

Microbial Etiology of Bacteremia in Controlled and Uncontrolled Type-2 Diabetes in Eastern Part of India

E. Premprakash Patra¹, Dayanidhi Meher², Mrutyunjay Suar¹, Jyoti Panigrahi¹ and Srilekha Mishra^{1*}

¹KIIT school of Biotechnology, KIIT University, Campus-11, Patia, Bhubaneswar, Odisha

²Kalinga Institute of Medical Sciences, KIIT University, Patia, Bhubaneswar, Odisha

*Corresponding author: Dr. Srilekha Mishra, KIIT School of Biotechnology, Bhubaneswar, Odisha, Tel: +919937443314; E-mail: mishra.srilekha@gmail.com

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Abstract

Bacteremia in Type 2 Diabetes (T2D) is the most serious public health issue. Very limited information is available in this aspect with reference to complications. We hypothesized that abnormal increase in blood sugar levels may result in different bacterial infection associated with different categories of complications. Therefore, the aim of the present study was to evaluate and investigate the possible causes of bacteremia in T2D patients and its related complications in eastern region of the country where the data is unavailable to the best of our knowledge. The study included a total of 244 established T2D patients and 20 healthy controls in and around Bhubaneswar region, Odisha. Staging of T2D was done as per standard criteria into controlled and uncontrolled T2D. Blood samples were collected from all subjects including healthy controls and culture was done for bacterial isolation, identification. Prevalence of bacteremia was more among the uncontrolled cases (70.1%) than the controlled ones (29.8%) compared to healthy controls with $p < 0.0001$. Occurrence of both gram positive and gram negative bacteria were found which comprised of *Staphylococcus sp.*, *Streptococcus*, *Bacilli*, *E. coli*, *Klebsiella*. These groups of bacteria were thought as the causes of T2D complications. Eastern region of India being called as sweet region have a great affinity towards food and entertainment. So, we expect a higher intensity of sugar level in their blood and culture which often remains undiagnosed and develop to complications. Therefore, in our results we evaluated a strong connection of hyperglycemia with bacteremia providing a key for good empiric treatment in such high risk population.

Keywords: Type-2 diabetes; bacteremia; diabetic complications; Hyperglycemia

Introduction

Diabetes mellitus is the most common, debilitating, chronic, attenuating endocrine disease that results in increased public health and clinical problems. Approximately 346 million people suffer from this disorder annually [WHO, 2016]. The number of people suffering from diabetes were estimated to be 439 million or 7.7% of the total population by 2030 out of 346 million of total population with an increment of 20% in developed countries and 69% in developing countries [1]. Diabetes is the leading cause of many macro and micro vascular complications. It affects the functions of multiple organ systems like kidney, heart, foot, blood vessels, nerves, etc. A fourfold increased risk in cardio- and cerebrovascular disease is found in diabetics [2]. It is the leading cause of end stage kidney disease, lower extremity amputations, and also adult blindness [3].

Earlier reports suggested that, diabetes contribute to 6.8% of total global death in all age groups [4]. During the last few years, there has been increasing incidence of infections in diabetic individuals with diabetic complications due to opportunistic infections [5]. It was noted that, the mortality rate was 13% due to diabetes and the rate was increased to 87% due to diabetic complications with infections [6] suggesting an the risk of infections due to this disease. Until 2000, the same was supported by several authors [7,8]. There are evidences of bacteremia arising in diabetics, although in some areas the evidence is still scanty. Infections in diabetic population can be intense and deadly

masque by chronic complications leading to late perception and medical addressability.

Generally, diabetes is divided into two categories, type-1 (T1D) and type-2 (T2D). Most individuals with T2D can manage their illness with perfect lifestyle, food practices etc. They are called as controlled diabetics while other subjects are diagnosed with uncontrolled state of the disease with obesogenic environment, probability of socioeconomic status, depression, race and hypertension [9,10]. Uncontrolled diabetics have been proved to be the source of major complications affecting heart and blood vessels. High sugar concentration damages capillaries causing neuronal defect. This affects different organs such as heart, kidney, eye, teeth, digestive system etc., [11,12]. Uncontrolled diabetes is more painful and needs an expensive treatment. According to opinion of American Diabetes Association, T2D diabetes is a state of hyperglycemic condition that causes blood glucose levels to rise higher than normal. Due to increased blood sugar level, the individuals are more susceptible to infections especially bacteremia [13].

Patients with diabetes have 4.4 times greater risk of systemic infection caused by bacteria compared to non-diabetics [14]. The major cause of infection is impaired cellular and humoral immune defense system caused by hyperglycemia. Phagocytosis being the major defense mechanism against microorganisms is disrupted due to inability of WBC to break the phagocytosed microorganism. Hyperglycemic state hinders the mechanism of phagocytosis leading to increased incidence of bacterial infection in T2D individuals [15]. Hyperglycemic state serves as a defined pathway for bacteria as they grow better in the presence of sugar [16]. Therefore, more adherences of bacteria in blood cells in addition to the expression of virulence

factors may be a possible reason for the increased rate of severe infections with microbial etiology.

In the current study, we made an approach to identify the microbial species that contribute to bacteremia in patients with T2D cases comprising controlled and uncontrolled groups and correlated the prevalence pattern with clinical complications in T2D diabetic subjects.

Research Design and Methods

Patients and sample collection

Endocrinology clinic is certainly the busiest departments concerning the number of infection conditions in patients. Study was commenced after ethical clearance from institutional board and written consent obtained from individuals. Samples from patients with established T2D were selected by simple random sampling with the age ranging from 25 to 65 years who attended the outpatient department of KIMS hospital in Bhubaneswar.

The study population comprised of 244 established T2D subjects according to ADA criteria. The staging and classification were based on Fasting blood sugar (FBS) and Post Prandial blood sugar (PPBS) level with reference to HbA1c. The subjects were divided into controlled and uncontrolled diabetics based on glycemic status and Glycosylated hemoglobin (HbA1C) level on the same day and same time of examination.

Controlled Type-2 Diabetes-FBS \leq 150, PPBS \leq 200, HbA1c $<$ 7

Uncontrolled Type-2 diabetes-FBS \geq 150, PPBS \geq 200, HbA1c $>$ 7

Demographic details, details of medical history such as duration of disease, glycemic status (HbA1c), levels of FBS and PPBS, dietary habits etc. were collected from patients and also from medical records. Clinical features along with the risk factors were also recorded carefully. The risk factors also included Hypercholesterolemia, Hypertension and Family history. Anthropometric parameters were taken according to standardized procedures. To address the presence of complications, a detailed report was obtained from certified clinicians.

The complication occurred in both controlled and uncontrolled subjects. Microvascular complications included diabetic nephropathy, neuropathy, ophthalmic and periodontal infections. Examination by certified ophthalmologists, dentists and neurologists were done for confirmation. Macro vascular complications included foot disease, skin problems, cardiac disease etc.

Isolation and identification of bacterial isolate

The blood samples were directly introduced to Brain heart infusion broth bottles with a ratio of 1:10 of blood to the medium used. The bottles were kept at 37°C for 7 days with shaking condition for the first 48 hrs. Repeated observation of positive bacterial culture was taken from day 1 to day 7 [17]. Suspected positive cultures were sub-cultured to MacConkey agar, Bile Esculin Agar, Manitol salt Agar, Blood Agar and Chocolate Agar. All the plates were incubated at 37°C.

Urine samples were also collected simultaneously from patients with UTI and diabetic nephropathy. Urine samples were streaked on UTI

agar (HIMEDIA) for culture analysis. The bacteria were identified according to colour of the colonies on the chromogenic media following the standard chart. Similarly swabbing of affected areas of teeth pockets were also done to isolate bacteria from patients with periodontal infection.

Isolates recovered from positive blood culture, urine culture and teeth swabs were classified by colony morphology, gram staining, sugar tests and basic biochemical tests. Further confirmation of the isolates was carried out through sugar test kits (HIMEDIA).

The results were put on the online system (ABIS online /PIBWIN) [18] for probabilistic identification of the bacterial isolates. Organisms that are commonly recovered from the environment or skin were considered as contaminants unless associated with clinical sepsis or results of culture from other body sites. Evidence of clinical sepsis such as bacteremia fever, systolic blood pressure $<$ 90 mm Hg or oliguria ($<$ 20 ml/h were recorded) [19]. The episode of bacteremia was recorded after 7 days post blood culture of individuals.

Statistical analysis

Student's t-test was performed to compare between groups. Statistical Package for Social Science (SPSS version 10) for windows was employed for data analysis. p value less than 0.05 was considered to be significant.

Results

The present work illustrates a total of 244 T2D cases, among which 144 (59%) were males and 100 (41%) females. The age ranged between 25 to 65 yrs with a mean age of 50.29 \pm 10.76 (Controlled) and 52.27 \pm 10.52 (Uncontrolled).

Epidemiological and demographic information

The duration of Diabetes ranged from 1-20 yrs. Of the total diabetic subjects, majority 123 (50%) had newly diagnosed diabetes upto 5 years, followed by 79 (32.3%), with 5-10 yrs duration and 37 (15.1%) within 10-20 yrs duration. 5 (2%) subjects were found to have long term diabetes greater than 20 yrs. The status of controlled diabetics was 34% (n=83) compared to the uncontrolled groups (66%) (n=161). Glycemic control was poor in uncontrolled diabetes. All study groups were taking antidiabetic medicine as all of them have established Type-2 diabetes. 165 (67.6%) subjects were on oral antidiabetic medicine. Results also showed a combination therapy due to some complications. 20 (8.1%) were taking other medicine with insulin therapy. 46 (18.8%) subjects had oral medicine with other therapeutics. 13 (5.3%) subjects were on insulin therapy due to high blood sugar level (Table 1).

The proportion of bacteremia occurred with duration of diabetes. Out of total positive cases (97 of 244), individuals with $<$ 5yrs of T2D were traced to be showing higher incidence of bacteremia (43 of 97, 44.33%), followed by 5-10 yrs (39 of 97, 40.21%), 10-20 yrs (12 of 97, 12.37%), and $>$ 20 yrs (3 of 97, 3.09%) (Figure 1). The numbers gradually decreased with subsequent increase in duration.

| Parameters | Controlled n=83 | Uncontrolled n= 161 | Total n=244 | p value |
|--|----------------------------|-------------------------------|------------------------|----------------|
| Gender Distribution | | | | |
| Female | 38 (45.7%) | 62 (38.5%) | 100 | 0.169 |
| Male | 45 (54.2%) | 99 (61.4%) | 144 | |
| Total | 83 (100%) | 161 (100%) | 244 (100%) | |
| Age of Patients | | | | |
| Mean ± SD years | 51.89 ± 10.10 | 52.27 ± 10.52 | | |
| Range in years | 25-65 | 25-65 | | |
| Anthropometric parameters (Mean ± SD) | | | | |
| BMI (kg/m ²) | 25.68 ± 3.81 | 24.59 ± 3.36 | | |
| Duration of T2D (years) | | | | |
| <5 yrs | 55 (66.2%) | 68 (42.2%) | 123 (50%) | 0.004*** |
| 5-10 yrs | 20 (24%) | 59 (36.6%) | 79 (32.3%) | |
| 10-20 yrs | 7 (8.4%) | 30 (18.6%) | 37 (15.1%) | |
| >20 yrs | 1 (1.2%) | 4 (2.4%) | 5 (2%) | |
| Total | 83 (100%) | 161 (100%) | 244 (100%) | |
| Disease Complications | | | | |
| | Controlled n=43 | Uncontrolled n=94 | Total 137 | p value |
| Microvascular (eye problem, kidney disease, neuroproblm, periodontal) | 21 | 42 | 63 | 0.05** |
| Macrovascular (CAD, PVD, cardiac disease, dyslipidemia/ hyperlipidemia, hyperthyroidism, skin infections) | 22 | 52 | 74 | |
| Negative | 41 | 67 | 108 | |
| Status of treatment of Diabetes mellitus | | | | |
| | Controlled n=83 | Uncontrolled n=161 | Total n=244 | p value |
| On Insulin | 5 | 8 | 13 (5.3%) | 0.009** |
| Oral antidiabetic drugs | 55 | 110 | 165 (67.6%) | |
| Insulin & Combination | 1 | 19 | 20 (8.1%) | |
| Oral & Combination | 22 | 24 | 46 (18.8%) | |
| No treatment | 0 | 0 | 0 | |
| ***P value<0.05 is considered to be significant | | | | |

Table 1: Demographic information of study population.

Microbiological features

Results of blood culture reports (Table 2) revealed that bacteremia or positive blood cultures were observed in uncontrolled and controlled T2D cases. A group of healthy controls (n=20) were also compared. The healthy individuals included in the study were all culture negative and free of infection. The result was statistically significant (p=0.0001) between the T2D subjects and control groups which justifies the incidence of higher bacterial infection in T2D study subjects. 42.2 % of uncontrolled subjects were positive for bacteremia

while 34.9% of controlled ones were found to be bacteremic. The results suggested a higher percentage of bacteremia in uncontrolled diabetics.

Similarly the occurrence of infection with a single species of bacteria and more than one species in cultures of subjects has been compared between the individuals with difference in glycemic status (Table 3). 93.1% of controlled diabetics were bacteremic due to single species of bacteria and 6.8 % with more than one strain of bacteria in culture. Similarly the picture of bacteremia in uncontrolled T2D shows

66.17% with monomicrobial bacteremia and 33.8% cases with polymicrobial bacteremia. The percentage was more in uncontrolled cases as compared to controlled ones suggesting higher frequency of presence of bacteria in culture of T2D individuals with abnormal blood sugar level. Both mono and poly microbial bacterial infections were found in higher proportion in uncontrolled T2D cases. The results were found to be statistically significant ($p=0.005$).

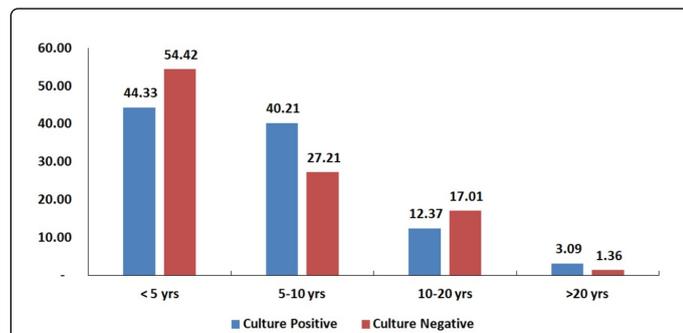


Figure 1: Proportion of bacteremia with duration of T2D. The culture positive cases decreased gradually with the increase duration of the disease. The frequency appeared to be more with the cases within 5 years (44.33%) and between 5-10 years (40.21%). So newly diagnosed cases are more prone to bacterial infections.

| Study Groups | | | | | | |
|-----------------|------------------------|------|---------------------|-------|-----------------------|-----|
| Culture pattern | Type-2 Diabetes (244) | | | | Healthy controls (20) | |
| | Uncontrolled T2D (161) | | Controlled T2D (83) | | | |
| | n | % | n | % | n | % |
| Bacteremia | 68 | 42.2 | 29 | 34.9 | 0 | 0 |
| Non-bacteremia | 93 | 57.7 | 54 | 65.06 | 20 | 100 |

***P value<0.01 is highly significant with respect to healthy controls

Table 2: Percentage of Uncontrolled and Controlled T2D subjects having bacteremia with respect to healthy controls.

| Patients having bacterial infection | Controlled T2D | Uncontrolled T2D | Total | p value |
|-------------------------------------|----------------|------------------|------------|----------|
| Mono microbial | 27 (93.1%) | 45 (66.17%) | 72 (74.2%) | 0.005*** |
| Poly microbial | 2 (6.8%) | 23 (33.8%) | 25 (25.7%) | |
| Total | 29 (100%) | 68 (100%) | 97 (100%) | |

P value<0.05 is highly significant

Table 3: Percentage of Uncontrolled and Controlled T2D individuals with proportion of bacteremia.

The pattern of gram positive (93 of 127, 73.2%) and gram negative bacteria (34 of 127, 26.8%) in both the controlled and uncontrolled T2D are seen (Table 4). The predominance of gram positive bacteria in the bacteremic type-2 diabetic individuals which consisted of both cocci and rods. The frequency of cocci was again higher over the rods and the number significantly increased in uncontrolled T2D cases.

| Gram positive | Uncontrolled T2D | Controlled T2D | Total no. of isolates n=127 |
|-------------------------------------|-------------------|-------------------|-----------------------------|
| <i>Staphylococcus epidermidis</i> | 11 (8.6%) | 1 (0.8%) | 12 (9.4%) |
| <i>Staphylococcus aureus</i> | 15 (11.8%) | 4 (3.1%) | 19 (15%) |
| <i>Staphylococcus hominis</i> | 1 (0.8%) | 2 (1.6%) | 3 (2.4%) |
| <i>Staphylococcus saprophyticus</i> | 7 (5.5%) | 2 (1.6%) | 9 (7.08%) |
| <i>Enterococcus</i> | 9 (7%) | 1 (0.8%) | 10 (7.8%) |
| <i>Streptococcus Sp.</i> | 7 (5.5%) | 6 (4.7%) | 13 (10.2%) |
| <i>staphylococcus sp.</i> | 10 (7.8%) | 2 (1.6%) | 12 (9.4%) |
| <i>clostridium</i> | 5 (3.9%) | 0 | 5 (3.9%) |
| <i>Micrococcus</i> | 4 (3.1%) | 1 (0.8%) | 5 (3.9%) |
| <i>Bacilli</i> | 4 (3.1%) | 1 (0.8%) | 5 (3.9%) |
| Total | 73 (57.5%) | 20 (15.7%) | 93 (73.2%) |
| Gram negative | | | |
| <i>Escherichia coli</i> | 2 (1.6%) | 23 (18.1%) | 25 (19.7%) |
| <i>Klebsiella</i> | 0 | 7 (5.5%) | 7 (5.5%) |
| <i>Citrobacter</i> | 2 (1.6%) | 0 | 2 (1.6%) |
| Total | 4 (3.1%) | 30 (23.6%) | 34 (26.8%) |

Table 4: Spectrum of bacterial isolates from type-2 diabetic patients.

The results of identification of bacterial isolates (n=127) according to their physiological biochemical and cultural characteristics stated that the isolates were species specific, although some of them belong to the same genera. *Staphylococcus aureus* (19 of 127, 15%) was found to be the major and important gram-positive bacteria followed by *S. epidermidis* (12 of 127, 9.4%), *S. saprophyticus* (9 of 127, 7.08%), *Staphylococcus sp.* (12 of 127, 9.4%), *Streptococcus sp.* (13 of 127, 10.2%), *Enterococcus sp.* (10 of 127, 7.84%) and *S. hominis* (3 of 127, 2.36%). Gram-positive rods including *Clostridium* (5 of 127, 3.9%) and *Bacilli* (5 of 127, 3.9%) were lesser in numbers. *Micrococci* (5 of 127, 3.9%) presumed to be as potential skin contaminants were also selected in the study as these isolates were isolated repeatedly from blood culture of the uncontrolled patients with complications. Gram negative bacteria *E. coli* (25 of 127, 19.7%), *Klebsiella* (7 of 127, 5.5%) and *Citrobater* (2 of 127, 1.6%) were also isolated from the hyperglycemic individuals. The percentages of different bacterial isolates were found to be comparatively more in uncontrolled cases as compared to controlled ones.

We correlated the frequencies of bacterial isolates with clinical complications in T2D cases. The results are mentioned in Table 5. Major organisms associated with UTI and nephropathy were *E. coli*, *S. aureus* and other *Staph sp.* followed by *Streptococcus*. *Streptococcus* and *Clostridium* were also the causes of bacteremia in patients with no

complications. They were found to be present sub clinically. Bacteria were also isolated from patients with underlying disorders like Hypertension and Hypercholesterolemia. *S. saprophyticus*, *Enterococcus* and *Streptococcus sp.* were also found in cases with periodontal infection.

| | Nephropathy | | Periodontal Infection | | Skin Infection | | UTI | | Hyper-cholesterolemia | | HTN | | Subclinical | |
|-------------------------------------|-------------|--------------|-----------------------|--------------|----------------|--------------|------------|--------------|-----------------------|--------------|------------|--------------|-------------|--------------|
| | Controlled | Uncontrolled | Controlled | Uncontrolled | Controlled | Uncontrolled | Controlled | Uncontrolled | Controlled | Uncontrolled | Controlled | Uncontrolled | Controlled | Uncontrolled |
| Isolates Microorganism (%) | 16(13.4%) | 19(15.9%) | 2 (1.6) | 7 (5.8%) | 1 (0.8%) | 2 (1.6%) | 2 (1.6%) | 24 (20.1%) | 3 (2.5%) | 0 | 0 | 8 | 8 | 27 |
| <i>Staphylococcus epidermidis</i> | 0 | 1 (5.2%) | 0 | 1(14.2%) | 0 | 0 | 0 | 0 | 1(33.3%) | 0 | 0 | 1(12.5%) | 0 | 4(14.8%) |
| <i>Staphylococcus aureus</i> | 4 (25%) | 4 (21%) | 0 | 0 | 0 | 1 (50%) | 0 | 2(8.3%) | 0 | 0 | 0 | 3(37.5%) | 0 | 4(14.8%) |
| <i>Staphylococcus hominis</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2(25%) | 1(3.7%) |
| <i>Staphylococcus saprophyticus</i> | 0 | 0 | 1 (50%) | 3(42.8%) | 0 | 0 | 0 | 3(12.5%) | 1(33.3%) | 0 | 0 | 0 | 0 | 1(3.7%) |
| <i>Enterococcus</i> | 0 | 0 | 1 (50%) | 1 (14.2%) | 0 | 0 | 1(50%) | 5(20.8%) | 0 | 0 | 0 | 1(12.5%) | 0 | 1(3.7%) |
| <i>Streptococcus Sp.</i> | 2 (12.5%) | 1 (5.2%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1(12.5%) | 4(50%) | 4(14.8%) |
| <i>staphylococcus sp.</i> | 0 | 2 (10.4%) | 0 | 1 (14.2%) | 1(100%) | 1 (50%) | 0 | 1(4.1%) | 0 | 0 | 0 | 0 | 1(12.5%) | 5(18.5%) |
| <i>clostridium</i> | 0 | 0 | 0 | 1(14.2%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1(12.5%) | 0 | 3(11.1%) |
| <i>Micrococcus</i> | 0 | 1 (5.2%) | 0 | 0 | 0 | 0 | 0 | 1(4.1%) | 1(33.3%) | 0 | 0 | 1(12.5%) | 0 | 1(3.7%) |
| <i>Bacilli</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1(12.5%) | 3(11.1%) |
| <i>E.coli</i> | 9 (56.2%) | 8 (42.1%) | 0 | 0 | 0 | 0 | 1(50%) | 7(29.1%) | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Klebsiella</i> | 1 (6.2%) | 2(10.4%) | 0 | 0 | 0 | 0 | 0 | 3(12.5%) | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Citrobacter</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2(8.3%) | 0 | 0 | 0 | 0 | 0 | 0 |

Table 5: Percentage of microorganism isolated from blood culture of controlled and uncontrolled Type-2 diabetic patients according to complications.

Among the study population (n=244), 58(23.7%) individuals assumed they had any infection while coming to clinic, but 148(60.6%) were clinically suspected by questionnaires and clinician observation. They were all subjected to blood culture analysis and about 97 (39.7%) were bacteriologically confirmed for presence of bacteremia (Figure 2).

Discussion

It is known that onset diabetes is a risk factor for bacteremia [20]. The incidence of bacterial infection increases with up regulation of blood sugar level [21]. Diabetes, especially T2D cases are related to several long term micro & macro vascular complications and there is also an association between diabetes & systemic complications like

heart disease, kidney disease, eye problem, neuro problem in those subjects [22,23]. In our study, bacteremia along with the clinical complications was found to be significantly higher in T2D cases as compared to healthy controls.

Distribution of bacterial isolates revealed the predominance of gram positive bacteria over Gram negative bacteria as observed in our study. The contribution of gram positive bacteria causing bacteremia in diabetes subjects is supported by various authors [24,25]. However, it was also suggested that diabetes is neither related to Enterobacteriaceae bacteremia, nor affected by the outcome of gram negative bacteria [26]. However, our study indicates the presence of gram negative bacteria in cultures of patients and this finding is

supported by previous reports [20]. Our results also depicted a greater incidence of *Staphylococcal* isolates in T2D cases than T1D. The bacteremia caused by *Staphylococcus sp.* is comparatively higher and most of these species are resistant to Methicillin in individuals with an abnormal blood sugar level. This is in accordance with previous reports of Davenport, 2016 who found methicillin resistant *Staphylococcus* in foot ulcer in T2D diabetic patients.

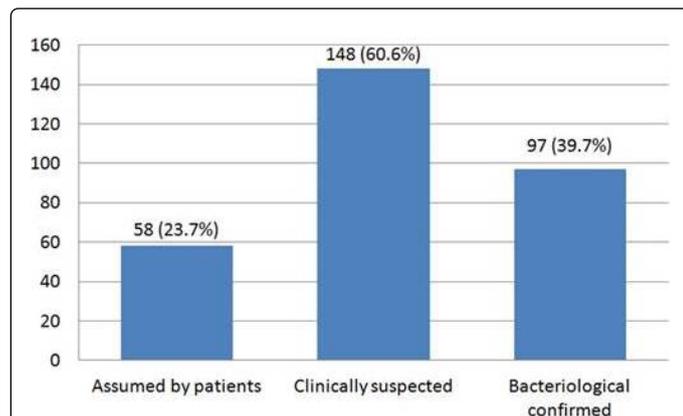


Figure 2: The relationship between patient assessment on bacteremia, clinical finding and bacteriological results. The outpatients coming for checkup are asymptomatic and carry the infections within them. Out of the total assumed cases (23.7%), most of the cases were clinically suspected (60.6%) by the clinicians due to various reasons. But only 39% of them were confirmed culturally by the standardized procedures.

Micro and macro vascular complications are associated with T2D. Most of the bacterial infections are observed in individuals with micro and macro vascular complications. Our results are supported by this observation describing the increased percentage of kidney diseases in individuals with poor controlled state of blood sugar level. There is a significant difference between diabetic linked complications of individuals with glycemic state [27]. Different microorganisms are responsible for causing bacteremia irrespective of different complications. Our observation shows *Staphylococcus sp.* and *E. coli* were found to be the major source of bacteremia in T2D cases which links to major complications like kidney infection, periodontal infection and skin infection. Gram positive cocci, such as *Enterococci* and *Staphylococcus sp.* are the sources of bacteremia in kidneys and consequently resulting renal damage in diabetic subjects has been found out in our study. While some reports described the occurrence of *Staphylococcus sp.* and *Enterococci* in the etiology of renal disease [28,29]. Few authors have suggested an increased incidence of *Staphylococcal* and *Streptococcal* infections in diabetic patients with complications like periodontitis, skin infections, foot ulcers [12,30]. Although it has long been a common clinical belief that diabetes increases the risk of *S. aureus* infection, until now there has been little evidence to support this. In our study we got higher percentage of *Staphylococcus aureus* (11%) in uncontrolled individuals with kidney disease as compared to controlled cases. Whereas, *E. coli* constitutes about 18.1% in controlled cases with UTI infection. These findings indicate that bacteria persist inside the body sub clinically without any symptoms and with increase in blood sugar level these bacteria grow favorably and travel through blood to different parts of the body causing complications. Although, extensive reports have been made on

role of microbes in causing infections in diabetic subjects, contribution of these organisms in controlled and uncontrolled cases are not known. Further, *Streptococcus viridians* were isolated in 40.3% of chronic periodontitis cases with diabetes [31]. Our results showed 5% of *Streptococcus sp.* and *Streptococcus lactis* in uncontrolled diabetic patients having periodontal infection. These results partly correlate with on the studies by [32] who cultured *Staphylococcus* in uncontrolled diabetic individuals having periodontal infection but could not isolate any *Streptococcus sp.* We found that 7% of *Enterococcus* isolates in uncontrolled study groups which was the major cause of periodontal infection. Although, *Enterococcus* is considered as a contaminant and is not harmful for the host, recent reports suggested *Enterococcus* to be a major risk factor in nosocomial infections and its prophages are described as important elements in competition between strains during colonization as well as pathogenicity of strains. *Enterococcus* was mainly observed during end stage renal failure individuals [33]. Identified a range of the species causing bacteremia that includes *Pseudomonas*, *S. epidermidis*, *S. hominis*, *coagulase negative Staphylococcus* and *E. coli* incases with ulcer out of which 20% were found as diabetic. The authors found *Pseudomonas* and *E. coli* to be prominent organisms, although *Staphylococcus aureus* was also found as the dominant one in other cases of ulcer [34]. Importantly, this bacterium causes a wide range of clinical infections (e.g., bacteremia, endocarditis, skin and soft tissue, osteoarticular, pulmonary and device-related infections) [35]. However, in our study we detected all these bacteria related to different clinical complications.

Bacteremia with *S. epidermidis* was associated with kidney disease and periodontal disease [36-38]. We also found the same group of bacteria in T2D subjects, preferably uncontrolled T2D. However, our results showed that *Bacilli* (17.3%) and *Clostridium* (13%) caused bacteremia in periodontal and subclinical infection. This finding is in contrast to the reports made by [16], who found only 4.6% of bacilli causing bacteremia in diabetic patients. Interestingly, in our study, few species of gram negative bacteria were also isolated in T2D patients with urinary tract infection, nephropathy and blood stream infections. *E. coli* and *Klebsiella* are major organisms isolated from these subjects, which supports the work of where the predominant strains isolated from 148 bacteremic episodes were *E. coli*, *Klebsiella*, *S. aureus*, *Enterococcus*, *S. epidermidis* etc. in hemodialysis and UTI patients.

It is assumed that Skin infection, UTI and periodontal infection are the most important link between T2D and increase risk of bacteremia. They may serve as the key roles for the transmission of microbes from affected parts to blood causing bacteremia. Similarly cases suffering from UTI may serve as the portal entry for the microbes to cause nephropathic complication. The most common cause of skin infection was *Staphylococcus aureus* and *S. epidermidis*. Skin can also turn into a medium of entry for microbes causing bacteremia.

Among diabetics, subclinical cases refer to those which are undiagnosed. Interestingly, our results showed around 33% of bacterial isolates in controlled cases without any clinical complications. The most dominant organism was *S. hominis* that constitutes 16% of total. This is novel data and no such report is made before. It was confirmed after regular culture and continuous observation. *S. hominis* has been shown to cause nosocomial or community acquired infection in immunocompromised patients [36]. To our knowledge T2D normally occurs in those subjects. Due to MDR of *Staphylococcus sp.*, *S. hominis* has also gained importance in this respect, which was also interpreted in our study.

From the demographic study, it is clear that the numbers of uncontrolled T2D cases were significantly higher than the controlled ones. One reason may be due to shifting of many subjects in controlled diabetic group to uncontrolled group after gaining an imbalanced blood sugar level. Other reason may be due to lifestyle and environmental factors. Further, increased levels of sugars in uncontrolled cases lead to presence of higher incidence of bacteremia as compared to controlled cases, which is a novel finding in our study. We also observed a significantly higher incidence of clinical complications in uncontrolled cases as compared to controlled ones indicating a higher association of clinical complications with uncontrolled subjects. It has been thoroughly reviewed before that lifestyle factors, biochemical status basically regulate the uncontrolled status of diabetic individuals. However, we for the first time showed that the bacteremia status also contribute to staging of T2D into two categories. This means that, by getting bacterial infection, a subject in controlled group can go to an uncontrolled state. Therefore, social awareness, cautions and choice to prevent bacterial infections in different sources is absolutely necessary to avoid more severe and chronic complications in diabetic patients. We made a through microbiological analysis in controlled and uncontrolled diabetic individuals to examine the factors that can contribute to staging of T2D cases. We find that, hyperglycemia referred to as uncontrolled state of T2D leads to occurrence of higher incidence of polymicrobial bacteremia and onset of chronic complications. We further examined the distribution of bacterial species in T2D cases with clinical complications such as nephropathy, periodontal and urinary tract infections where *S. aureus*, *E. coli*, *Enterococcus*, *Klebsiella*, *Streptococcus* were the common causes in varying degrees which in turn specify a specific organism is responsible for the cause of a particular complication. Hence careful clinical evaluation, improvement of nutritional status and successful management of Diabetes mellitus considerably can reduce bacteremia. Finally, surveillance of local microbiology is of utmost importance for appropriate empirical antimicrobial treatment.

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Compliance with Ethical Standards

Ethics Approval and Consent to Participate Study is approved by the local Ethics Committee.

Competing Interests

The authors declare that they have no conflict of interest.

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