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Isolation and Identification of *Staphylococci* and *Pseudomonas* from Diabetic Foot Ulcer in Perambalur District Hospitals in Tamil Nadu

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Diabetic foot ulcers are still a problem for the health care system and are majorly influencing quality of life. Infected foot ulcer is one of the most feared complications of diabetes mellitus, leading to gangrene and it needs to be amputed. Diabetic foot lesions are major medical, social and economic problem and leading cause of hospitalization for patients with diabetes worldwide. The risk of a diabetic patient for developing a foot ulcer was estimated to be ~25%. Samples were collected from 65 infected patients of diabetic foot ulcer male and female. The mean age group was found to be 40-80 years with the clinical history such as age, sex, types of diabetes duration of diabetes, size of ulcer and duration of ulcer were observed and recorded. The Specific mediums were used for the isolation and identification using classical methods based on their morphology, Grams stain reaction, oxidase and catalase tests were performed to confirm the isolates were both gram-negative and gram-positive organisms Bergey's manual of Determinative Bacteriology. Further, MALDI- TOF **(**Matrix-Assisted Laser Desorption/Ionization) was used to confirm the isolates identified in classical methods were *Staphylococci* (79%) and *Pseudomonas* (21%) In this study

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Among 68 isolates, identified in out of 41 isolates *Pseudomonasaeruginosa* (21%) *Staphylococcus aureus* (54%) *Staphylococcus hominis* (20%)and *Staphylococcous hemolytic*(5%). Although in gram positive organism *Staphylococcus aureus* (54%) was the most predominant isolate found in diabetic foot ulcer.

Keywords: Diabeticfoot ulcer; Staphylococcus aureus; maldi-tof and protein extraction.

1. INTRODUCTION

The chronic disorder in diabetes is leads to very serious damage to many systems of body. Globally 2010 it is period 285 million people affected in estimated, so that 90% of the cases constituting type II diabetes. 2011 had 366 million people have in diabetes. In the year of 2013, International diabetes federation estimates 381 million peoples have diabetes. The diabetes for all age groups prevalence of estimated worldwide 2.8% in 2000 and 4.4% in 2030. The 2000 in 171 million people with diabetes rise in 2030 for 366 million people affected. India the prevalence of diabetes more pronounced in urban areas and roughly doubles then rural areas [1].

The world over is major health problem increasing the globally in the danger of alarming rate for diabetes mellitus [2]. The Diabetes mellitus is a chronic disease one of the disorder in depend many factors such as etiological age sex heredity and economic in physical activity environmental factor in life style and various factors act in complex manner [3]. In suffering from majority of patient affecting on long term complication in diabetes foot ulcer worldwide prevalence in the diabetes mellitus to compare there all most sudden surge increase in associated diabetic foot infection [4].The multi-drug resistant association of diabetic foot ulcer challenge faced the physician and surgeon in treating for amputation [5].

The diabetes foot ulcer leading cause the hospitalization in patient on one of the most feared complication Pathological based on the characteristic such on difficulty in diabetes mellitus in neuropathy, peripheral vascular disease, and most importance of diabetic foot ulcer infection without infection in leading development in gangrene, osteomyelitis as necessitating limp amputation [6]. The development of infection in favoring on limits access of phagocytes and impaired micro-vascular circulation in patient for diabetic foot [7]. The foot infections persons in diabetes are treated in initially an empirically, to directed therapy knows as outcome is improve the causative organisms [8].

Taxonomy is systematic classification in microbes the basic required quantifiable properties the example for bacterial classification in macroscopic and microscopic properties used in organisms into related groups, followed the metabolic and antigenic properties in more than genomic relationships approach still in value for the clinical microbiologists. Example the gram stain is a classification in bacterial isolates in powerful tool. Classify organisms is based on rules an define arbitrary division although scheme in relationship for genus, species, subtype so that genome analysis standard in currently accepted for new technique as matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) used in classified organisms must be compare with genomic classification [9].

Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) recently used to identification markers in directly profiling and quantifying the peptide and proteins in biological samples under different physiological or experimental conditions in Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry (MS) has been important to evaluate and reduce error from technical variation [10]. Currently available in MALDI-TOF MS system are commercially used epidemiological classification and identification of bacteria that can be provide in directed antibiotic treatment for microorganisms more rapidly identification directly as rearrangement for earlier antibiotic, the demonstrated when MALDI-TOF MS was applied in culture in from blood culture [11]. The unusual pathogen for more precise in identification reference laboratory using protein fingerprints by MALDIP-TOF MS, the confirmed was later by 16s rRNA gene sequencing in our laboratory performed molecular biology [12]. The powerful tool in clinical isolates emerged as a recently identification MALDI-TOF MS. The whole cell proteins were extracted using ethanol formic acid extraction method and direct smear method on MALDI plate. In the present research

work, we planned to isolation and identification of *Staphylococci* and *Pseudomonas* species from the pus samples of diabetic foot ulcers in the Perambalur district.

2. MATERIALS AND METHODS

2.1 Sample Collection

The pus samples from collected from the Diabetic wound bearing patients Fig. 1 on the Government Head Hospital, Perambalur in Tamil Nadu. The wound sites DFU of 65 diabetic patients among the different regions of Perambalur district were selected and the written consent was given to these patients. After getting permission from these patients as well as hospital head, the pus samples were collected with at most care under the supervision of doctors. After the collection, samples were sealed in air tight container and transported to the laboratory for further experiments. Patients history collected from the subject detailed demographic data include age, sex, occupation, socio economic status from types of treatment were gathered. The duration in for ulcer ranged from cases Wagner's grading 0 to V.

2.2 Wagner's Grading 0 to V Classification

Grade 0 No open lesion
Grade I Superficial ulcer
Grade II Probing to tendon or capsule
Grade III Deep ulcer with osteiomyelitis, abscess and joint sepsis
Grade I Local gangrene in Frient foot whole ulcer
Grade V Gangrene of entire foot ulcer

In our study, approximately 70% of patients were in Grade III. This study of the all diabetic foot ulcers were respective ulcer grading. Prepared the according to the questions in this study, subjects for suffering from patient other diseases such as cardiovascular disease, renal disease, cancer and auto immune disease are excluded. The sample pus collected from the patients after ulcer base debridement. To avoid contamination, foot wounds and tissue debris were cleaned thoroughly sterile water for slow by gentle rubbing of the site of DFI wound and 70% of alcohol to swabbing. Pus sample collected in sterile cotton swab sticks moist end in sterile water in dipping and before sample collection. Then swab deeply extended into depth of the wound site in majorly avoid the wound surroundings from without contamination. The pus copious volumes existed collected from aseptically needle aspiration to avoid major exogenous contamination. Sample pus collected after peptone water (20ml) sterile tube to transport properly labeled and maintains aseptic condition from laboratory.

2.3 Samples Processing and Isolation of MDR Microbial Strains

Then various differentials selective media in specific mediums were used for the isolation of Mannitol salt agar and cetrimide agar. Preservation of the strains and for further experiments Brain Heart infusion Agar (BHIA) and Nutrient Agar (NA) were used. Preservation of the strains and for further experiments Brain Heart infusion Agar (BHIA) and Nutrient Agar (NA) were used. Identification using classical methods based on their morphology, Grams stain reaction, oxidase and catalase tests were performed to confirm the isolates were both gram-negative and gram-positive organisms confirm the isolates were gram-positive organisms such as *Staphylococcus aureus*, *Staphylococcus hominis, Staphylococcous hemolytic* and gram-negative *Pseudomonas aeruginosa* according to the Bergey's manual of Determinative Bacteriology. Further, MALDI-TOF **(**Matrix-Assisted Laser Desorption/Ionization) was used to confirm the isolates identification. The whole cell proteins were extracted using ethanol formic acid extraction method and direct smear method on MALDI plate.

3. RESULTS

3.1 Study of Profile Subjects

The study profile of the patients with diabetic foot ulcers were illustrated in the Table 1. The more number of diabetic patients are belonged in the age group of 51-60, when compared with other age groups Fig. 3. The 46% of diabetic patients were falls under this age group than other age groups Table 1.

In the other hand, the male populations contribute to the major percentage of diabetes. In our study, the male patients are 72%, when compared with female population those has only 28%. The most of the patients has the grade points III, when compared with others Table 1. The diabetic patients with grade point III are the 49% followed by the grape point IV 32% Fig. 2.

Diabetic foot ulcer is predominantly polymicrobial infection with ability of form in biofilm, important virulence factor and also associated in treatment failure. Out of 65diabetic foot ulcer patients including the study there were patient for 47 (72%) males and 18 (28%) females. The mean age of for patient was 31± 80 years. So that 41 isolated in confirm the isolates total were both gram-negative 9 (21%) and gram-positive 32 (79%) organisms such as *Staphylococcus aureus* 22 (54%), *Staphylococcus hominis* 8 (20%), *Staphylococcous hemolytic* 2 (5%) and *Pseudomonas aeruginosa* 9 (21%) in MALDI plate smear method in direct whole cell proteins were extracted using for ethanol and formic acid Fig. 3. The findings of our study clearly demonstrated that the *S.aureus* is majorly present in the pus samples of diabetic foot ulcer patients, when compared with other strains. *S.aureus* showed the presence in 54% followed by the *S.hominis* (20%). The microscopic observation of gram-stained *S.aureus* was depicted in the Fig. 5.

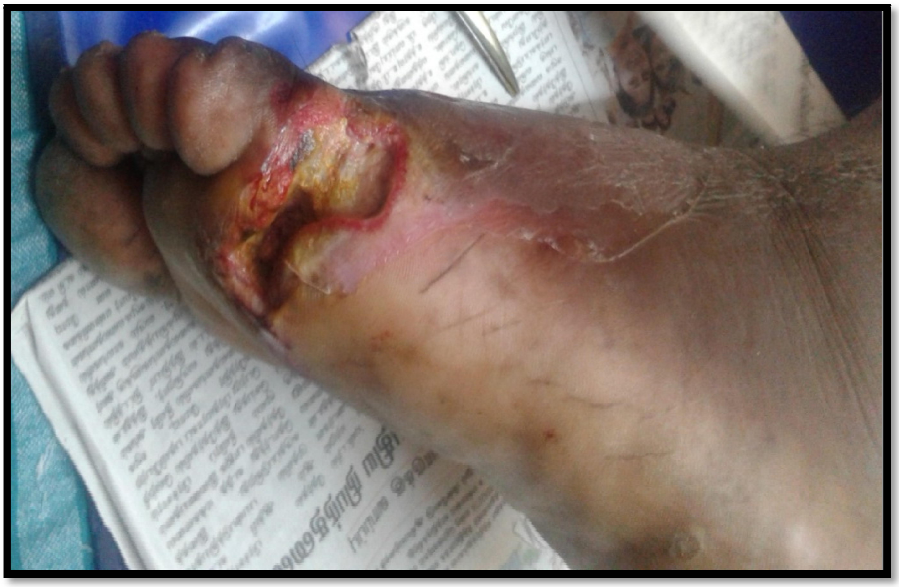

Fig. 1. The diabetic foot ulcer infection site

Table 1. Study of profile subjects

S. No	Variables	Frequency	%
1.	**Age in years**		
	31-40	11	17
	41-50	10	15
	51-60	30	46
	61-70	10	15
	71-80	4	6
2.	**Sex**		
	Male	47	72
	Female	18	28
3.	**Grade**		
	0	-	-
	I	-	-
	II	3	5
	III	32	49
	IV	21	32
	V	9	14

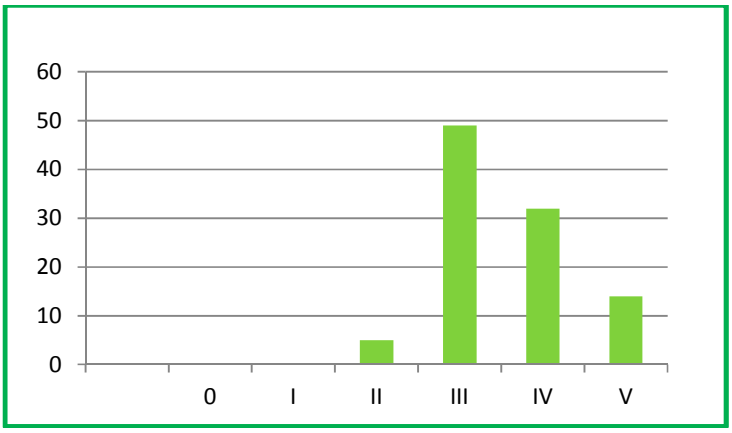

Fig. 2. Infection grade of diabetic foot ulcer patients in this study

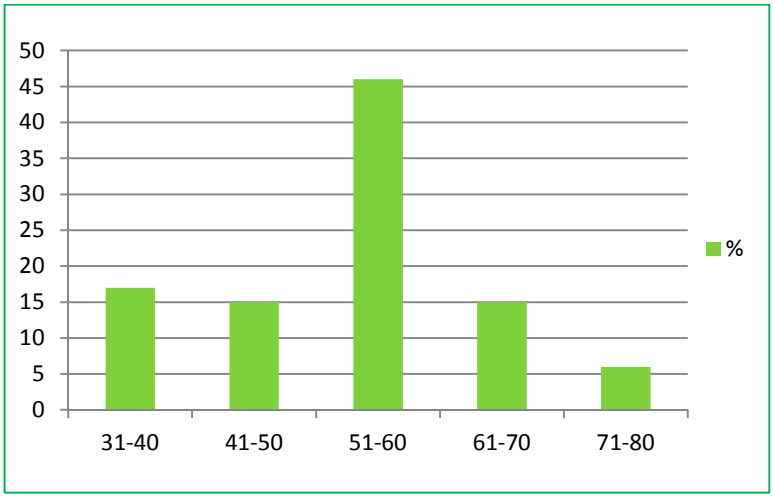

Fig. 3. The age and infection profile of diabetic foot ulcer patients

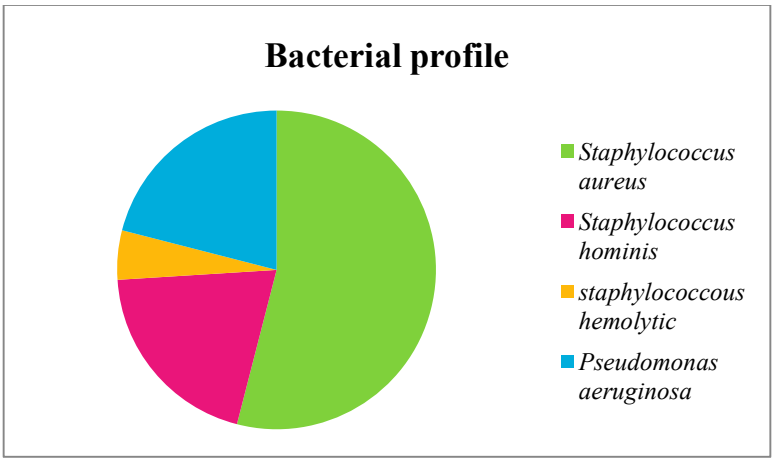

Fig. 4. Bacterial profile of pus samples from the diabetic foot ulcer patients

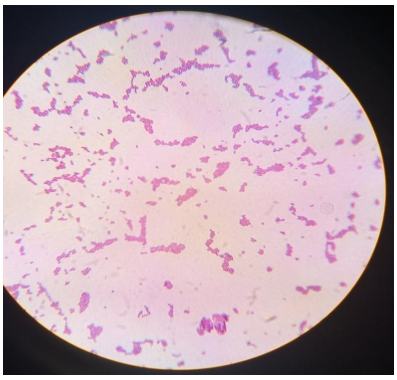

Fig. 5. Microscopic observation of isolated *Staphylococcous aureus*

4. DISCUSSION

Diabetic foot ulcers for more bacterial infections rapidly tissue damage irreversible and ultimately leads. Treatment is essential to prevent amputation of infected foot. This study comprises of the data 65 diabetic foot ulcer patients.

Table 1 proved that among the majority of 30(46%) patient for 51 – 60 age group male and female predominant in the study of population also having type II diabetes obtained in other similar findings. The majority of patients with diabetic foot ulcers in this study were suffering from diabetes longer than 5 years more. It is diabetes patient most under the oral hypoglycemic therapy [13]. The ulcer of classification followed in wagner classification system assess ulcer and depth presence of osteomyelitis or Gangner based diabetic ulcer in predominantly 32(49%) grade III in deep ulcer followed poly microbial infection and similar presented study [14].

The southern India, diabetic foot infection studies obtained very result from poly microbial infection and shown varying pattern of microorganism distribution and sensitivity based contradictory severity of the infection [15]. The most previous studies predominantly showed in gram positive such as *Staphylococcus aureus* and gram negative organism *Pseudomonas aeruginosa* in diabetic foot infection to frequent isolates [16].

Most common bacteria isolates in gram negative bacilli as *Pseudomonas aeruginosa* as predominant isolates in 27% as followed by *E.coli* and *Klebsiella* spp 22% in total isolates the gram positive cocci, *S.aureus* in respectively 7% DFU known as polymicrobial infection [17]. Zubair study regarding for polymicrobial infection in monomicrobial results and our findings were slightly in similar [18].

5. CONCLUSION

Identification using classical methods based on their morphology, Grams stain reaction, and oxidase and catalase tests were performed to confirm the isolates were both gram-negative and gram-positive organisms such as *Staphylococcus aureus*, *Staphylococcus hominis, staphylococcous hemolytic* and *Pseudomonas aeruginosa* according to the Bergey's manual of Determinative Bacteriology. Further, MALDI- TOF **(**Matrix-Assisted Laser Desorption/Ionization) was used to confirm the isolates identified in classical methods were *Staphylococci* and *Pseudomonas*. The whole cell proteins were extracted using ethanol formic acid extraction method and direct smear method on MALDI plate. The patient treatment for easy to identified microbial infection based to wound heal. In cost of identification very low and short time period.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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