Original Article

J Nepal Health Res Counc 2023 Jan-Mar;21(58): 122-8

Microbial Spectrum of Complete Denture Wearer in Old Age People of Chitwan

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ABSTRACT

Background: Human oral cavity contains many microorganisms, the habitat of which may be changed by complete denture among edentulous people. The complete dentures favor aggregation of microorganism. The aim of this study was to identify the microorganisms present in the complete dentures of old age people of Chitwan and assess the sensitivity pattern of the microorganisms to the common antibiotics.

Methods: A descriptive cross-sectional study was conducted at Chitwan Medical College, Chitwan, Nepal among 45 old age people who have been wearing dentures above one year. The duration of the study was from 18th Nov 2021 to 12th May 2022. Swab was taken from the polished and tissue surfaces of both maxillary and mandibular dentures in the Department of Prosthodontics while the laboratory-based experiments were conducted in the Department of Microbiology. Antibiotic sensitivity was also done. The data was analyzed using Statistical Package for Social Science (SPSS) version 16.0. Descriptive statistics were used. The data was presented in form of frequency, percentage, mean and standard deviation.

Results: *Streptococcus* spp. was predominant microorganism followed by Coagulase-negative Staphylococci and *Staphylococcus aureus*. The highest sensitivity pattern was observed to Amikacin, Nalidixic acid and Ciprofloxacin while the most resistant antibiotics were Amoxicillin/clavulanic acid and Cefixime. *Escherichia coli* was sensitive to all the tested antibiotics.

Conclusions: In this study, *Streptococcus* spp. followed by Coagulase-negative Staphylococci and *Staphylococcus aureus were* the most frequently identified microorganisms from the dentures of old age people. Amikacin, Nalidixic acid and Ciprofloxacin were highly sensitive among the people of old age.

Keywords: Antibiotic sensitivity; complete denture; microorganisms

INTRODUCTION

An increasing number of people in the old age have lost their teeth due to dental caries, periodontal diseases or trauma.[1] The prevalence of edentulism was reported as 8.58 % in a study conducted in Kathmandu.[2] The completely edentulous state is restored most commonly by complete dentures, fabricated using synthetic resins which are means for adhesion and colonization of microorganisms.[3-5]

During the old age, due to reduced salivary flow and self-cleansing activity of tongue, ageing factor and need of special care, the denture hygiene is not maintained.[4,6] As a result, there is formation of denture plaque. The end result is denture stomatitis, denture malodor and prevalence of systemic diseases.[5, 7]

Studies related to presence of microorganisms among the long-term denture use and the antibiotic sensitivity pattern is sparse in literature. This study was conducted to identify the microorganisms present in the complete dentures of old age people of Chitwan and assess the antibiotic sensitivity patterns.

METHODS

A descriptive cross-sectional study was conducted at Chitwan Medical College, Chitwan, Nepal. The duration of the study was from 18th Nov 2021 to 12th May 2022. The study was approved by Nepal Health Research Council (Ref No 1087). The sample size was calculated using formula $n=Z^2pq/e^2$, Where, p = 97.14% = 0.9714[8] q = 1-p = 0.0286 and e (margin of error) = 5%,

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The sample size was calculated as 42.67, however, in the study 45 participants were included. A convenient sampling technique was used. Participants within the age group of 55-80 years, wearing dentures for more than one year, who could visit the Department of Prosthodontics, Chitwan Medical College, who did not take any antifungal and antimicrobial therapy for past three months were included in the study. Very old denture wearers who did not remove the dentures for several years, with known systemic diseases and unwilling to participate in the study were excluded.

The samples were collected in the Department of Prosthodontics while the laboratory-based experiments were conducted in the Department of Microbiology Chitwan Medical College, Bharatpur, Nepal. All the participants were explained about the study objectives, and also asked if they could come to the department. Those who gave verbal consent to participate were enlisted in an order in Microsoft excel. The participants were divided into three groups with each group containing 15 participants. Each group was invited in three different days.

After they arrived, the detail objectives and procedures were explained and then the written consent was taken. They were then seated in the dental chair, clinical examination was done and the required information was recorded in the predesigned proforma. An identification number was given to each participant. Then each polished and the tissue surface of both maxillary and mandibular arch was given a code. The same codes were also marked on the sterilized test tubes. Care was taken to avoid mix-ups and contamination. The complete dentures after taking out from participant's mouth were gently cleaned with normal saline. Swab were at first soaked in nutrient broth and rubbed gently over the polished surface and tissue surface of both maxillary and mandibular arch. Each of these swabs were then transferred into a labelled sterilized test tubes containing 2.0 ml of nutrient broth, transported to Department of Microbiology within one hour and incubated at 37°C for 18-24 hours.

The next day, the test tubes were shaken well. With the help of sterilized wire loop, subculture was done in Nutrient agar, MacConkey agar and Blood agar respectively and again incubated at 37°C for 24-48 hours.

The microorganisms were identified on the basis of their gram staining, colony morphology in different media and biochemical test such as, catalase test, oxidase test, triple sugar iron test, Simmon's citrate agar, Sulphide indole motility, Christensen's urease medium. All the yeast grown after subculture were further inoculated in SDA and incubated at 37°C for 24-48 hours. Identification of yeast were done by sugar assimilation and fermentation.

Antibiotic sensitivity test was done by Kirby-Bauer disk diffusion method on Muller Hinton agar using antibiotic discs (HiMedia Laboratories Pvt. Limited, India). The following antibiotics were used: Amikacin (AK: 30 µg), Amoxicillin/clavulanic acid (AMC: 20/10 µg), Cefixime (CFM: 5 µg), Cefotaxime (CTX: 30 µg), Ceftazidime (CAZ: 30 µg), Ceftriaxone (CTR: 30 µg), Ciprofloxacin (CIP: 5 µg), Cotrimoxazole (COT: 32.7 µg), Nitrofurantoin (NIT: 50 µg) and Nalidixic acid (NA: 30 µg). All the isolates were classified as sensitive (S), Resistant (R) and Intermediate (I) in accordance with the standardized table supplied by CLSI 2016.[9]

Data were entered in Microsoft excel and then exported and analyzed using Statistical Package for Social Science (SPSS) version 16.0. Descriptive statistics were used. The data was presented in form of frequency, percentage, mean and standard deviation. Tables were constructed to present the results.

RESULTS

In total 45 people of age 55-80 years (mean age 72.42 years ± 6.7 SD) participated in this study. Among them, 17 (37.8%) were females and 28 (62.2%) were males (Table 1).

Table 1. Mean age and gender wise distribution of participants.

Particulars of participants	Frequency (%)
Mean age (years) ± SD	72.44±6.7
Female	17 (37.8)
Male	28 (62.2)

Among the 180 samples, Gram-positive cocci were isolated in 109 (60.6%), Gram-negative bacilli in 56 (31.1%) (Table 2).

Table 2. Characteristics of microorganisms after gram staining.

Microorganisms characteristics	Frequency (%)
Gram Negative Bacilli	56 (31.1)
Gram Positive Cocci	109 (60.6)
Gram Positive Rod	7 (3.9)
Yeast	8 (4.4)

Among the microorganisms, *Streptococcus* spp. was predominantly isolated from both the dentures, followed by Coagulase-negative Staphylococci and *Staphylococcus aureus*. Apart from this *Candida albicans* was also isolated from 2 participants samples (Table 3).

An antibiotic sensitivity test was also performed on bacterial isolates. *Acinetobacter anitratus* was sensitive to Amikacin (100%), Ciprofloxacin (100%) and Nalidixic acid (100%). However, it was resistant to Amoxicillin/clavulanic acid (75%), Cefixime (75%), Nitrofurantoin (75%). *Citrobacter freundii* was sensitive to Cefotaxime (85.7%) and Nalidixic acid (85.7%), but it was resistant to Amikacin (71.4%) and Cefixime (71.4%). CONS was sensitive to Amikacin (91.4%), Ciprofloxacin (74.3%) and Nalidixic acid (77.1%). *Enterobacter cloacae* was sensitive to Ciprofloxacin (85.7%). It showed 100% resistant to Amoxicillin/clavulanic acid and Cefixime. *Escherichia coli* was sensitive to all the tested antibiotics. Gram positive rods showed sensitivity to Amikacin (100%), Amoxicillin/clavulanic acid (71.4%), Ciprofloxacin (85.7%). However, it was resistant to Cefixime (100%) and Ceftazidime (100%). *Klebsiella pneumoniae* was 100% sensitive to Amikacin and Ciprofloxacin and resistant to Amoxicillin/clavulanic acid (71.4%), Cefixime (100%) and Ceftazidime (71.4%). Except Ciprofloxacin and Nalidixic acid, *Proteus vulgaris* was resistant to all the tested antibiotics. *Pseudomonas aeruginosa* was 100% resistant to Cotrimoxazole and Nitrofurantoin. *Staphylococcus aureus* was sensitive to all the tested antibiotics except Ceftazidime (36.7%) and Cotrimoxazole (40%) (Table 4).

Table 3. Microorganisms identified from polished and tissue surfaces of mandibular and maxillary dentures.

Microorganisms isolated	Maxillary denture		Mandibular denture	
	Polished surface n(%)	Tisssue surface n(%)	Polished surface n(%)	Tisssue surface n(%)
Streptococcus spp.	10 (22.7)	12 (27.3)	12 (27.3)	10 (22.7)
CoNS*	9 (25.7)	7 (20)	11 (31.4)	8 (22.9)
Staphylococcus aureus	8 (26.7)	7 (23.3)	7 (23.3)	8 (26.7)
Escherichia coli	3(25)	3(25)	3(25)	3(25)
Citrobacter freundii	2(28.6)	1(14.3)	2(28.6)	2(28.6)
Klebsiella pneumoniae	2(28.6)	2(28.6)	2(28.6)	1(14.3)
Proteus vulgaris	3(42.9)	3(42.9)	2(28.6)	3(42.9)
Pseudomonas aeruginosa	2(28.6)	2(28.6)	2(28.6)	2(28.6)
Candida albicans	2 (25	2 (25	2 (25	2 (25
Enterobacter cloacae	2(28.6)	2(28.6)	1(14.3)	2(28.6)
Acinetobacter anitratus	1 (25)	1 (25)	1 (25)	1 (25)
Gram Positive Rods	1(14.3)	3(42.9)	0	3(42.9)

**Coagulase-negative Staphylococci*

Table 4. Antibiotic sensitivity pattern of bacterial isolates.

Microrganisms		AK	AMC	CFM	CTX	CAZ	CTR	CIP	COT	NIT	NA
Acinetobacter anitratus	S	4(100)	-	1(25)	2(50)	1(25)	2(50)	4(100)	3(75)	-	4(100)
	R	-	3(75)	3(75)	1(25)	3(75)	2(50)	-	1(25)	3(75)	-
	I	-	1(25)	-	1(25)	-	-	-	-	1(25)	-
Citrobacter freundii	S	2(28.6)	3(42.9)	2(28.6)	6(85.7)	1(14.2)	3(42.9)	3(42.8)	4(57.1)	7(100)	6(85.7)
	R	5(71.4)	4(57.1)	5(71.4)	1(14.3)	3(42.9)	4(57.1)	2(28.6)	3(42.9)	-	1(14.3)
	I	-	-	-	-	3(42.9)	-	2(28.6)	-	-	-
CoNS*	S	32(91.4)	13(37.1)	3(8.6)	8(22.9)	2(20.0)	20(57.2)	26(74.3)	21(60.0)	16(45.7)	27(77.1)
	R	-	21(60.0)	29(82.9)	25(71.4)	28(80.0)	11(31.4)	8(22.9)	12(34.3)	14(40.0)	6(17.1)
	I	3 (8.6)	1(2.9)	3(8.6)	2(5.7)	-	4(11.4)	1(2.8)	2(5.7)	5(14.3)	2(5.7)
Enterobacter cloacae	S	3(42.9)	-	-	2(28.6)	3(42.9)	2(28.6)	6(85.7)	5(71.4)	3(42.9)	5(71.4)
	R	3(42.9)	7(100)	7(100)	4(57.1)	4(57.1)	3(42.8)	-	2(28.6)	4(57.1)	2(28.6)
	I	1(14.2)	-	-	1(14.3)	-	2(28.6)	1(14.3)	-	-	-

Escherichia coli	S	11(91.7)	11(91.7)	8(66.7)	12(100)	11(91.7)	10(83.3)	12(100)	12(100)	12(100)	12(100)
	R	1(8.3)	1(8.3)	4(33.3)	-	1(8.3)	-	-	-	-	-
	I	-	-	-	-	-	2(16.7)	-	-	-	-
Gram Positive Rods	S	7(100)	5(71.4)	-	4(57.1)	-	4(57.1)	6(85.7)	1(14.3)	5(71.4)	5(71.4)
	R	-	2(28.6)	7(100)	2(28.6)	7(100)	3(42.9)	1(14.3)	6(85.7)	2(28.6)	2(28.6)
	I	-	-	-	1(14.3)	-	-	-	-	-	-
Klebsiella pneumoniae	S	7(100)	2(28.6)	-	3(42.9)	2(28.6)	3(42.9)	7(100)	3(42.9)	4(57.1))	6(85.7)
	R	-	5(71.4)	7(100)	4(57.1)	5(71.4)	4(57.1)	-	3(42.9)	2(28.6)	-
	I	-	-	-	-	-	-	-	1(14.3)	1(14.3)	1(14.3)
Proteus vulgaris	S	5(45.45)	-	-	1(9.1)	-	2(18.2)	10(90.9)	3(27.3)	1(9.1)	11(100)
	R	5(45.45)	11(100)	10(90.9)	7(63.6)	11(100)	5(45.4)	-	8(72.7)	9(81.8)	-
	I	1(9.1)	-	1(9.1)	3(27.3)	-	4(36.4)	1(9.1)	-	1(9.1)	-
Pseudomonas aeruginosa	S	5(62.5)	-	4(50)	8(100)	8(100)	3(37.5)	6(75)	-	-	8(100)
	R	3(37.5)	7(87.5)	4(50)	-	-	3(37.5)	2(25)	8(100)	8(100)	-
	I	-	1(12.5)	-	-	-	2(25)	-	-	-	-
Staphylococcus aureus	S	27(90)	21(70)	15(50)	19(63.3)	11(36.7)	18(60)	26(86.6)	12(40)	24(80)	29(96.7)
	R	2(6.7)	6(20)	15(50)	10(33.3)	19(63.3)	10(33.3)	2(6.7)	11(36.7)	5(16.7)	-
	I	1(3.3)	3(10)	-	1(3.4)	-	2(6.7)	2(6.7)	7(23.3)	1(3.3)	1(3.3)
Streptococcus spp.	S	43(97.7)	28(63.6)	16(36.4)	21(47.7)	5(11.4)	29(65.9)	38(86.3)	19(43.2)	28(63.6)	35(79.5)
	R	1(2.3)	13(29.6)	28(63.6)	18(40.9)	38(86.3)	14(31.8)	5(11.4)	23(52.3)	12(27.3)	6(13.6)
	I	-	3(6.8)	-	5(11.4)	1(2.3)	1(2.3)	1(2.3)	2(4.5)	4(9.1)	3(6.8)

**Coagulase-negative Staphylococci*

DISCUSSION

More than 700 different types of bacterial species are present in our oral cavity.[10] Their bio habitat are always changing inside our oral cavity.[11] This is due to the fact our oral cavity have lot of narrow crevices, all of which favor bacterial colonization.[5] However, it should be noted that they all maintain healthy status of our oral cavity.[10, 11] Any material added into our mouth becomes an excellent site for colonization of microorganisms.[5,12,13]

A person in a part of his/her lifetime becomes either partially or completely edentulous. Dentures are the most commonly used in such case. Dentures are prosthesis made in dental labs to replace the missing hard and soft tissues of the oral cavity. They consist of two components: denture base and teeth. The denture bases are mostly made up of acrylic materials (polymethylmethacrylate), cobalt-chromium alloys and polymers. The teeth component is fabricated from acrylic resins, composite resins or porcelain.[4] The dentures thus made in the lab, once it gets inserted into the oral cavity are quickly colonized by microorganisms.[5]

The colonization of microorganisms in dentures are dependent upon various factors including the type of materials added to construct of dentures, age and health of denture wearer, denture hygiene habits.[5] The dentures first get coated with an acquired pellicle. Immunoglobulins and salivary glycoproteins such as salivary amylase, mucins are the main component of acquired pellicle.[14] The main drawback of these salivary proteins is to provide adhesion receptors enhancing the accumulation and adhesion of the microorganisms.[15] Studies have shown presence of different microorganisms in dentures.[16-18] The primary colonizers such as *S. oralis, S. mutans, S. mitis,* and other bacterial species such as *Veillonella spp., Neisseria spp., Rothia spp.* have been identified in dentures.[10, 19] Secondary colonizers then adhere on the primary colonizers and later they form denture plaque.[5, 20]

In the present study, most of the organisms were gram positive cocci and gram-negative bacilli. This was in consistent with the findings of other authors.[8, 21] In this study the different types of microorganisms isolated from the polished surface and the tissue surface were same. *Streptococcus* spp was the constant followed by followed by Coagulase-negative Staphylococci and *Staphylococcus aureus*. The other microorganisms were *E. coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*. All the bacteria identified were in line with the other studies performed in Nepal.[8,22] Sharma et al showed that there was not significant difference in the isolation of

microorganisms between the polished and the tissue surfaces of the dentures at 24 hours.[8] The present study showed *Streptococcus* spp more on polished surface of mandibular dentures and on tissue surface of maxillary dentures. *Staphylococcus aureus* was identified more on tissue surface of mandibular denture and polished surface of maxillary dentures. Coagulase-negative Staphylococci was identified more on polished surface of mandibular dentures. The differences in identification may be due to the time period of isolation as the present study was conducted among the participants who had worn dentures for more than a year.

Literatures have also revealed the presence of facultative anaerobes in the dentures.[23, 24] Many of the authors have also shown *Candida* spp. too, with Candida albicans as the most common species.[20,25,26] Other *Candida* spp. such as *C. glabrata*, *C. tropicalis* have also been identified.[27,28]

Sharma et al. in their study showed the presence of *Candida albicans* in two samples at week one.[3, 8] In the present study, *Candida albicans* was also isolated from 2 participants. *Candida* spp. are said to be one of the secondary colonizers and have also the role in biofilm formation among the denture wearers. They are mainly found in the tissue fitting surfaces of the dentures.[5, 27] *Candida* spp. together with *Streptococcus* spp. also form biofilm.[29] The presence of Candida spp. have also been reported its association with denture related stomatitis.[7, 30] However, in our case they were also isolated from the polished surface of both the dentures. The presence of *Candida* spp. may be further associated with denture hygiene practice, duration of denture uses and smoking.[27] In the present scenario, the swab were collected from old age people with dentures more than a year. Their poor denture hygiene habits cannot be denied too due to the old age. This study mainly aimed at the presence of microorganisms, so the denture related stomatitis was not observed.

Ideally an appropriate antibiotic should be given after observing the antibiotic susceptibility pattern. However, it takes normally 24-48 hours to get the results. In this situation a broad-spectrum antibiotic is given. Due to this inappropriate use of antibiotics, the antimicrobial resistance has increased in Nepal. Over the counter purchase, self-medication, lack of knowledge and awareness are the means to blame.[31,32] In a study conducted in Chitwan, Amoxycillin was the most commonly prescribed antibiotic by the dentists followed by amoxicillin/clavulanic acid.[33] Apart from the different bacterial isolates this study also evaluated their antibiotic susceptibility pattern. Among the tested antibiotics, the present study revealed the highest sensitivity pattern of the bacterial isolated to Amikacin, Nalidixic acid and Ciprofloxacin while the most resistant antibiotics were Amoxicillin/clavulanic acid and Cefixime. Other antibiotics showed intermediate effect. Many studies have shown conflicting results in the sensitivity patterns of the antibiotics. The source of samples taken also makes the result different. It should be noted that in the present study the samples were obtained from the dentures of the geriatric populations. Among the odontogenic infection cases, Bahl et al. reported that the aerobic microorganisms were most sensitive to Amoxicillin/clavulanic acid and azithromycin.[34] Among the oral cancer patients, Kanadan et al. reported that all of the patients were 80% susceptible to the tested antibiotics.[35] Chunduri et al. in their study reported high sensitive value for Amoxicillin/clavulanic acid, amoxicillin, clindamycin.[36]

This study is a baseline study conducted among the complete denture wearer people on the basis of antibiotic susceptibility testing. However, the study had less sample size and the generalizability of the study to other parts of Nepal are the limitations of the study.

CONCLUSION

Based on the findings from this study, *Streptococcus* spp. followed by Coagulase-negative Staphylococci and *Staphylococcus aureus* are the most frequently identified microorganisms from the dentures of old age people. This is the first study on the antimicrobial susceptibility pattern of microorganisms isolated from the dentures. Amikacin, Nalidixic acid and Ciprofloxacin seems to be highly sensitive among the people of old age.

ACKNOWLEDGEMENT

The authors would like to thank all the participants of the study. The study was funded by the University Grant Commission (Ref No-211/077/078, Award No FRG-76/77-HS-06).

CONFLICT OF INTEREST

The authors declare no conflict of interest

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