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NL Journal of Dentistry and Oral Sciences

(ISSN: 3049-1053)

Volume 3 Issue 2 April 2026

Research Article

Antibiotic Resistance in Oral Microbiota of Diabetic and Non-diabetic Populations in the Northern part of Bangladesh

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doi: 10.71168/NDO.03.02.147

Received Date: March 25- 2026 **Publication Date:** April 30- 2026

Abstract: Oral infections are common and can be more serious in people with diabetes because their bodies do not fight infections well. Antibiotic resistance, where bacteria no longer respond to medicines, is becoming a big problem worldwide. In Bangladesh, there is little information about how oral bacteria in diabetic and non-diabetic patients resist antibiotics. This study will help understand these differences to improve treatment.

Methods: A cross-sectional comparative study was conducted where initially 120 samples were distributed equally between diabetic and non-diabetic respondents and 10 gram positive and negative catalase samples were collected from the both group for this research.

Results: It was found that the concentrations among antibiotics are significantly different for both groups. It was also noted that the bacterial inhibitions are different on the basis of different concentrations of antibiotics as well as the bacterial inhibitions are decreased as the concentrations of the antibiotics were decreased.

Conclusion: The study emphasizes that diabetic patients require adjusted antibiotic regimens, often involving higher dosages, to effectively manage oral biofilm-associated infections. Azithromycin and gentamicin emerge as promising antibiotics due to their effectiveness at lower concentrations in this population. Tailoring antibiotic treatment strategies for diabetic individuals is crucial to improve clinical outcomes and combat oral infections effectively.

Keywords: Antibiotic resistance, Diabetes mellitus, Periodontal disease, Oral biofilm, Periodontitis, Diabetic, Non-diabetic patients.

Introduction

Bangladesh has neglected to address the serious public health issue of antibiotic resistance for a long time. The ability of a bacterium to develop immunity to one or more conventional drugs is known as antibiotic resistance. Through a variety of processes, including mutational adaptations, the acquisition of genetic materials, and changes in gene expression, bacteria can develop resistance to conventional antibiotics. Antibiotic resistance has grown to be a significant global public health concern as a result of its rising prevalence and the decline in the number of new antibiotics breakthroughs [1]. This has now led to increased risk and prevalence of mortality and morbidity, recurrent infections, treatment failure, and higher healthcare costs [2,3]. According to estimates, antibiotic resistance directly caused 1.27 million deaths worldwide in 2019 and led to around 5 million fatalities overall, more than deaths from AIDS, malaria, and other causes combined [4]. Antimicrobial resistance is also predicted to contribute to over 10 million deaths per year by 2050 [5]. Antibiotic resistance in Bangladesh has risen by 11% in the last five years, according to the National Antimicrobial Resistance Surveillance, Bangladesh, 2016–2023. At the same time, the efficiency of certain regularly used antibiotics has dropped by up to 82% in 2023, down from 71% in 2016 [6].

The main causes of antibiotic resistance in Bangladesh are the lack of defined guidelines for their use and the ease with which they can be purchased without a prescription. For minor ailments like colds, fevers, or food poisoning, many people purchase antibiotics from neighborhood pharmacies and take them independently without consulting a physician [7,8]. In Bangladesh, there are 7.2 pharmacies for every 10,000 people, public hospitals are overcrowded [9], and private physicians are frequently expensive. In order to feel well more quickly, this leads many to decide to self-medicate with antibiotics [10]. Antibiotics were also widely used throughout the COVID-19 pandemic, even when they weren't necessary. In addition to lacking the necessary skills, many healthcare professionals occasionally break the law. In Bangladesh, these problems lead to an increase in antibiotic resistance [11].

An absolute or relative lack of the anabolic hormone insulin results in diabetes mellitus (DM), a chronic metabolic disease. The pancreatic beta cells of the islets of Langerhans are responsible for producing insulin. Type 1 diabetes (insulin-dependent diabetes mellitus) is caused by the loss, destruction, or lack of these cells [12]. Type 2 diabetes, also known as non-insulin-dependent diabetes mellitus, is a chronic illness that impacts people's life.

Plaque, also called "oral biofilm," is a group of tiny living things called bacteria that grows on the teeth and gums. These bacteria can cause gum disease and tooth decay. For a long time, people thought these germs only affected the mouth. But now, scientists have found many connections around 200 between mouth health and other body illnesses, according to the American Dental Association [13]. One important link is between gum disease and diabetes. Gum disease can make it harder to control blood sugar in people with diabetes [14,15]. Treating gum disease can help improve blood sugar control [16]. This shows that the bacteria in our mouths are part of a complex system that affects overall health. Patients with diabetes mellitus who have hyperglycemia have higher salivary glucose levels, which provide cariogenic bacteria in the tooth biofilm with an ideal food source [17].

Azithromycin is a very effective bacteriostatic antibiotic that exhibits strong activity against gram-negative bacteria. It is often regarded as the safest option among the macrolide class of antibiotics [18,19]. The medicine is not recommended as the initial treatment for odontogenic infections and is typically provided as a substitute for penicillin in patients with allergies [20,21]. Clindamycin is a broad-spectrum antibiotic that inhibits the growth of a wide range of bacteria, including those that can survive with or without oxygen [21]. This medicine belongs to the latest generation of lincomycin and exhibits significant efficacy against infections affecting the bones, joints, and teeth [21]. Ciprofloxacin belongs to the second generation of fluoroquinolone antibiotics and has the ability to effectively combat both Gram-positive and Gram-negative infections [22]. Prior research has shown that approximately 12% of dentists appropriately prescribe antibiotics for both preventive and therapeutic purposes [23].

The unwarranted administration of antibiotics may lead to other significant complications, such as bacterial resistance, gastrointestinal and hematological issues, and alteration of bacterial flora [24]. Antibiotic resistance in mouth bacteria is a serious problem. To prevent this, antibiotics should be used only for serious infections and with a narrow focus. More research and education are needed [25]. In people with diabetes, mouth bacteria change and increase the risk of gum disease. Good oral hygiene, blood sugar control, and regular dental visits are essential for their health. The data show that careful antibiotic use and good oral care are especially important for people with diabetes. For this reason, this study attempted to identify the oral issues linked to diabetes by focusing on patients with and without diabetes who had various dental issues. In order to treat diabetic patients after increasing their dosage and preserve the remaining antibiotics for their future health.

Methodology

Study design, place: This is a cross-sectional type of comparative study conducted at the Institute of Bioogical Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh.

Study population and sample size: All the patients with orodental problems with and without diabetes attending outpatient department of Chapainawabgonj Diabetic Hospital, Chapainawabgonj, Bangladesh constituted the study population during the study period. The total sample size was 120, divided into two groups, where 60 were from diabetic patients and another 60 from healthy individuals and purposive sampling is used for this investigation.

Inclusion criteria

- Participants having orodental problems with or without diabetes mellitus type-2.

Exclusion criteria

- Patients with type-1 diabetes, gestational diabetes.
- Patients with oral carcinoma.
- Unwilling to participate the study.

Sample collection and preparation: Sample were collected from orodental problems related patients with and without diabetes. A sterile swab stick inserted inside the mouth and biofilm were collected from the tooth surface [Fig-1] and a healthy oral cavity will be free from biofilm or oral disease [Fig-2].

Collected samples were taken inside the test tube, patient name and number were written in every test tube and carefully taken into the laboratory.

Collected biofilm were transfer on agar filled Petri dish. Inside the biosafety cabinet, wire loop was burn for sterilization then biofilm smear on the media evenly [Fig-3]. Organism are then placed in an incubator for 24 hours at 37^0C. Incubated organism collected through toothpick and inserted into hydrogen peroxide fill test tube, if there is a bubble then it is catalase (+) ve bacteria otherwise it is catalase (-) ve bacteria. Then serial dilution was performed for extensive research.

The purpose of a serial dilution [Fig-4] is to estimate the concentration of a sample. From the diabetic group five catalase-positive and five catalase-negative samples were taken, and the same was done for the non-diabetic group. From these 20 samples, 10 are randomly chosen for the extensive experiment. Six antibiotics viz. ciprofloxacin, clindamycin, azithromycin, ceftriaxone, gentamycin, and fluconazole each with 7 concentrations [Table-1] were considered for this investigation. All the sterilized test tube were stockpiling in to a test tube holder. For the first concentration, 1 µg/ml of antibiotics is taken to an Eppendorf tube and mixed with 9 ml of distilled water, making a total of 10 ml of solution. Again, from the 1st solution, 1 µg/ml is taken and mixed with 9 ml of water to make another concentration; this way, further dilution is done up to the 6th time [Table-1]. This method allows for a systematic reduction in the concentration of antibiotics, facilitating the assessment of their efficacy. Each dilution can be tested to determine the minimum inhibitory concentration required for effective bacterial growth inhibition.

After adjusting the pH to 7, antibiotics were dissolved in medium at the concentration used in the final test. Columns 1-5 and 6-10 each had 100 ml of non-diabetic and diabetic lab broth media, respectively. Column 12 had a sterile lab broth media. Each row was treated with one serially diluted antibiotic solution up to the 7th row with 7 different serial dilutions of the same antibiotics. The 96-well plate [Fig-5] was then left to incubate for a full day. Then optical density was measured at 600 nm wavelength to check their growth and inhibition.

Spectrophotometric analysis work as, spectrophotometer measures the amount of light transmitted or absorbed directly through a sample and thus quantifies the turbidity. As the cell population increases the amount of transmitted light decreases. The amount of light energy transmitted through the suspension is measured as percentage of transmission or % on the spectrophotometer as 0% to 100%. This transmitted light is now converted to electrical energy, and this is specified on a galvanometer. The reading, is termed as absorbance or optical density, indirectly indicates the number of bacteria. Absorbance is a logarithmic value and is used to plot bacterial growth on a graph. This method is rapid in comparison to standard plate count but is limited as the sensitivity is confined to bacterial suspensions of 107 cells or greater.

Table 1: Concentration of serial dilution.

Name of the antibiotics	Concentrations						
	1st dilution	2nd dilution	3rd dilution	4th dilution	5th dilution	6th dilution	7th dilution
Ciprofloxacin	5.0x10µg/ml	5.0µg/ml	$5.0x10^{-1}$µg/ml	$5.0x10^{-2}$µg/ml	$5.0x10^{-3}$µg/ml	$5.0x10^{-4}$µg/ml	$5.0x10^{-5}$µg/ml
Clindamycin	40.0x10µg/ml	40.0µg/ml	$40.0x10^{-1}$µg/ml	$40.0x10^{-2}$µg/ml	$40.0x10^{-3}$µg/ml	$40.0x10^{-4}$µg/ml	$40.0x10^{-5}$µg/ml
Azithromycin	0.04x10µg/ml	0.4µg/ml	$0.4x10^{-1}$µg/ml	$0.4x10^{-2}$µg/ml	$0.4x10^{-3}$µg/ml	$0.4x10^{-4}$µg/ml	$0.4x10^{-5}$µg/ml
Ceftriaxone	0.1µg/ml	0.01µg/ml	$1x10^{-3}$µg/ml	$5.0x10^{-4}$µg/ml	$5.0x10^{-5}$µg/ml	$5.0x10^{-6}$µg/ml	$5.0x10^{-7}$µg/ml
Gentamycin	0.04x10µg/ml	0.4µg/ml	$0.4x10^{-1}$µg/ml	$0.4x10^{-2}$µg/ml	$0.4x10^{-3}$µg/ml	$0.4x10^{-4}$µg/ml	$0.4x10^{-5}$µg/ml
Fluconazole	0.04x10µg/ml	0.4µg/ml	$0.4x10^{-1}$µg/ml	$0.4x10^{-2}$µg/ml	$0.4x10^{-3}$µg/ml	$0.4x10^{-4}$µg/ml	$0.4x10^{-5}$µg/ml

Statistical analysis

The statistical analysis was performed using the SPSS statistical package for WINDOWS v.21.0. which are described under the following sub-head as:

(a) Mean ($\overline{X}$): Data of individual population were added together then divided by the total number of observation and then the mean was obtained as follows:

Mean: $$\overline{X} = \frac{\sum_{i=1}^{n} x_i}{n}$$

Where, $\bar{X}$ = mean of all the readings; Xi = individual reading of the recorded data; ∑= summation, i = 1, 2, 3, 4...,n; and n = number of observations.

(b) Standard deviation (SD): Standard deviation is an average deviation of the individual observations from the mean. It was calculated as the square root of the variance as follows:

$SD = \sqrt{S^2}$

Where, SD = standard deviation, and S2 = variance

(c) Analysis of variance (ANOVA): Variance is a measure of dispersion of a population. Thus, the ANOVA is done for testing the significant difference among the populations. Variance analysis for each if the characters was carried out separately on mean value. The variance due to different sources such as concentration (C), sample (S), interaction C×S and within error of a population were calculated as per the following skeleton:

Table 2: Skeleton of ANOVA.

Sources	df	SS	MS	F
Concentration (C)	C-1=7-1= 6	SS1	MS1	MS1/MS4
Sample (S)	S-1= 5-1 = 4	SS2	MS2	MS2/MS4
Interaction (CS)	(C-1) (S-1) = 6 * 4 = 24	SS3	MS3	MS3/MS4
Error	CS (r-1) = 7 *5 * (2-1) = 35	SS4	MS4	
Total	CSr-1= 69			

(d) Duncan multiple range test (DMRT): DMRT is a statistical method that compares all pairs of means and group means that are not significantly different. It's a multiple comparison procedure that uses the studentized range statistic to compare sets of means.

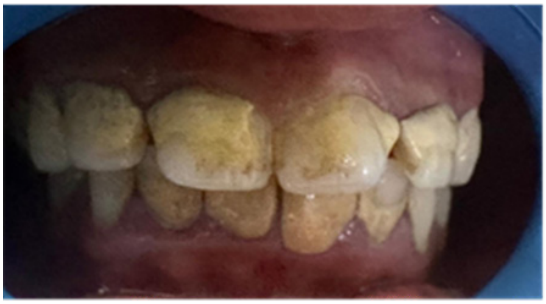

Figure 1: Biofilm in the oral cavity.

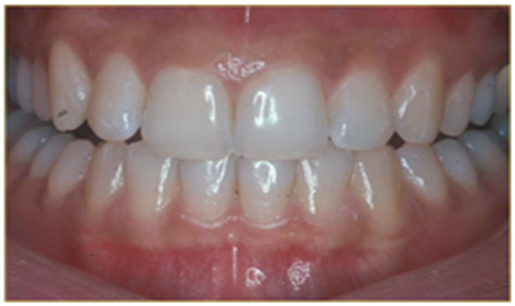

Figure 2: Healthy oral cavity.

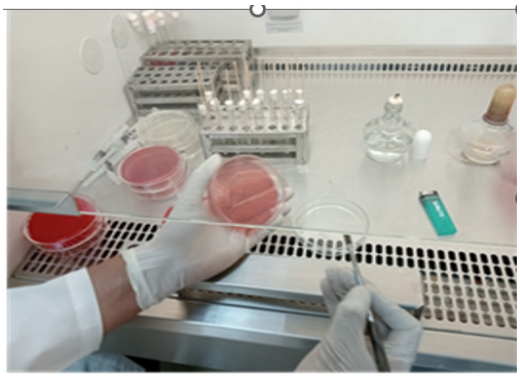

Figure 3: Smearing biofilm.

Figure 4: Serial dilution preparation.

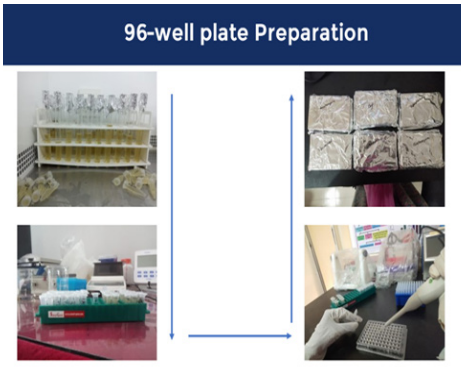

Figure 5a: 96-well plate preparation.

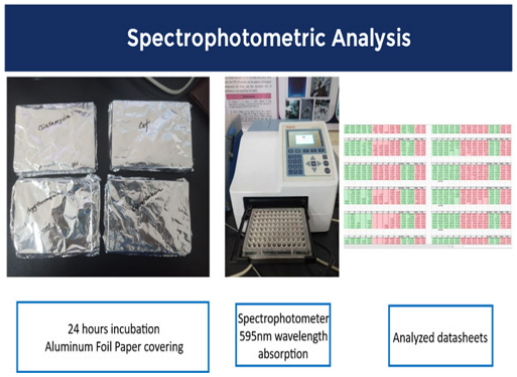

Figure 5b: Spectrophotometric analysis.

Results

The present study evaluated the effects of various antibiotics on oral microbiota isolated from diabetic and non-diabetic patients, focusing on differences in antibiotic resistance patterns between these groups. Statistical analyses were conducted to assess the significance of concentration, sample type, and their interaction on the efficacy of six commonly used antibiotics. The findings reveal distinct variations in antibiotic effectiveness, highlighting a trend of greater susceptibility in the non-diabetic group compared to their diabetic counterparts. Detailed results for each antibiotic are summarized below in the following sub-heads.

Graphical distribution of the responds based on the effects of different antibiotics

A typical broad-spectrum antibiotic used to treat a variety of bacterial illnesses is ciprofloxacin. It can affect the normal flora (microorganisms) in the body, including the mouth cavity, much like all antibiotics do. Ciprofloxacin's broad-spectrum action against a variety of bacteria can result in a reduction in the diversity of oral microbial species. The effects of ciprofloxacin were estimated on 7 concentrations of the drug among the two groups viz diabetic population (Dp) and control population (Cp). At the same concentration, ciprofloxacin was more effective on the non-diabetic population than the diabetic patient in order to inhibit bacterial growth. As the concentration decreased, the bacterial inhibition was also decreased. Bacterial inhibition was lowest at the 7th concentration due to the application of the lowest concentration gradually (Fig-6).

An antibiotic called clindamycin is frequently used to treat bacterial infections, particularly those that affect the mouth. Seven concentrations given both diabetic non-diabetic groups. In diabetic group 1st treatment showed more than 70% growth inhibition. Then when concentration lowered their efficacy also lowered. At 4th and 5th treatment though their concentration different their efficacy was same, which also seen in 6th and 7th treatment. In non-diabetic group 1st treatment of clindamycin was effective more than 80% and then when concentration lowered for 2nd treatment its inhibition was also lowered. From 3rd to 6th treatment through their concentration was different but their efficacy was same and 7th treatment its inhibition was lowest (Fig-7).

Diabetic patients may be more sensitive to the effects of varying azithromycin concentrations on their oral and systemic microbiome. First treatment seen at same concentration azithromycin was more effective on the non-diabetic population than the diabetic patient. As the concentration was decreased the bacterial inhibition was also decreased. When concentration decreases the bacterial growth increases as showed in the graph. In this case, the bacterial inhibition was lowest at the 7th concentration. Compared to others drug, it showed more inhibition at lower concentration (Fig-8).

A wide variety of bacterial infections can be effectively treated with ceftriaxone, a broad-spectrum cephalosporin antibiotic. Oral bacteria, including both commensal and harmful species, can be killed by high concentrations of ceftriaxone, which exhibits considerable bactericidal activity. Ceftriaxone inhibited bacterial growth more effectively in the non-diabetic group than in the diabetic patient at the same dose. Bacterial inhibition decreased in tandem with the concentration. Bacterial growth increases while inhibition reduces. As the lowest concentration was applied progressively, the bacterial inhibition was lowest at the seventh concentration (Fig-9).

Both gram-negative and certain gram-positive bacteria were susceptible to the action of the aminoglycoside antibiotic gentamicin. Due to the specific oral and systemic conditions caused by diabetes, the impact of varying doses of gentamicin on dental bioflora, especially in diabetic individuals, can be considerable. For gentamycin it was found that, even in lowered concentration it was effective on both group that was, it could inhibit bacterial growth even in low concentration. So, it was strong antibiotic than others (Fig-10).

When it comes to treating fungal infections, fluconazole is a go-to drug, especially for those that manifest in the mouth. Diabetic patients, with their impaired immune systems and changed oral environment, are at increased risk for fungal infections, making its use all the more pertinent for this population. Seven concentrations were used to estimate the effects of fluconazole in the two groups. At the same concentration, fluconazole inhibited bacterial growth more effectively in the non-diabetic group than in the diabetic patient. As the concentration decreased, bacterial inhibition lessened, allowing the bacteria to grow more (Fig-11).

Results of the analysis of variance

Result of the analysis of variance (ANOVA) is shown in Table-3. Table-3 shows that for ciprofloxacin, items concentration (C) and sample (S) were highly significant and the interaction (CS) found to be non-significant when tested by the error. For the clindamycin, concentration (C) was significant at 1% level and sample (S) was significant at 5% level. The interaction (CS) item showed non-significant. When analysis was done for azithromycin, the concentration (C) and sample (S) were highly significant. The other item of interaction (CS) was non-significant. For ceftriaxone, concentration (C) and sample (S) were highly significant, but the interaction item (CS) was non-significant. In case of gentamycin items concentration (C) and sample (S) were found to be highly significant, when tested by the error and the other item (CS) found to be non-significant.

Concentration (C) and sample (S) were found to be significant at 1% and 5% level, respectively while interaction item found to be non-significant for fluconazole.

Significant differences of seven doses of antibiotics through Duncan Multiple Range Test (DMRT)

The dental bioflora significantly impacted by ciprofloxacin, which also promote opportunistic infections and antibiotic resistance. Other possible consequences of ciprofloxacin include changes in microbial balance and composition. When administering ciprofloxacin, medical practitioners should be aware of these effects and take precautions to lessen the possibility of negative consequences for dental health. It was exposed that between the diabetic and non-diabetic group, ciprofloxacin found more effective among the non-diabetic group in T1 to T7 concentrations indicated that this drug would have more effect among the non-diabetic group during the treatment of oral problems (Fig-12). In diabetic group 1st treatment "a" shows more than 70% growth inhibition. Then when concentration lowered in 2nd and 3rd treatment though their concentration was different but efficacy was same "b". For 4th and 5th treatment concentration was different but efficacy was same "bc" which also seen in 6th and 7th treatment "c". In non-diabetic group 1st "a" treatment of Ciprofloxacin was effective more than 80% and then when concentration lowered for 2nd "b" treatment its inhibition was also lowered. From 3rd to 6th "bc" treatment through their concentration was different but their efficacy was same and 7th "c" treatment its inhibition was lowest significantly.

The impact of clindamycin on dental bioflora can vary across concentrations, from mild suppression to complete eradication of vulnerable bacterial species. When doctors prescribe clindamycin to treat mouth infections, they should think about how it can affect microbial diversity, the likelihood of resistance developing, and the harmony of the oral microbiota as a whole. Optimizing therapeutic outcomes while minimizing adverse effects on oral health requires individualized treatment regimens, concepts of antimicrobial stewardship, and regular patient monitoring. Clindamycin was found to be more effective in the non-diabetic group in T1 to T7 concentrations when compared to the diabetes group. It suggested that while treating oral issues, this medication might work better for the non-diabetic population (Fig-13). Clindamycin on diabetic group 1st treatment "a" showed more than 70% growth inhibition. Then when concentration lowered in 2nd,3rd and 4th treatment "b" "bc" "cd" organism growth inhibition was greatly lowered, at 5th and 6th treatment "de" though concentration was different but efficacy was same. 7th treatment "e" growth inhibition was lowest. In non-diabetic group 1stand 2nd treatment "a" Clindamycin showed 80% inhibition through their concentration was different. When concentration was lowered efficacy was also lowered at 3rd and 4ththreatment "b, bc". 5th and 6th treatment "cd" concentration was different inhibition was seen at 7th treatments "e" inhibition was lowest significantly.

Complex and warranting of meticulous investigation were the effects of varying azithromycin concentrations on the oral bioflora of diabetes individuals. High concentrations pose the danger of upsetting the oral microbiota and encouraging resistance, whereas low to moderate concentrations successfully decrease harmful bacteria and enhance periodontal health. To maximize dental health outcomes and enhance overall health in this susceptible group, it was necessary to tailor antibiotic treatment to the specific requirements of diabetes patients, in addition to frequent monitoring and supportive therapies. Azithromycin, in low concentrations, was found to be more effective on the diabetic group than other drugs, which suggested that while treating oral issues, this medication might work better for the diabetic population. (Fig-14). Azithromycin had great role for both groups. In diabetic group 1st treatment "a" of azithromycin can inhibit 80% organism growth. 2nd treatment "ab" though concentration was different but efficacy was very near to 1st treatment. 3rd and 4th treatment "abc" concentration was not same but efficacy was same which was also seen in 5th and 6th treatment "bc" at 7th treatment "d" in very low concentration azithromycin was very active which was not seen others drugs. For non-diabetic group, 1st, 2nd and 3rd treatment "a" though their concentration was different but efficacy was same, which was also seen at 4th and 5th treatment "ab". At 6th treatment "b" when concentration lowered inhibition was also lowered seen at 7th treatment "c". Here founded, when concentration was lowered their efficacy was lowered.

Dental bioflora in diabetes patients can be affected differently by different ceftriaxone concentrations. At low to moderate concentrations, harmful bacteria can be efficiently reduced and periodontal health can be improved. On the other hand, large concentrations eliminate germs quickly but put the oral microbiome at danger. To maximize oral health benefits while minimizing adverse effects in diabetes patients, personalized antibiotic medication was required, along with regular monitoring and supportive care. In T1 to T7 concentrations, it was discovered that ceftriaxone was more effective in the non-diabetic group compared to the diabetic group. It suggested that this medication might be more effective in treating oral health issues in the non-diabetic group (Fig-15). Ceftriaxone had a great role for both groups. In diabetic group 1st treatment "a" of ceftriaxone can inhibit 65% organism growth. From 2nd to 7th treatment though their concentration lowered but their efficacy was nearly same. For non-diabetic group, 1st treatment 'a' is 80% effective and 2nd,3rd,4th treatment "ab", "abc", "bc" concentration was lowered significantly. Then from 5th to 7th treatment "c" though their concentration was different but efficacy was same. Here found when concentration was lowered their efficacy was lowered significantly.

The effects on diabetic oral bioflora of gentamicin at different concentrations can vary. At low to moderate concentrations, harmful bacteria can be efficiently reduced and periodontal health can be improved. On the other hand, large concentrations eliminate germs quickly but put the oral microbiome at danger. To maximize oral health benefits while minimizing adverse effects in diabetes patients, personalized antibiotic medication was required, along with regular monitoring and supportive care. Careful consideration should be given to the use of gentamicin in dental applications due to its possible toxicity and the need for parenteral administration. It was normally reserved for serious infections where other antibiotics have failed. Though gentamicin had a great effect on both groups, in low concentration it was more effective on the diabetic group, so this suggests that the diabetic group would benefit more from this medication while treating oral issues (Fig-16). Gentamycin on diabetic group 1st treatment "a" shows more than 80% growth inhibition and 2nd, 3rd treatment concentration was lowered gradually but efficacy same. At 4th, 5th and 6th treatment "ab" though concentration was different but efficacy was same. 7th treatment "b" growth inhibition was lowest. In non-diabetic group Gentamycin showed 80% inhibition and from 1st to 7th treatment though concentration was lowered gradually but their efficacy was nearly same.

The effects on diabetic oral bioflora of fluconazole at different doses can vary. Oral health can be improved and fungal burdens reduced at low to moderate concentrations, while serious infections can be rapidly eradicated at high concentrations, but this comes at the cost of potentially upsetting the delicate balance of the oral microbiome. To maximize oral health benefits while minimizing adverse effects in diabetes patients, personalized antifungal medication was required, along with regular monitoring and supportive care. Effective use of fluconazole in this susceptible population requires careful supervision and specific treatment strategies. In T1 to T7 concentrations, it was discovered that fluconazole was more effective in the non-diabetic group than in the diabetic group. It suggested that this medication might be more effective in treating oral health issues in the non-diabetic group (Fig-17). Fluconazole on diabetic group 1st treatment "a" showed more than 70% growth inhibition. 2nd and 3rd treatment "ab" concentration different but efficacy was same. From 4th to 7th treatment when concentration was lower their efficacy was significantly lowered. In non-diabetic group Fluconazole showed 80% inhibition. From 1st to 7th treatment though concentration was lowered gradually but their efficacy was nearly same.

Table 3: ANOVA of different antibiotic.

Sources	df	SS	MS	F
Ciprofloxacin				
Concentration (C)	6	20869.06	3478.17	13.47**
Sample (S)	4	7519.39	1879.84	7.28**
Interaction (CS)	24	3268.74	136.19	0.52NS
Error	35	9036.28	258.17	
Total	69	34693.47		
Clindamycin				
Concentration (C)	6	41203.83	6867.30	34.67**
Sample (S)	4	2941.83	735.32	3.71*
Interaction (CS)	24	2995.28	124.80	0.63NS
Error	35	6931.09	198.03	
Total	69	54072.03		
Azithromycin				
Concentration (C)	6	4644.27	774.04	18.22**
Sample (S)	4	1241.50	310.37	7.30**
Interaction (CS)	24	652.02	27.16	0.63NS
Error	35	1486.28	42.46	
Total	69	8024.08		
Ceftriaxone				
Concentration (C)	6	2098.79	3497.63	7.63**
Sample (S)	4	25286.44	6321.61	13.79**
Interaction (CS)	24	5139.99	214.16	0.46NS
Error	35	16035.41	458.15	
Total	69	48560.63		

Sources	df	SS	MS	F
Gentamycin				
Concentration (C)	6	3558.37	593.06	3.53**
Sample (S)	4	4284.54	1071.13	6.36**
Interaction (CS)	24	613.17	25.54	0.15NS
Error	35	5890.83	168.30	
Total	69	14346.92		
Fluconazole				
Concentration (C)	6	26811.72	4468.62	4.13**
Sample (S)	4	8137.70	2043.42	1.89*
Interaction (CS)	24	3519.99	146.66	0.13NS
Error	35	37835.25	1080.02	
Total	69	76304.66		

Where, * and ** indicate significance at 5% and 1% levels, respectively and NS indicates non-significance.

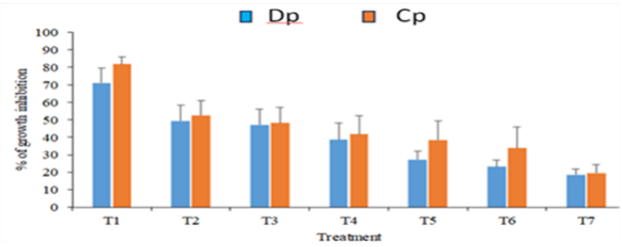

Figure 6: Distribution of the respondents by effects of Ciprofloxacin among diabetic and non-diabetic group.

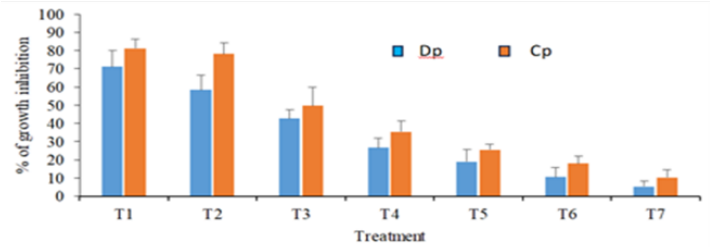

Figure 7: Distribution of the respondents by effects of Clindamycin among diabetic and non-diabetic group.

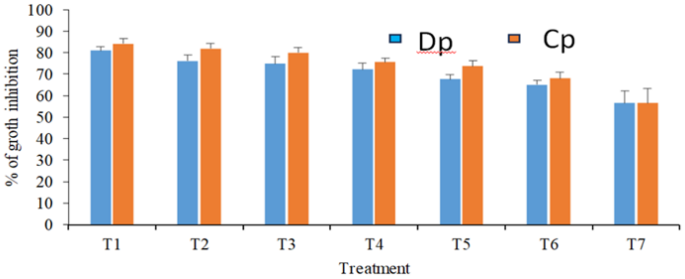

Figure 8: Distribution of the respondents by effects of Azithromycin among diabetic and non-diabetic group.

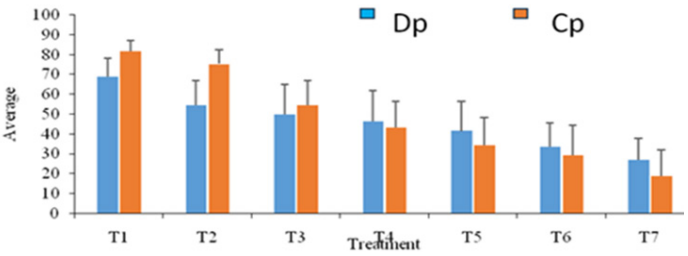

Figure-9: Distribution of the respondents by effects of Ceftriaxone among diabetic and non-diabetic group.

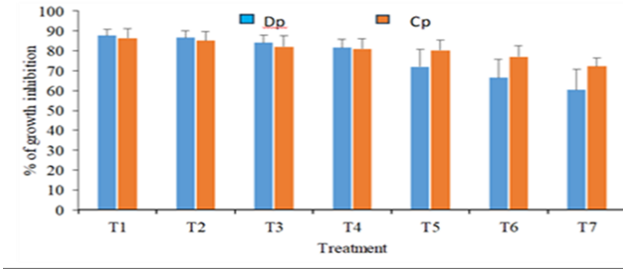

Figure 10: Distribution of the respondents by effects of Gentamycin among diabetic and non-diabetic group.

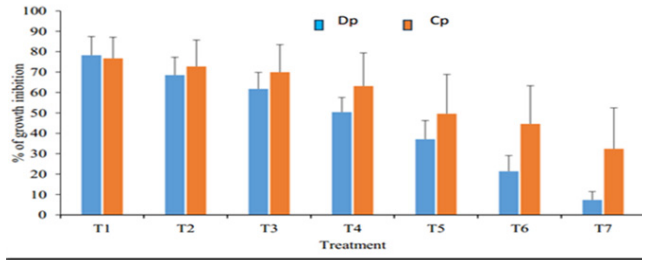

Figure 11: Distribution of the respondents by effects of Fluconazole among diabetic and non-diabetic group.

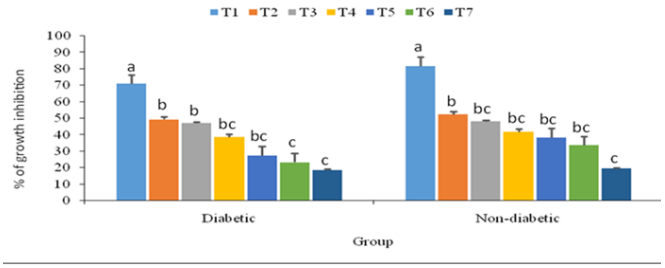

Figure 12: Effect of Ciprofloxacin on diabetic and non-diabetic patient shown by DMRT graph.

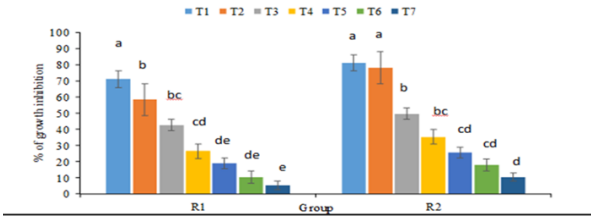

Figure-13: Effect of Clindamycin on diabetic and non-diabetic patient shown by DMRT graph.

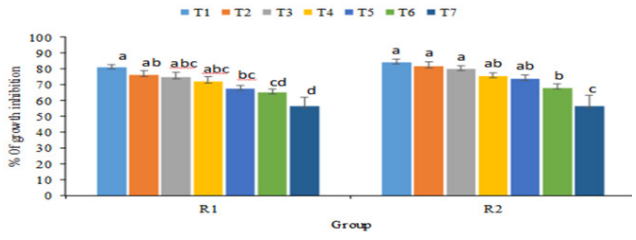

Figure-14: Effect of Azithromycin on diabetic and non-diabetic patient shown by DMRT graph.

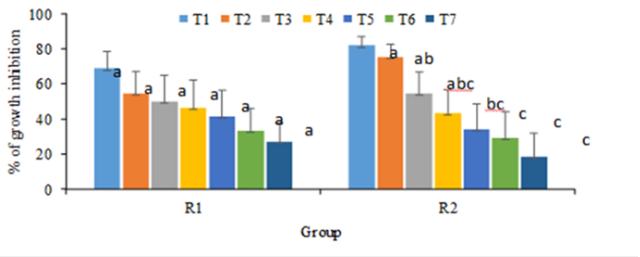

Figure 15: Effect of Ceftriaxone on diabetic and non-diabetic patient shown by DMRT graph.

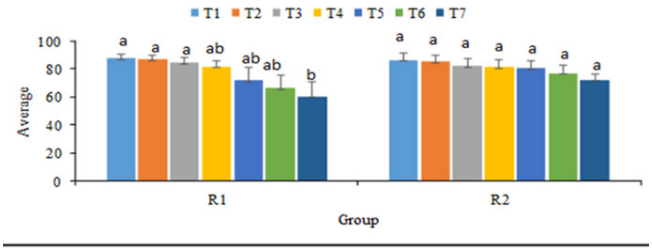

Figure 16: Effect of Gentamicin on diabetic and non-diabetic patient shown by DMRT graph.

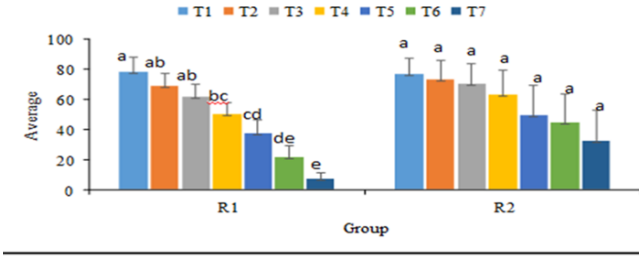

Figure 17: Effect of Fluconazole on diabetic and non-diabetic patient shown by DMRT graph.

Discussion

This study presents a comprehensive evaluation of the efficacy of various antibiotics against oral biofilm-associated pathogens in diabetic and non-diabetic populations, highlighting significant differences in antimicrobial response attributable to diabetic status. The findings underscore the public health implications in Bangladesh, where rising diabetes prevalence compounds the burden of oral infections.

Our analysis showed the critical role of antibiotic concentration in bacterial inhibition, with significant differences observed across all tested antibiotics viz. ciprofloxacin, clindamycin, azithromycin, ceftriaxone, gentamicin, and fluconazole. Notably, non-diabetic samples consistently exhibited greater susceptibility to these antibiotics compared to diabetic samples, reflecting the immunocompromised state associated with diabetes and its impact on infection control.

In this study, ciprofloxacin sensitivity rate of 83.33% against oral infections was in good agreement with Umeshappa et al [26], who found that ciprofloxacin was just as effective as cefotaxime. Present finding showed lower ciprofloxacin efficacy in diabetic patients despite its broad antimicrobial spectrum, which is in line with the pharmacokinetic findings of Mujahid et al [25] that ciprofloxacin saliva concentrations rarely reach the minimum inhibitory concentration (MIC) necessary to effectively suppress oral bacteria. The observed decreased efficacy is probably due to this pharmacological constraint combined with immunological dysfunction associated with diabetes. Ciprofloxacin has demonstrated a high level of effectiveness against Gram-negative bacteria, such as Escherichia coli and Pseudomonas aeruginosa, as well as numerous Staphylococcus and Streptococcus species, as reported by Umeshappa et al [26], Chang et al [27], and Chandra et al [28].

Clindamycin was also more successful in non-diabetic people. Clindamycin is known for its strong action against methicillin-resistant Staphylococcus aureus, Streptococci, and anaerobic germs. According to Levi and Eusterman's [29] research, it is as effective as penicillin in treating odontogenic infections and can be used as a second-line treatment for those who are allergic to it. Although there is less activity in diabetic subjects, our results are consistent with these findings, indicating that the therapeutic efficacy of clindamycin may be lessened by diabetes immunological dysfunction.

Even at lower dosages, azithromycin demonstrated strong bacterial inhibition, beating a number of other antibiotics in both groups. This is in line with Beganovic et al [30], who found that azithromycin inhibited oral pathogens by 69.8%. Furthermore, our data showed that inhibition was somewhat lower in diabetic patients, supporting earlier findings by Buset et al [19]. that azithromycin's effectiveness is impaired in diabetes. These results highlight the potential of azithromycin as a first-line treatment for oral infections.

Ceftriaxone showed strong antibacterial properties at all doses, but it worked better on people without diabetes. In contrast, Casarin et al [31] stated that ceftriaxone was mostly used for major surgical procedures and rarely utilized in the normal treatment of oral infections. According to our findings, ceftriaxone may be used more broadly to treat oral infections, particularly in individuals without diabetes.

At low dosages, gentamicin showed remarkable efficacy, retaining high inhibitory rates in people with diabetes. Gentamicin's greater activity against dental infections was similarly confirmed by Dantas et al [32], and Dias et al [33], supporting its potential as a potent treatment alternative. However, its decreased effectiveness in diabetic individuals highlights the difficulties in treating infections in this population.

In line with Patel et al [34], who noted enhanced oral candidal carriage in people with type-2 diabetes mellitus (T2DM), fluconazole's antifungal activity was noticeably higher in non-diabetic participants. The need for attentive antifungal medication to avoid persistent oral candidiasis is highlighted by the increased fungal burden in diabetics.

This work shows that oral infections in diabetic patients are less susceptible to antibiotics than those in non-diabetics. This phenomenon is probably caused by immunosuppression linked to diabetes, changes in medication pharmacokinetics, and the ability of biofilms to withstand high blood sugar levels. In order to improve therapeutic success and reduce antibiotic resistance, the treatment of oral infections in diabetic populations necessitates specialized antibacterial regimens in addition to strict glycemic control.

At last, present findings emphasize the critical need for integrated approaches that address both metabolic control and oral health in diabetic populations. Tailored antimicrobial therapy, informed by susceptibility profiles and patient-specific factors, will be essential to improving clinical outcomes and reducing the public health burden of oral infections and antibiotic resistance in Bangladesh.

Conclusion

This study demonstrates that diabetic patients, due to their compromised immunity, require higher concentrations of antibiotics to effectively inhibit oral biofilm-forming bacteria compared to non-diabetic individuals. Among the antibiotics tested, azithromycin and gentamicin were notably effective in inhibiting bacterial growth even at low concentrations in diabetic patients, suggesting their potential as preferred treatments for oral infections in this group. To achieve optimal therapeutic outcomes in diabetic patients, increasing antibiotic dosages may be necessary. These findings highlight the importance of tailored antibiotic strategies for managing oral infections in diabetic populations.

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Citation: Anil Chandra Deb.,et al. "Antibiotic Resistance in Oral Microbiota of Diabetic and Non-diabetic Populations in the Northern part of Bangladesh". NL Journal of Dentistry and Oral Sciences 3.2 (2026): 12-23.