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A PRELIMINARY STUDY ON THE ISOLATION AND IDENTIFICATION OF AEROBIC BACTERIA FROM DIABETIC FOOT INFECTION AND ITS ANTIBIOGRAM

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ABSTRACT

Microbiology study in diabetes is metabolic disorder affecting the Langher islets of pancreas affecting the insulin and glucogan production and in turn affecting the sugar (glucose) metabolism which in turn affecting the overall metabolism the disease may be classified in type I diabetes mellitus and type II diabetes mellitus. Type I diabetic mellitus It is formerly known as insulin- dependent diabetic mellitus type II diabetes mellitus It is characterized by hyperglycemia due to an individual's resistance to insulin with an insulin secretory defect. Treatment the oral hypoglycemic agents are sulphonyl urea and biguanides. The sulphonyl urea drugs (tolbu – tamide and glibenclamidea) stimulate insulin secretion. Diabetic foot infections are infections that can develop in the skin, muscles or bones of the foot as a result of nerve damage and poor circulation that is associated with diabetes. Diabetes mellitus is a disorder that primarily affects the microvascular circulation. Collection of sample diabetic patients included in this study was of age group (35-45). The foot sample of both sexes were collected from government hospitals in Cheyyar , a sterile swab was used for collecting sample from diabetic patients study in sample processing, Microscopic examination, Antibiotic sensitivity test, Statistical analysis, the samples were processed according to the standard microbiological techniques the aerobic organisms were isolate from pus culture statistical analysis was done. *Staphylococcus aureus* was the predominant organism among the isolation the gram negative organism *Pseudomonas aeruginosa* was predominant other organism included in our study was *Escherichia coli* and *Proteus mirabilis.*

Keywords: Diabetic foot infection, Antibiotic sensitivity test, Microscopic examination, Sample processing.

INTRODUCTION

Diabetes is metabolic disorder affecting the Langher islets of pancreas affecting the insulin and glucogan production and in turn affecting the sugar (glucose) metabolism which in turn affecting the overall metabolism the disease may be classified in type I diabetes mellitus and type II diabetes mellitus. Type I diabetic mellitus It is formerly known as insulin- dependent diabetic mellitus type II diabetes mellitus It is characterized by hyperglycemia due to an individual's resistance to insulin with an insulin secretory defect [1]. The resistance results in a relative. The changes are mainly the result of low insulin glucagons ratio. The effects of insulin on carbohydrate lipid and amino acid metabolism have been described in diabetic Mellitus all these effects are reversed [2].

Treatment the oral hypoglycemic agents are sulphonylurea and biguanides. The sulphonyl urea drugs (tolbu – tamide and glibenclamidea) stimulate insulin secretion [3]. They are used in Type -2 disease. Insulin is injected as replacement therapy in Type 1 disease, as well as in Type 2 disease. Where oral drugs are not sufficient. There are many hypoglycemic drugs, which belong to the group sulfonamide, are tolbutamide (Orinase),

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chlorpropamide (diabinese) and tolazamide (tolinase) [4].

Diabetic foot infections are infections that can develop in the skin, muscles or bones of the foot as a result of nerve damage and poor circulation that is associated with diabetes. Diabetes mellitus is a disorder that primarily affects the microvascular circulation [5]. In the extremities, microvascular disease due to "sugar-coated capillaries" limits the blood supply to the superficial and deep structures Pressure due to ill-fitting shoes or trauma further compromises the local blood supply at the microvascular level, predisposing the patient to infection the infection may involve the skin, soft tissues, bone, or all of these tissues [6].

MATERIALS AND METHODS

Collection of sample diabetic patients included in this study was of age group (35-45). The foot sample of both sexes were collected from government hospitals in Cheyyar, a sterile swab was used for collecting sample from diabetic patients. The sample was collected by simply rolling the tip of the swab on its side for one full rotation over the infected area. Dried surface was premoistened with a saline swab which improves the yield, transportation of sample the collected swab was placed in Stuart's media and transported to the lab.

Processing Of Sample

Microscopic examination, Staining method, Hanging drop method, Culture, Biochemical parameter**,** Catalase , Oxidase, Coagulase, Indole , Methyl red, Voges-proskauer, Citrate ,Triple sugar iron agar, Urease, Gelatin Hydrolysis, Nitrate reduction, Sugar fermentation, Selective media, Antibiotic sensitivity study. All the analysis are carried out by the method of Sigma Diagnostic kits (Sigma Chemical Company Catalogue, 1997) [7].

Statistical Analysis

All the data were analyzed as per the method of Pillai and Sinha HC. (1968) [8].

RESULTS AND DISCUSSION

Plate 1, Tables1-10 and Fig 6 indicate the results obtained in the present investigation. Diabetics patients 25 sample had been collected from Government Hospital in cheyyar 16 sample were collected from male and 9 sample were collected from female, the number of different isolates from total number of specimens and microscopic examination such as staining motility test and cultural characters, colony morphology, biochemical characters in different isolation specimen in the microorganism for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E.coli* and *Proteus mirabilis* identified.

The isolated bacteria were tested for their susceptibility to different antibiotics. *Staphylococcus aureus* were found to be highly [9], sensitive to ampicillin, amikacin, gentamicin, vancomycin and resistant to methicillin, *Pseudomonas aeruginosa* were found to be highly [10], sensitive to amikacin, gentamicin, ceftazidime and moderately sensitive to ciprofloxacin, *Proteus mirabilis* highly sensitivity to ciprofloxacin and moderately sensitive to tetracycline it was found to ampicillin, amoxycillin, cefotaxime, *Escherichia coli* highly tetracycline and moderately [11], sensitive to ampicillin, streptomycin and polymyxin-B, our study demonstrated the large number and variety of organism can be isolated from properly obtained specimens that are optimally processed.

Samples were collected from the nearby hospital the samples were processed according to the standard microbiological techniques the aerobic organisms were isolate from pus culture statistical analysis was done. *Staphylococcus aureus* was the predominant organism among the isolation the gram negative organism *Pseudomonas aeruginosa* was predominant other organism included in our study was *Escherichia coli* and *Proteus mirabilis* [12].

Plate. 1 Diabetic Foot Infection

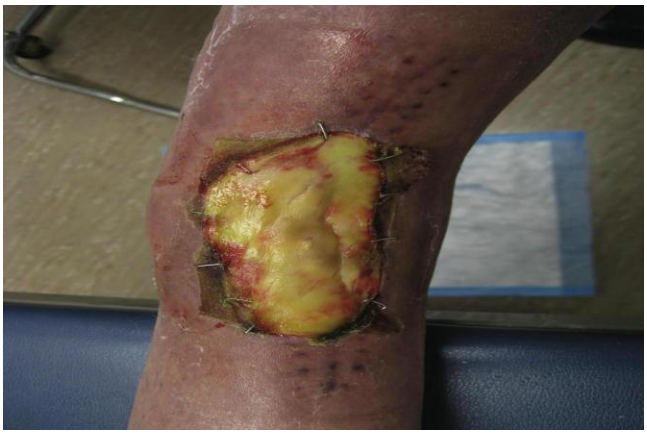
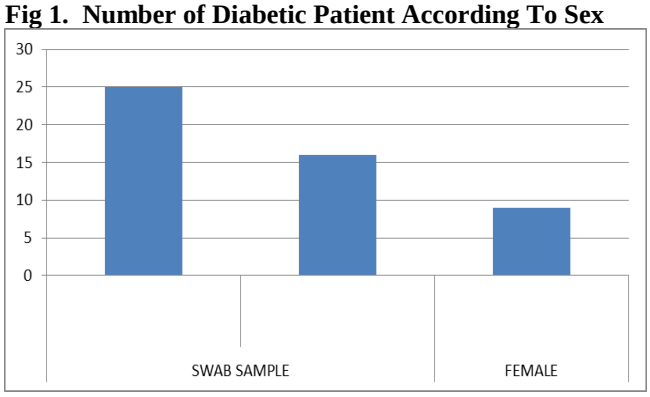


Table 1. Number of diabetic patient according to sex

Swab Sample	Male	Female
25±1.00	16±1.52	9±1.00

Values are mean ± SD of 6 individual observations.
Values are significant at $P < 0.001$.

Fig 1. Number of Diabetic Patient According To Sex



CHARACTERIZATION OF *STAPHYLO COCCUS AUREUS*

Microscope: Gram positive with more pus cells
Coloney Morphology: Circular convex smooth golden yellow coloney in nutrient agar Beta haemolytic coloney blood yellow colouration in mannitol salt agar

Table 2. Biochemical Characterzation of *Staphylo coccus aureus*

Name of The Test	Result
Catalase	Positive
Oxidase	Negative
Coagulase	Positive
Indole	Negative
Methyl red	Positive
Voges Proskauer	Positive
Citrate	Positive
Urease	Positive
Gelatinase	Positive
Nitrate	Positive
Mannitol fermentation	Positive

CHARACTERIZATION OF *PSEUDOMONAS AERUGINOSA*

Microscope: Gram negative rod with more pus cells
Motility: Motile
Cultural characters: Large opaque irregular colonies with a distinctive musty or earth smell
On Nutrient agar: Green colour coloney with diffused Pigmentation
On MacConkey agar: Forming NFL colonies
On Blood agar: Beta haemolytic colonies
Triple sugar iron: Alkaline butt alkaline slant

Table 3. Biochemical Characterzation of *Pseudomonas aeruginosa*

Name of The Test	Result
Catalase	Positive
Oxidase	Positive
Coagulase	Not Done
Indole	Negative
Methyl red	Positive
Voges Proskauer	Negative
Citrate	Positive
Urease	Positive
Gelatinase	Positive
Nitrate	Positive

CHARACTERIZATION OF *PROTEUS MIRABILIS*

Microscope: Gram negative rod with more pus cells
Motility Test: Motile
Cultural Characters: Fishy or seminal odour
On nutrient agar: Pale colour swarming growth
On MacConkey agar: Smooth colour less separate colonies
On Blood agar: Non haemolytic colonies
Triple sugar iron: Acid butt alkaline slant gas Positive H2s(+)

Table 4. Biochemical Characterization of *Proteus mirabilis*

Name of The Test	Result
Catalase	Positive
Oxidase	Negative
Coagulase	Not Done
Indole	Negative
Methyl red	Positive
Voges Proskauer	Negative
Citrate	Positive
Urease	Positive
Gelatinase	Positive
Nitrate	Positive
Sugar fermentation	
Glucose	+
Lactose	-
Sucrose	-
Mannitol	-

CHARACTERIZATION OF *ESCHERICHIA COLI*

Microscope: Gram negative straight rod with a few pus cells
Motility Test: Motile
Cultural Characters: The colonies are large thick grayish white moister smooth opaque or partially translucent discs
On nutrient agar: Large circular convex smooth white moist colonies
On MacConkey agar: pink colonies due to lactose fermentation
On Blood agar: Non haemolytic colonies
Triple sugar iron: Acid butt alkaline slant gas production

Table 5. Biochemical Characterization of *Escherichia coli*

Name of The Test	Result
Catalase	Positive
Oxidase	Negative
Coagulase	Not Done
Indole	Positive
Methyl red	Positive
Voges Proskauer	Negative
Citrate	Negative
Urease	Negative
Gelatinase	Positive
Nitrate	Positive
Sugar fermentation	
Glucose	+
Lactose	+
Sucrose	-
Mannitol	+

Table 6. Aerobic Bacteria Isolated From Diabetic Foot Infection

Bacterial Types	Number of The Cases	Percentage (%)
Staphylococcus aureus	15±2.00	60±6.00
Pseudomonas aeruginosa	10±1.00	36±5.40
Proteus mirabilis	6.0±0.50	24±4.00
Escherichia coli	2 .0±0.45	8.0±0.50

Values are mean ± SD of 6 individual observations.
Values are significant at P < 0.001.

Table 7. Antibiotic Sensitivity for *Staphylo coccus aureus*

Name of the antibiotic	Diameter of zone result
Ampicillin	28.0±1.50 (S)
Amikacin	20.0± 1.20 (S)
Gentamicin	20.0±1.54 (S)
Vancomycin	15.0± 0.50 (S)
Methicillin	Resistant

(S) - Sensitive (I) - Intermediate (R) - Resistance
Values are mean ± SD of 6 individual observations.
Values are significant at P < 0.001.

Table 8. Antibiotic Sensitivity for *Pseudomonas aeruginosa*

Name of the Antibiotic	Diameter of Zone Result
Amikacin	20.0±1.20 (S)
Gentamicin	20.0± 1.00 (S)
Ciproflaxcin	19.0± 1.10 (I)
Ceftazidime	20.0± 1.24 (S)

(S) - Sensitive (I) - Intermediate (R) - Resistance
Values are mean ± SD of 6 individual observations.
Values are significant at P < 0.001.

Table 9. Antibiotic Sensitivity for *Proteus mirabilis*

Name of the antibiotic	Diameter of zone result
Ampicillin	`12.0± 0.50 (R)
Amoxycillin	13.0± 1.00 (R)
Cefotaxime	12.0±1.00 (R)
Ciprofloxacin	28.0± 1.52 (S)
Tetracycline	18.0± 1.45 (I)

(S) - Sensitive (I) - Intermediate (R) - Resistance
Values are mean ± SD of 6 individual observations.
Values are significant at P < 0.001.

Table 10. Antibiotic Sensitivity for *Escherichia Coli*

Name of the antibiotic	Diameter of zone result
Streptomycin	19.0± 1.00 (S)
Ampicillin	20.0± 1.52 (S)
Polymyxin-B	19.0± 1.00 (S)
Tetracycline	20.0± 1.15 (S)

(S) - Sensitive (I) - Intermediate (R) - Resistance
Values are mean ± SD of 6 individual observations.
Values are significant at P < 0.001.

Fig 2. Aerobic Bacteria Isolated From Diabetic Foot Infection

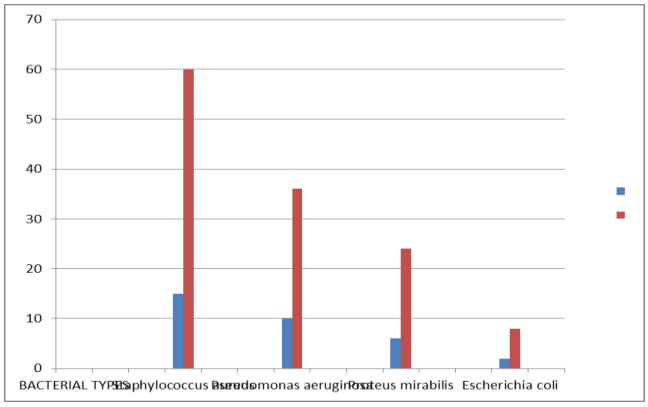


Fig 3. Antibiotic Sensitivity for *Staphylo coccus aureus*

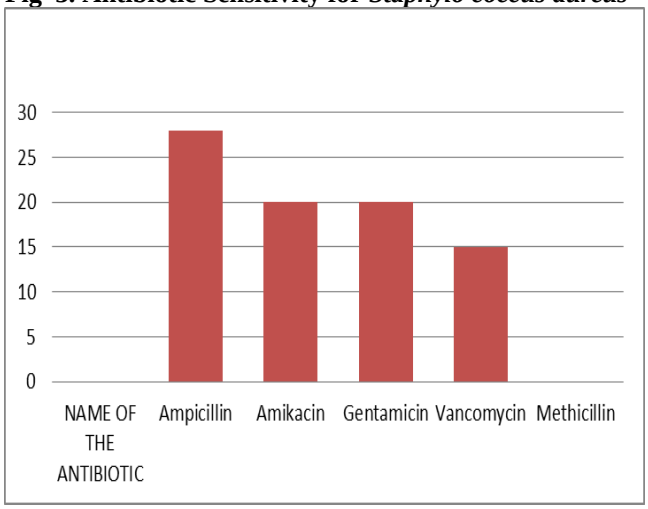


Fig 4. Antibiotic Sensitivity for *Pseudomonas aeruginosa*

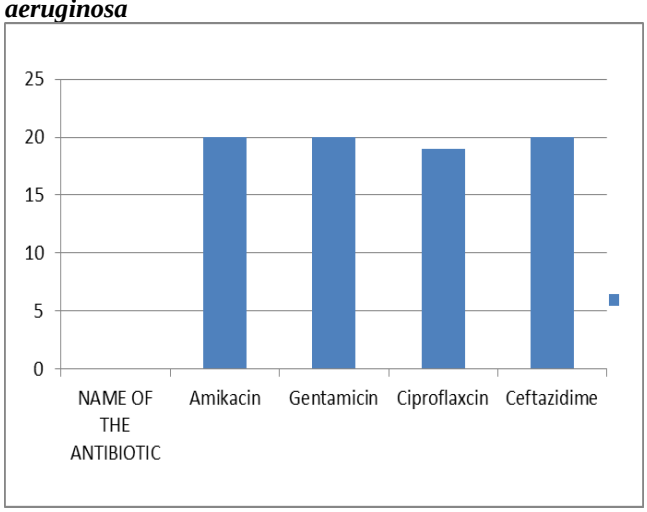


Fig 5. Antibiotic Sensitivity for *Proteus mirabilis*

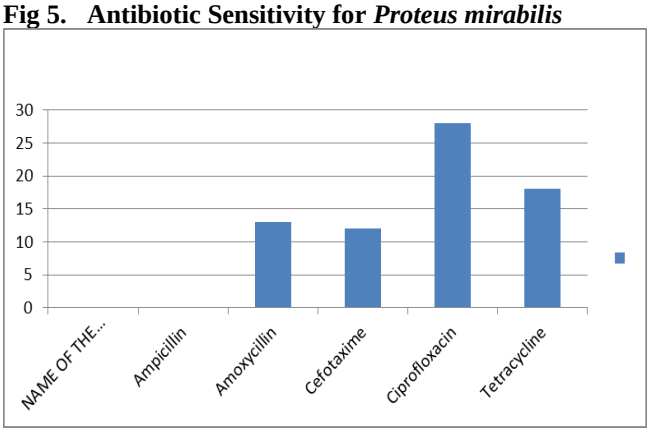
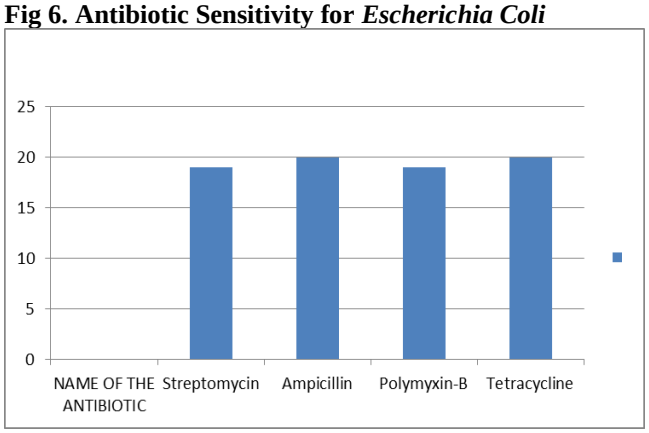


Fig 6. Antibiotic Sensitivity for *Escherichia Coli*



CONCLUSION

The human preliminary study on the diabetic foot infected wound isolation of microorganisms and morphological arrangement in aerobic bacteria identified.

REFERENCES

1. Papanas N and Maltezos E. Growth Factors in the Treatment of Diabetic Foot Ulcers New Technologies. *International Journal of Lower Extremity Wounds*, 6 (1), 2007, 37-53.
2. Varaiya AY, Dogra JD, Kulkarni MH and Bhalekar PN. Extended-spectrum beta-lactamase-producing *Escherichia coli and Klebsiella pneumoniae* in diabetic foot infections. *Indian J Pathol Microbiol*, 51, 2008, 370-2.
3. Ihsan Edan Abdulkareem Alsaimary. Bacterial Wound Infections in Diabetic Patients and Their Therapeutic Implications. *Journal of Microbiology*, 7 (2), 2009, 1-5.
4. Shazi Shakil Asad U and Khan. Infected foot ulcers in male and female diabetic patients a clinico-bioinformative study. *Annals of Clinical Microbiology and Antimicrobials*, 9(2), 2010, 1476-0711.
5. Graham P and Leese. The Varied Attractions of the Diabetic Foot. *British Journal of Diabetes and Vascular Disease*, 9(4), 2009, 155-159.
6. Samir Paul, Lovely Barai, Ashraf Jahan and Ashraful Haq J. A Bacteriological Study of Diabetic Foot Infection in an Urban Tertiary Care Hospital in Dhaka City. *Journal of Ibrahim Med*, 3(2), 2009, 50-54.
7. Gutr. Leonard Hills (Books) Ltd., London, 1959.
8. Pillai SK and Sinha HC. In: Statistical Methods For Biological Workers Pubs.Ramprasad And Sons. Agra, India, 1968.
9. Diane M, Citron Ellie J, Goldstein C, Vreni Merriam C, Benjamin A, Lipsky Murray A and Abramson. Bacteriology of Moderate-to-Severe Diabetic Foot Infections and In Vitro Activity of Antimicrobial Agents. *Journal of Clinical Microbiology*, 45(9), 2007, 2819-2828.
10. Anandi C, Alaguraja D, Natarajan V, Ramanathan M, Subramaniam CS, Thulasiram M and Sumithra S. Bacteriology of diabetic foot lesions. *Indian J Med Microbiol*, 22, 2004, 175-8.
11. Raja and Nadir Sajjad. Microbiology of diabetic foot infections in a teaching hospital in Malaysia a retrospective study of 194 cases. *Journal of Microbiology. Immunology and Infection*, 14(1), 2007, 45-49.
12. Abolfazl Shojaiefard, Zhamak Korari and Bagher Larijani. Independent risk factors for amputation in diabetic foot. *PMID Medical sciences Endocrinology*, 28 (2), 2008, 32-37.