prevalence pattern of dental caries varies with age, sex, socio economic status, race, geographical location, food habits and oral hygiene practices.

Dental caries is an infectious disease where bacterial metabolic processes damage dental hard tissue structure and is marked by localized progressive demineralization of the crown and root surfaces of the tooth [3]. It is one of the most important chronic disease that progresses slowly in most of the people [4] which results from an ecological imbalance in the equilibrium between tooth minerals and oral biofilms [5]. The biofilm is characterised by microbial activity, resulting in fluctuations in plaque pH. This is a result of both bacterial acid production and buffering action from saliva and the surrounding tooth structure. The tooth surface is therefore in a dynamic equilibrium with its surrounding environment. As the pH falls below a critical value i.e. 5.5, the demineralisation of enamel, dentine or cementum occurs, while the pH increases above critical value, remineralisation occurs [6]. The process of demineralisation and remineralisation takes place frequently during the day. Over time, this process leads to either caries lesions or the repair and reversal of a lesion [7].

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*,

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Enterococcus faecalis, *Scardovi*, *Prevotella*, *Selenomonas*, *Dialister*, *Fusobacterium*, *Pseudoramibacter*, *Veillonella*, *Atopobium*, *Granulicatella*, *Leptotrichia* and *Thiomonas* [9-12]. *Streptococcus mutans* is considered to be the principle etiological agent of dental caries [9, 13].

The classic description of the cause of dental caries includes three factors: host, bacteria and diet. Dental caries occurs when a susceptible tooth surface is colonized with cariogenic bacteria and dietary source of sucrose or refined sugar is present. Bacterial pathogens produce lactic acid from fermentation of carbohydrates and this acid dissolves the hydroxyapatite crystal structure of the tooth which causes caries [14].

The experience of pain, problem with eating, chewing, smiling and communication due to missing, discolored or damaged teeth have a major impact on people's daily lives and well-beings [15]. Dental disease has been associated with low self-esteem, adverse pregnancy outcomes, increased risk of myocardial infarction, cardiovascular, respiratory, erectile, diabetes complications [16], cavernous sinus thrombosis and Ludwig angina which can be life-threatening [17].

In recent years, multiple drug resistance have been developed in both human and plant pathogenic microorganisms due to the indiscriminate use of commercial antibacterial drugs commonly used in the treatment of infectious diseases [18]. Resistance of numerous bacterial pathogens to many antibiotics continues to increase globally. Frequencies, pattern, and distributions of resistant bacteria vary significantly with geographic regions and often reflect the usage patterns of antibiotics [19]. 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 usage [18].

Inappropriate antibiotic prescribing and its use have been identified as major factors in the emergence 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 [20]. Fluoroquinolones are quinolone antimicrobials which are active against many β -lactam resistant bacteria. Amoxicillin/clavulanic acid, combination of a β -lactam antibiotic (amoxicillin trihydrate) and a β -lactamase inhibitor (potassium clavulanate) has broad antimicrobial spectrum and effective against amoxicillin-resistant bacteria that produce β -lactamase [21]. Such antimicrobial agent may prove valuable for managing dental infections.

Production of β -lactamase is, however, unusual for most of streptococci, where resistance is happening by slight alteration in penicillin binding proteins [22]. Bacterial resistance to antibiotics such as penicillin and other β -lactam is a health issue in numerous parts of the world. Therefore, the present study aims to determine polymicrobial pathogens causing dental caries and to determine the antibiotic sensitivity pattern of bacterial isolates. The findings will help in the necessary intervention program to monitor the spread of resistant bacteria and identify the common bacterial agent involved in caries. Moreover, the results will help health care authorities for further planning, implementation and evaluation in public dental health service by building the effective management program for it.

2. Materials and Methods

The present research work was a cross-sectional study which was conducted in the Microbiology laboratory of Janaki Medical College and Teaching Hospital (JMCTH), Janakpur,

Nepal from April to December, 2014. All the patients were examined clinically in the dental OPD by Dental Surgeon in the Department of Dentistry. Informed consent was obtained from the participants prior to the study and work approval was taken from the institutional ethical committee. The patients reported with complaint of teeth cavities associated with or without pain, pus discharge and biofilm deposits or staining in the teeth were included. Caries free dental patients were excluded from the study.

2.1 Specimen Collection

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. In case of staining teeth, samples were collected by scrubbing the staining area of teeth by curette and dipped in glucose solution and the tube was labelled with name, age and sex. Collected specimens were immediately incubated at 37 °C for 3-4 hours.

2.2 Specimen Processing

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. The significant growth of isolates were sub-cultured on Nutrient Agar (NA) and BA plates and incubated at 37 °C for 24 hours.

2.3 Identification of the Isolates

After incubation, visual growth on the inoculated plates was observed and colony morphology was noted. Identification of the isolates was done by using standard microbiological techniques as described in the Bergy's Manual which involved morphological appearance of the colonies, Gram's staining and biochemical properties.

2.4 Identification of Gram Positive Isolates

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.

2.5 Identification of Gram negative Isolates

The identification of various Gram negative isolates was done by using standard microbiological techniques described in Bergey's manual of Systematic Bacteriology (2nd edition). The isolates were identified on the basis of various biochemical tests such as catalase test, oxidase test, O/F test, MR/VP test, SIM test, citrate test, urease test, TSI test.

2.6 Antibiotic Susceptibility Testing of the Bacterial Isolates

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 for those organisms that were Gram negative 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 then streaked on

entire Muller-Hinton agar (MHA) plate. For *Streptococcus* spp, bacterial inoculum was prepared by suspending the freshly grown bacteria in 2 ml of sterile Brain Heart Infusion broth (BHIB) with yeast extract and the turbidity of tube was matched with 0.5 Mc Farland turbidity standard. It was then streaked onto MHA plate with 5% blood. Antibiotic discs were placed around the outer edge of the plate and incubated overnight at 37 °C. Diameter of zone of inhibition was measured and zone diameter criterion was used to interpret the level of susceptibility to each antibiotic CLSI (2013) [23].

2.7 Statistical Analysis

The data were analyzed using statistical package for social science (SPSS) 16.0 version statistical software and Microsoft excel 2007. The p-value 0.05 was considered statistically significant.

3. Results

In present study, total numbers of patients attending dental OPD, JMCTH were 528 of which 226 were male and 302 were

female. Among them, 330 patients had dental caries where of 43.93% were male and 56.06% were female. Female had more dental caries than male. This was found to be statistically insignificant. The results are shown in table 1.

Table 1: Incidence of dental caries among total OPD patients

Gender	Dental caries		Total OPD patients	Statistics
	Positive	Negative		
	No. (%)	No. (%)		
Male	145 (43.93)	81 (40.90)	226	$\chi^2= 0.46$ $p = 0.65$
Female	185 (56.06)	117 (59.09)	302	
Total	330 (100)	198 (100)	528	

The highest numbers of dental caries patients were found to be in 16-20 years age group where of 36 (42.9%) patients were male and 48 (57.1%) patients were female. It was followed by under 5 years children in which 23 (46%) were male and 27 (54%) were female. The results are shown in figure 1.

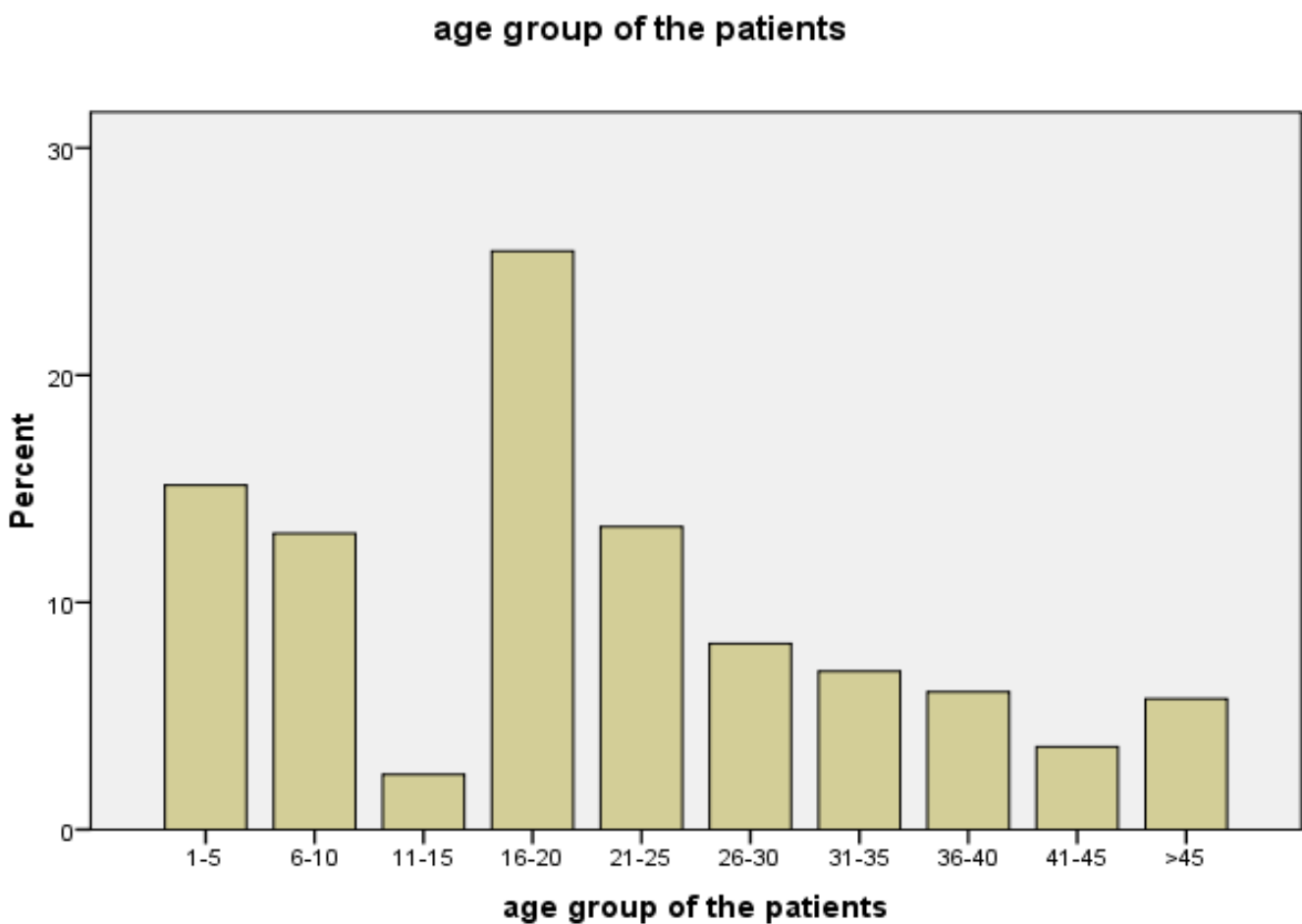


Fig 1: Age wise distribution of dental caries patients

3.1 Macroscopic Examination of Patient’s Teeth

Three hundred thirty caries patient’s teeth were examined macroscopically. Among them, 198 (60%) had visible holes, 35 (10.6%) had brown staining, 64 (19.4%) had black staining, 33 (10%) had white staining and pus discharge respectively from their teeth surface. Similarly, 15 (4.5%), 14 (4.2%), 24 (7.3%), 27 (8.2%) and 35 (10.6%) had lower central incisor caries, lateral incisor caries, canine caries, first molar caries and second molar caries respectively. Thirty three (10%), 28 (8.5%), 31 (9.4%), 58 (17.6%) and 65 (19.7%) had upper central incisor caries, lateral incisor caries, canine caries, first

molar caries, and second molar caries respectively. Teeth caries was found to be more on upper second molar followed by upper first molar tooth.

3.2 Microbial Growth among total specimen

A total of 330 dental swab samples were collected from patients for culture in whom 145 samples were collected from male and 185 samples from female patients. The microbial growth was observed higher in female (56.61%) than male (43.38%). This was found to be statistically insignificant. The results are shown in table 2.

Table 2: Microbial growth among total specimen

Gender	Microbial growth		Total samples	Statistics
	Positive	Negative		
	No. (%)	No. (%)		
Male	141 (43.38)	4 (80)	145	$\chi^2=2.68$ $p=0.78$
Female	184 (56.61)	1 (20)	185	
Total	325 (100)	5 (100)	330	

3.3 Pattern of Microbial isolates from Dental Caries Patients

A total of 325 bacterial isolates were identified in which 297 isolates were Gram positive and 28 isolates were Gram negative. Of 90% Gram positive bacterial isolates, 43.77% were *S. mutans*, 31.64% were *S. aureus*, 10.77% were *S. mitis*,

8.08% were *S. albus*, 5.72% were *S. vestibularis*. Similarly, among 8.48% of Gram negative bacterial isolates, 39.28% were *Pseudomonas* spp., 32.14% were *K. pneumoniae*, 17.85% were *P. vulgaris* and 10.71% were *Enterobacter* spp. Among all bacterial isolates, *S. mutans* was found to be predominant organism to cause dental caries followed by *S. aureus*.

Among all bacterial isolates, 27.69% bacteria were isolates from incisor, 16.92% bacteria were isolates from canine, 26.15% bacteria were isolated from 1st molar and 30.76% bacteria were isolated from 2nd molar. The bacterial isolates were found more in 2nd molar tooth followed by others. The results are shown in table 3.

Table 3: Toothwise distribution of isolated organisms

Isolated organisms	Types of tooth				Total
	Incisor	Canine	1 st molar	2 nd molar	
	No. (%)	No. (%)	No. (%)	No. (%)	
<i>S. mutans</i>	33 (25.38)	14 (10.76)	33 (25.38)	50 (38.46)	130
<i>S. aureus</i>	29 (30.85)	18 (19.14)	23 (24.46)	24 (25.53)	94
<i>S. mitis</i>	12 (37.5)	7 (21.87)	6 (18.75)	7 (21.87)	32
<i>S. albus</i>	5 (20.83)	7 (29.16)	8 (33.33)	4 (16.66)	24
<i>S. vestibularis</i>	4 (23.52)	4 (23.52)	6 (35.29)	3 (17.64)	17
<i>Pseudomonas</i> spp	2 (22.22)	2 (22.22)	0	5 (55.55)	11
<i>K. pneumoniae</i>	3 (27.27)	2 (18.18)	6 (54.54)	0	9
<i>P. vulgaris</i>	2 (40)	0	0	3 (60)	5
<i>Enterobacter</i> spp	0	0	0	3 (100)	3
Total	90(27.69)	55 (16.92)	85 (26.15)	100 (30.76)	325

3.4 Distribution of bacterial isolates fermenting Sucrose

Among 325 bacterial isolates, 305 (93.84%) bacterial isolates were sucrose fermenter and 20 (6.15%) bacterial isolates did not ferment sucrose. The sucrose non-fermenter isolates were *Pseudomonas* species and *K. pneumoniae*.

Among all Gram positive isolates, bacteria was found to be most sensitive towards ciprofloxacin (94.27%) followed by gentamicin (51.85%) and erythromycin with 49.49%. The results are shown in table 4.

Table 4: Antibiotic susceptibility pattern of Gram positive bacterial isolates

Antibiotic Used	Sensitive		Intermediate		Resistant		Total
	No.	%	No.	%	No.	%	
Ampicillin	-	-	-	-	118	39.73	297
Ceftriaxone	94	31.64	24	8.08	-	-	
Ciprofloxacin	280	94.27	17	5.72	-	-	
Cotrimoxazole	-	-	-	-	118	39.73	
Erythromycin	147	49.49	56	18.85	94	31.64	
Gentamicin	154	51.85	94	31.64	49	16.49	
Tetracycline	24	8.08	126	42.42	147	49.49	
Penicillin	-	-	17	5.72	162	54.54	

Among all Gram negative isolates, bacteria were found to be more sensitive towards ciprofloxacin followed by imipenem and gentamicin of 89.28% and ceftriaxone with 50% respectively. The results are shown in table 5.

Table 5: Antibiotic susceptibility pattern of Gram negative bacterial isolates

Antibiotic Used	Sensitive		Intermediate		Resistant		Total
	No.	%	No.	%	No.	%	
Ampicillin	5	17.85	9	32.14	14	50	28
Ceftriaxone	14	50	-	-	16	57.14	
Ciprofloxacin	28	100	-	-	-	-	
Cotrimoxazole	8	28.57	9	32.14	11	39.28	
Gentamicin	25	89.28	-	-	3	10.71	
Imipenem	25	89.28	-	-	3	10.71	
Tetracycline	-	-	9	32.14	19	67.85	

4. Discussion

Dental caries is the most studied oral disease in the world, the majority of studies concentrated in school children, with not enough research on the situation of the disease in young adults and old ages. The prevalence of dental caries was observed in many studies. Adhikari *et al* (2012) [24] reported the prevalence of dental caries in study population to be 47.1%. Similarly, the prevalence of dental caries was 60.3% in the study conducted by Bhagat and Shrestha (2014) [15]. The present study showed that the incidence of dental caries was found to be 62.5% which shows as the time goes on, the increasing pattern of caries patients among the population also raise as per the research work.

In this study, the occurrence of dental caries in female (56.06%) was found to be higher than in male (43.93%). It may be due to female’s dental eruption six months earlier than males, so they are exposed to cariogenic factors or may be due

to the increasing sugar consumption, low exposure to fluorides containing toothpaste and poor access to oral health care. Dikshit and Limbu (2013) [25] showed the prevalence of dental caries in male with 55% and in female with 44% which is different from this study which may be due to the high number of male patients.

The present study showed the frequency of dental caries was found to be higher in age group of 16-20 years old with 25.45% which is the pin point of adolescence period followed by 0-5 years old children with 15.15%. As the age advances there was rise in proportion of population affected by caries. The majority of adolescence (10-19 years) and children (1-9 years) had dental caries due to consumption of sugary foods such as chocolate, candy, jellies, soft drinks, etc. approximately 3-5 times daily, which may be considered one of the most important factors in high caries experience in these groups. Similar findings were obtained in other studies as well. According to Subedi *et al* (2011) [26], the prevalence of dental caries in 12-13 age groups was 53.23%. A study conducted by Khanal *et al* (2013) [27] reported 85.25% dental caries was found in children of 1-6 years old in Jorpati, Kathmandu. Caries was significantly higher in children with mothers of low education and low family income. Similarly, Adhikari *et al* (2012) [24] reported caries prevalence in the age group 11-14 years was 52.46% in tertiary care centre in western region of Nepal. Dikshit and Limbu (2013) [25] recognized caries prevalence in 12-13 year olds was 41% in Chepang school children of Chitwan district which is not concurring with this study which may be due to the least number of patients of 11-15 age groups.

The present study reported the pattern of teeth wise distribution of caries among the study population, which showed that the most severely affected teeth were maxillary molars (37.3%) followed by mandibular molars (18.8%) and maxillary incisors (18.5%). The mandibular incisors were the least affected teeth (8.7%). This may be due to the anatomical structures of molars tooth which have lots of grooves, pits and crannies that collects food particles. As a result they are harder to keep clean than front teeth where plaque can build and bacteria can thrive between back teeth as molars, producing the acids that destroy tooth enamel. The similar findings were also obtained in the study conducted by Dikshit and Limbu (2013) [25] who reported maxillary molars (32.11%), mandibular molars (36.92%) and maxillary incisor (22.55%) being predominantly affected teeth by caries while mandibular incisors (1.12%) were least affected. Both the studies showed maxillary and mandibular molars and maxillary incisors were more affected whereas the same studies showed mandibular incisors were least affected. This may be due to mandibular incisors are protected by the tongue and opening of major salivary ducts near the incisors which helps it to more resistance towards caries. Saliva maintains the super-saturation of calcium in plaque and also neutralises acids, raises pH and reverses the diffusion rate of calcium and phosphate towards the tooth surface.

The present study showed greater number of isolates were Gram positive bacteria (90%) whereas the contribution of Gram negative bacteria in dental caries was 8.48%. The incidence rate of Gram positive bacteria was found higher in caries than Gram negative bacteria because the most common bacteria that are found in the supragingival plaque are Gram positive cocci (*S. mitis*, *S. oralis*, *S. sanguis*, *S. mutans*, *S. gordonii*, *S. aureus* and *S. epidermidis*). These prominent bacteria are responsible for the plaque formation due to their interactions with each other and the tooth surface. Similar

results were obtained in a study conducted by Olajokun *et al* (2008) [28] in which 100% of bacterial isolates were Gram positive associated with caries.

Among all the bacterial isolates, *S. mutans* were predominant bacteria (43.77%) associated to caries. *S. mutans* collect around the teeth and gums in a sticky, creamy-coloured mass called plaque, which serves as a biofilm. Bacteria in a person's mouth convert sugars (glucose and fructose, and most commonly sucrose) into acids such as lactic acid through a glycolytic process called fermentation. If left in contact with the tooth, these acids may cause demineralization, which is the dissolution of its mineral content. The similar finding was also obtained in a study conducted by Olajokun *et al* (2008) [28] who reported 45.6% *S. mutans* was related to caries which is in accord with this study.

The present study showed 39.28% *Pseudomonas* spp. linked with caries followed by 32.14% *K. pneumoniae*, 31.64% *S. aureus* and 17.85% *P. vulgaris*. The rest were followed by 10.77% *S. mitis*, 10.71% *Enterobacter* spp, 8.08% *S. albus* and 5.72% *S. vestibularis*. Omolaja *et al* (2013) [29] found 53.13% of *Streptococcus mutans* and only 6.25% *P. aeruginosa* and *P. vulgaris* were isolated from caries. Similarly, Olajokun *et al* (2008) [28] reported 45.6% *S. mutans*, 41.2% *Lactobacillus* spp and 13.2% *S. aureus* were isolated from carious lesion.

In this study, *S. aureus* was found to be second most common Gram positive bacteria associated with caries which is similar to a study conducted at Department of the Rajah Muthiah Dental College and Hospital Annamalai University which reported *S. aureus* was found to be the most prevalent organism [30]. *S. aureus* in the aetiology of oral dysaesthesia and mucositis is complicated by the diversity of the normal oral flora and by healthy carriage of *S. aureus* in some patient groups. However, the high rates of *S. aureus* are suspected in patients presenting with symptoms of oral mucosa pain, burning, erythema and swelling. Isolates of *S. aureus* are capable of producing a wide range of exfoliative toxin and enterotoxin [31].

The present study showed that almost all bacterial isolates were fermenting sucrose fermenter except *Pseudomonas* spp and *K. pneumoniae*. Because these bacteria do not possess extracellular enzyme capable of cleaving the α -1 and α -2 glycosidic bond of sucrose. Sucrose is a fermentable carbohydrate which has been shown to be caries initiation and development. *K. pneumoniae* is classically thought of as community acquired and occurring in elderly and debilitated population with underlying alcoholism, chronic lung disease or diabetes. *Pseudomonas* spp is an opportunistic pathogen that significantly increases morbidity and mortality in nosocomial infections and its pathogenicity especially relies on the production of cellular and extracellular virulence factors associated with root caries.

In this study, all Gram positive bacterial isolates were found more sensitive towards ciprofloxacin (94.27%) followed by gentamicin (51.85%) and erythromycin with 49.49% whereas all Gram negative isolates were found to be more sensitive towards ciprofloxacin followed by imipenem and gentamicin of 89.28% and ceftriaxone with 50% respectively. The present study also revealed antibiotic susceptibility testing of the isolates showed high degree of resistance towards different antibiotics.

The key factor influencing the emergence of resistance to antibiotic is due to overuse of antibiotics in humans. In some developing countries antibiotics are available without prescription and this potentially facilitates overuse. Use of closely related drugs for other condition also plays a role in the

spread of resistance [32]. In the present study, a substantial resistance was observed to a number of commonly used antibiotics. This may be due to the indiscriminate and inappropriate use of antibiotics that is rampant in Nepal. Hence, it is important to periodically monitor the antibiotic resistance pattern in different regions.

5. Conclusion

Nepal is one of the developing countries facing a high prevalence of dental caries in all age groups which is life threatening infectious disease. The present study concluded that most commonly *S. mutans*, *S. aureus*, *S. mitis*, *S. albus*, *S. vestibularis* and *Pseudomonas aeruginosa* were directly associated with the establishment of dental infections. It was further concluded that prevalence of dental caries was found to be in an increasing trend, compared to previous studies. Moreover, 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. However, multiple drug-resistance patterns of some strains of bacterial isolates to chloramphenicol and tetracycline should be of interest. Thus, the current study pointed out that there is a great need to monitored antimicrobial resistance of bacterial isolates at time interval regularly. In turn, this study will allow the development of novel diagnostic and treatment methods in reducing periodontitis, gingivitis, cellulitis, malocclusion and others life threatening clinical conditions as the adverse pregnancy outcomes, increased risk of myocardial infarction, cardiovascular, respiratory, erectile, diabetes complications, cavernous sinus thrombosis and Ludwig angina globally.

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