

## SUSCEPTIBILITY ASSESSMENT OF STIPULATED ANTIBIOTICS VERSUS DIABETIC FOOT INFECTION

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### ABSTRACT

Purulent emanate samples from thirty diabetic foot valetudinarian were assembled to vet for fiendish *Clostridium perfringens*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. From the inspection, it was divulge that twelve samples enthralled the pathogenic diabetic foot bacteria that were explicable for bedeviling indispositions. Veritable bacterial colonies were then predisposed to aced antibiotics in which *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli* growth were staunched eminently. While mitigated to good impediment activity were validated against *Clostridium perfringens*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes*.

**KEYWORDS:** Antibiotics, Diabetic Foot Infection, Purulent

### INTRODUCTION

Diabetes is non-communicable metabolic disorder, in which the invalids endure copious systematic convolutions such as aloft blood sugar level, polyuria, polyphagia, polydipsia, retinopathy, slew of cardiovascular and renal dysfunctions etc. (David., et al 2011). In Pakistan, estimated diabetic population outreached to 7.1 million, as turning the plight to socio-economic burden (Amos et al., 1997).

A crucial Diabetes mellitus complication termed as Diabetic foot is a pathologic condition decrepit citizenry reverberating skin ulceration, bactermia and neuropathic osteoarthropathy. Bactermia façade serious profound commination of indisposition prevalently caused by pathogenic bacteria such as *Bacillus fragilis*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcal species* and *Staphylococcus aureus* etc.

These bacteria act as opportunistic entities that defile minor scratches, cuts and wounds and revolve it into severe integument tissue ulcers and suppurative infections (Lipsky et al., 2012). According to American Diabetes Association (ADA), 15 - 25% inhabitants defy diabetic foot complication and 14 - 24% suffers undergo surgical amputation world wide (Melmed and Morbach et al., 2012). The condition is customary in plebeians due to dearth of indoctrination and provision of preventive subsistence to organize germane lifestyle.

The cardinal notion of the research investigation was to isolate and characterize explicit diabetic foot borne bacteria expressively *Clostridium perfringens*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Staphylococcus epidermidis* and that were then laid open to antibiotic susceptibility trails to observe bacterial sensitivity in riposte to the commended antibiotics.

## MATERIALS AND METHODS

### Sample Collection

Suppurative exude samples were scraped together randomly from ulcerate foot region plying aseptic swabs from thirty hospitalized patients coping with chronic diabetic foot affliction.

### Bacterial Culturing

The specimen holding swabs were streaked uniformly on autoclaved selective media that is Columbia-CNA agar, Chocolate Agar, Mckonkey Agar, Mannitol salt agar and Sabouraud dextrose agar plates in laminar flow hood as per elucidated bacterial streaking stratagem (Cappuccino et al., 2008). Further all of the streaked petri-plates were placed in incubator at 37°C for 24 hours in transpose arrangement. At end of incubation phase, bacterial colonies were analyzed morphologically.

## BACTERIAL CHARACTERIZATION

Resultant bacterial colonies were pervaded threading on rash of biochemical tests comprising of Gram staining, Catalase test, Coagulase test, Oxidase test, Indole test, Mannitol test and Methyl red test, in order to distinguish aspired diabetic foot borne bacterial species specifically *Clostridium perfringens*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Staphylococcus epidermidis*.

## KIRBY-BAUER ANTIBIOTIC TESTING

Sterling cultures of preferred bacteria were prepared on autoclaved selective Muller-Hinton agar medium. Further five antibiotic discs scilicet Amoxicillin (10µg), Ciprofloxacin (10µg), Gentamicin (10µg), Penicillin (10µg), and Tetracycline (30µg) were assigned on the sterling bacterial cultured plates for sensitivity test as per described Kirby-Bauer antibiotic testing stratagem (Mohanty, 2010). All the plates were then positioned in incubator at 37°C for 24 hours. At end of incubation phase, coherent zones of inhibition were notified exposing antibiotic sensitivity against the selected bacteria.

## RESULTS

### Bacterial Culturing and Characterization

Cultured suppurative diabetic foot samples prior to characterization procedures substantiated that out of the fetched pus specimens, twelve samples influenced pined pathogenic bacterial species that are *Clostridium perfringens*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Staphylococcus epidermidis* which are wanted to enkindle critical diabetic foot symptoms.

These ill-reputed bacterial species were verified by Gram staining, Catalase test, Coagulase test, Oxidase test, Indole test, Mannitol test and Methyl red test, and results are condensed in (Table 1). From bacterial culturing and characterization, it was demonstrated that *Staphylococcus aureus* was predominant pathogenic bacterial inhabitant of diabetic foot ulcer, occurring both in isolated and co-infectious condition. Further *Streptococcus pyogenes* and *Escherichia coli* possessed higher infectious frequency but resided chiefly in co-infectious state. Remaining bacterial species owned less infection frequency but with exorbitant drastic symptoms. Results are condensed in (Table 2).

**Table 1: Tabular Illustration of Aimed Diabetic Foot Bacteria Delineated via Biochemical Tests**

List of Bacteria	Characterization							
	Shape	Gram Staining	Catalase Test	Coagulase Test	Oxidase Test	Indole Test	Mannitol Test	Methyl Red Test
<i>C. perfringens</i>	Bacilli	+	-	-	-	-	-	-
<i>E. coli</i>	Bacilli	-	+	-	-	+	-	+
<i>S. pyogene</i>	Cocci	+	-	-	-	+	-	+
<i>S. aureus</i>	Cocci	+	+	+	-	-	+	+
<i>S. epidermidis</i>	Cocci	+	+	-	-	-	+	+
<i>P. aeruginosa</i>	Bacilli	-	+	-	+	-	-	-

(+) intimation represents positive result, (-) intimation represents negative result

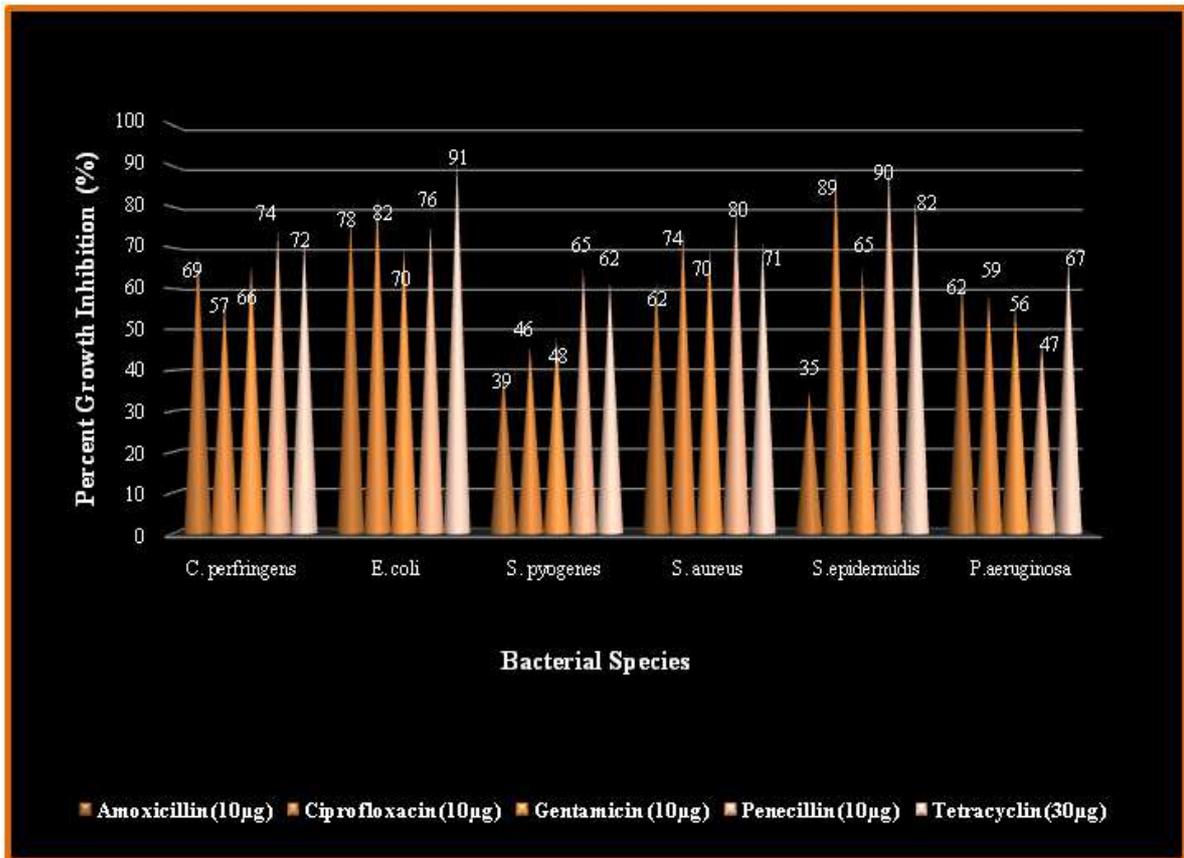
**Table 2: Tabular Illustration of Suppurative Samples Dominating Aimed Diabetic Foot Bacteria**

List of Bacteria	Bacteria Isolated from Test Samples											
	Number of Samples											
	1	2	3	4	5	6	7	8	9	10	11	12
<i>C. perfringens</i>	+	-	-	-	-	-	-	+	-	-	-	-
<i>E. coli</i>	+	+	-	+	-	-	-	-	-	-	-	+
<i>S. pyogene</i>	-	-	-	+	+	-	-	-	+	-	-	+
<i>S. aureus</i>	-	+	+	-	+	+	-	+	+	-	+	-
<i>S. epidermidis</i>	-	-	-	-	-	+	-	-	-	+	+	-
<i>P.aeruginosa</i>	-	-	+	-	-	-	+	-	-	-	-	-

(+) intimation represents presence of aimed bacteria, (-) intimation represents absence of aimed bacteria

## KIRBY-BAUER ANTIBIOTIC TESTING

From antibiotic susceptibility testing it was substantiated that all bacterial species were sensitive to the preferred antibiotics discs viz. Amoxicillin (10µg), Ciprofloxacin (10µg), Gentamicin (10µg), Penicillin (10µg), and Tetracycline (30µg). *Escherichia coli* were exceptionally inhibited by Tetracycline (91%) and Ciprofloxacin (82%). *Staphylococcus epidermidis* were also tremendously inhibited by Penicillin (90%), Ciprofloxacin (89%) and Tetracycline (82%). Subsequently abominable prominent diabetic foot *Staphylococcus aureus* growth was equally biased by Penicillin (80%), displaying excellent inhibition. Remaining bacterial species held moderate to good percent constraint against the preferred antibiotics. Results are condensed in (Graph 1).



**Graph 1: Graphical Illustration of Percent Growth Inhibition of Aimed Diabetic Foot Bacteria against Stipulated Antibiotics**

## DISCUSSIONS

From the survey conducted at Nepal Hospital, it was evidenced that *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the supreme infectious diabetic foot causing entities and afflicted invalids undergo combined antibiotic treatment and repeated wound dressings for recovery (Sharma et al., 2006). It was studied that both aerobic and anaerobic bacterial species have the aptitude to cause diabetic foot probs, for which credible antibiotic Imipenem was constructive against gram-negative organisms while Vancomycin was fruitful to halt growth of gram-positive bacteria (Louie et al., 1976). Combination antibiotic treatment was used to combat Methicillin-resistant *Staphylococcus aureus* (MRSA) which causes infection in oppressed patients that went under antibiotic treatment or were hospitalized earlier (Mazen et al., 2008).

## CONCLUSIONS

From the research interpretation, it was concluded that proliferating cases of Diabetes mellitus had mushroomed the exhibition of excruciating Diabetic foot infections might be due to compromised thrombocytosis and invasion of common epidermal microflora precisely *Clostridium perfringens*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. These species tends to contaminate petty wounds leading to severe lurgy and limb abscissions. To avoid such consequences, combination antibiotic therapy should be pertained along with proper sanitized health care practices. Proper antibiotic lore should be copped in order to circumvent the mutational resistance of pathogenic bacteria.

**REFERENCES**

1. David G, Gardner, Dolores (2011). Greenspan's basic and Clinical Endocrinology, 9<sup>th</sup> Edition, McGraw-Hill Medical., New York, pp. 17.
2. Amos J, McCarty J, Zimmet P (1997). The rising global burden of diabetes and its complications; estimates and projections to the year 2010. *Diabetic Medicine.*, 14:7:84.
3. Lipsky BA, Berendt AR, Cornia PB (2012). Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. *Clinical Infect Dis.*, 54:12:132-73.
4. Melmed S, Polonsky SK, Larsen PR, Kronenberg HM (2012). William's textbook of Endocrinology, 12<sup>th</sup> Edition, Philadelphia., pp. 1371–1435.
5. Morbach S, Furchert H, Gröblichhoff U, Hoffmeier H, Kersten K, Klauke GT (2012). Long-term prognosis of diabetic foot patients and their limbs: Amputation and death over the course of a decade. *Diabetes Care.* 35:10:2021-7.
6. Sharma K, Khadka B, Joshi A, Sharma R (2006). Common pathogens isolated in diabetic foot infection in Bir Hospital. *Kathmandu Univ Med J (KUMJ).*, 4:3:295-301
7. Louie J, Bartlett G, Tally P, Gorbach L (1976). Aerobic and anaerobic bacteria in diabetic foot ulcers. *Ann Intern Med.*, 85:4:461-3.
8. Mazen SB (2008). Diabetic Foot Infection. *Am Fam Physician.*, 78:1:71-79.
9. Cappuccino, J. and Sherman, N. (2008): *Microbiology a laboratory manual*, 8<sup>th</sup> Edition. Pearson/Benjamin Cummings, San Francisco, CA.
10. Mohanty, A. (2010): Physiochemical and antimicrobial study of polyherbal. *Pharmacie globale.*, 4:4:1-3.

