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## Delayed wound healing in diabetic patients with *Pseudomonas aeruginosa* biofilm challenge: Our experience at a tertiary care teaching hospital

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### Abstract

**Background:** Foot infections are a frequent complication of patients with diabetes mellitus, accounting for up to 20% of diabetes-related hospital admissions. Infectious agents are associated with the worst outcomes, which may ultimately lead to amputation of the infected foot unless prompt treatment strategies are ensued. The present study sought to reveal the bacterial etiology of diabetic foot ulcers in Bhubaneswar, the diabetic capital of India.

**Methods:** A 10-month-long descriptive study was carried out to analyse the aerobic and anaerobic bacterial isolates of all patients admitted with diabetic foot infections presenting with Wagner grade 2–5 ulcers. Bacteriological diagnosis and antibiotic sensitivity profiles were carried out and analysed using standard procedures.

**Results:** Diabetic polyneuropathy was found to be common (56.8%) and gram-negative bacteria (57.6%) were isolated more often than gram-positive ones (42.3%) in the patients screened. The most frequent bacterial isolates were *Pseudomonas aeruginosa*, *Staphylococcus aureus*, coagulase-negative staphylococci (CONS), and Enterobacteriaceae. Forty-nine cultures (68%) showed polymicrobial involvement. About 44% of *P. aeruginosa* were multi-drug-resistant, and MRSA was recovered on eight occasions (10.3%). *Bacteroides* spp. And *Peptostreptococcus* spp. were the major anaerobic isolates.

**Conclusions:** Our study supports the viewpoint put forth by previous South Indian authors that the distribution of gram-negative bacteria (57.6%) is more common than that of gram-positive ones (42.3%) and it is contrary to the viewpoint that diabetic foot infections are frequently monomicrobial. Furthermore, recovery of multi-drug-resistant *P. aeruginosa* isolates is of serious concern, as almost no one has reported the same from the Bhubaneswar.

**Keywords:** Diabetes, foot infections, Wagner grade

### Introduction

Endless injury contamination is a noteworthy clinical issue that prompts high horribleness, mortality, and cost. While there is no accord meaning of unending injuries, it is commonly concurred that a human injury is delegated endless in the event that it stays open for longer than 6–8 weeks<sup>[1, 2]</sup>. The injuries are portrayed by bacterial weight, ceaseless aggravation, and an uneven cell safeguard mechanism.<sup>2</sup> Multiple types of microorganisms, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacteroides* spp., *Peptostreptococcus*, *Enterococcus* spp., and *Streptococcus pyogenes*, have been secluded from constant injuries, despite the fact that the injury may not hint at any clinical confined disease. Numerous other bacterial species have been distinguished by utilizing progressed sub-atomic techniques<sup>[3]</sup>. Recent clinical information have appeared microbial biofilms are probably going to be available in unending injuries, and it has been suggested that biofilms are a noteworthy supporter of the improvement and upkeep of chronicity of wounds<sup>[4]</sup>. Biofilms are an organized network of microorganisms encased in a self-decreased framework that is follower to both a latent or living surface<sup>[5]</sup>. The diseases brought about by biofilms are portrayed by surface-related contaminating microorganism, microorganism bunches encased in an extracellular lattice (ECM), kept neighborhood contamination, and protection from anti-microbial treatment<sup>[6]</sup>. Current topical and fundamental anti-microbials are insignificantly successful in the treatment of these microbial networks. Furthermore, the host's fiery reaction is inadequate in battling the nearness of biofilm<sup>[7]</sup>.

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A noteworthy trouble in defining new treatments for interminable injuries is the absence of a palatable creature display in which constant injuries can be efficiently examined. Analysts have attempted a few techniques to instigate interminable wounds, for example, the ischemic rabbit ear model<sup>[8]</sup>, radiation impaired rats<sup>[9]</sup>, and diabetic mice<sup>[10, 11]</sup>. Studies, to date, have demonstrated that it is less demanding to harm the tissue than to actuate a reproducible model in which the ceaseless injury recuperating reaction is initiated<sup>[12]</sup>. Wound mending in these models is generally postponed just by a brief timeframe or advances so forcefully that the contamination ends up fundamental and results in high mortality<sup>[13, 14]</sup>. The db/db mouse has been broadly utilized for studies related with diabetes. The db/db mouse has an unconstrained hereditary transformation of the leptin receptor (LEPR) in the nerve center and loses control of satiation. The mouse indulges and in the end takes after human sort 2 diabetes mellitus, with stoutness, hyperglycemia, fringe neuropathy, and deferred wound mending. Notwithstanding the sort 2 diabetes phenotype, db/db mice take around twice as long to mend wounds as nondiabetic heterozygous littermates under specific conditions. Twisted conclusion in db/db mice is basically by epidermal relocation instead of wound contraction<sup>[15]</sup>. Much of the momentum inquire about on twisted mending in diabetic mice has concentrated on the impacts of such factors as incendiary cytokines, development factors, neuropeptides, network metalloproteinases, tissue inhibitor of metalloproteinases, low oxygen, and cell senescence<sup>[16, 17]</sup>. Targeting biofilms that are available on the outside of endless injuries might be a powerful technique to advance mending of ceaseless wounds. Our objective was to build up a diabetic mouse show in which we could examine the job of biofilm in incessant injuries. In this investigation, we built up a biofilm-tested injury display in the db/db mouse by immunizing the injury with *P. aeruginosa* biofilm and keeping up unhealed injury for 28 days. This model gives a reproducible mouse wound with confined cutaneous disease while maintaining a strategic distance from fundamental contamination. The model may empower the examination of explicit systems by which biofilms weaken recuperating, and encourage the screening of rising antibiofilm procedures on wound mending. Incessant injury contamination is a noteworthy clinical issue that prompts high bleakness, mortality, and cost. While there is no accord meaning of ceaseless injuries, it is commonly concurred that a human injury is delegated incessant in the event that it stays open for longer than 6-8 weeks<sup>[1, 2]</sup>. The injuries are portrayed by bacterial weight, perpetual irritation, and an unequal cell safeguard mechanism<sup>[2]</sup>. Multiple types of microbes, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacteroides* spp., *Peptostreptococcus*, *Enterococcus* spp., and *Streptococcus pyogenes*, have been secluded from constant injuries, despite the fact that the injury may not hint at any clinical restricted contamination. Numerous other bacterial species have been recognized by utilizing progressed atomic techniques<sup>[3]</sup>. Recent clinical information have appeared microbial biofilms are probably going to be available in interminable injuries, and it has been recommended that biofilms are a noteworthy supporter of the improvement and upkeep of chronicity of wounds<sup>[4]</sup>. Biofilms are an organized network of microorganisms encased in a self-decreased grid that is follower to both an inactive or living surface<sup>[5]</sup>. The contaminations brought

about by biofilms are portrayed by surface-related tainting microorganism, microorganism bunches encased in an extracellular lattice (ECM), restricted nearby disease, and protection from anti-infection treatment<sup>[6]</sup>. Current topical and fundamental anti-toxins are insignificantly compelling in the treatment of these microbial networks. Likewise, the host's fiery reaction is insufficient in fighting the nearness of biofilm<sup>[7]</sup>. A noteworthy trouble in detailing new treatments for interminable injuries is the absence of a tasteful creature demonstrate in which unending injuries can be methodically contemplated. Specialists have attempted a few strategies to instigate ceaseless wounds, for example, the ischemic rabbit ear model<sup>[8]</sup>, radiation impaired rats<sup>[9]</sup>, and diabetic mice<sup>[10, 11]</sup>. Studies, to date, have demonstrated that it is less demanding to harm the tissue than to actuate a reproducible model in which the unending injury mending reaction is initiated<sup>[12]</sup>. Wound recuperating in these models is normally deferred just by a brief timeframe or advances so forcefully that the disease ends up foundational and results in high mortality<sup>[13, 14]</sup>. The db/db mouse has been broadly utilized for studies related with diabetes. The db/db mouse has an unconstrained hereditary change of the leptin receptor (LEPR) in the nerve center and loses control of satiation. The mouse gorges and inevitably takes after human kind 2 diabetes mellitus, with weight, hyperglycemia, fringe neuropathy, and deferred wound mending. Notwithstanding the sort 2 diabetes phenotype, db/db mice take roughly twice as long to mend wounds as nondiabetic heterozygous littermates under specific conditions. Twisted conclusion in db/db mice is fundamentally by epidermal relocation as opposed to wound contraction<sup>[15]</sup>. Much of the ebb and flow examine on twisted recuperating in diabetic mice has concentrated on the impacts of such factors as fiery cytokines, development factors, neuropeptides, grid metalloproteinases, tissue inhibitor of metalloproteinases, low oxygen, and cell senescence<sup>[16, 17]</sup>. In this study, we evaluated delayed wound healing in diabetic patients with *Pseudomonas aeruginosa* biofilm at IMS and Sum hospital, Bhubaneswar.

## Materials and Methods

### Study subjects

Patients (n=77) admitted to a specific diabetes care focus in at IMS and Sum hospital, Bhubaneswar, from May 2016 to March 2017 were broke down to decide the etiology of their diseases and their seriousness on the Wagner scale. Age, sex, metabolic control, cardiovascular hazard factors, wound sort and its confinement and Wagner review were archived. The related co-dismal conditions in every one of the 77 patients were likewise recorded. Review 1 was characterized as cellulitis or a shallow ulcer, review 2 as a subcutaneous injury, fasciitis, or tendonitis without osteomyelitis, review 3 as osteomyelitis, review 4 as a confined gangrene, and grade 5 as boundless gangrene. Wagner review 1 patients were not considered for the investigation as these sores typically harbor skin contaminants that frequently lead to false-positive outcomes.

### Procedures

Culture materials from all the wounds were collected, either by washing the wound with 100 cm<sup>3</sup> of sterile physiological saline and then making a puncture-aspiration at the bottom of the wound or by applying a sterile cotton swab (Himedia, Mumbai) to the wound. The specimens were transported to

the processing laboratory on Cary and Blair media and Robertson's cooked meat media in a 0° mini cooler (Tarson Labs, Mumbai) and were cultured under standard microbiological conditions. Identification was made as per procedures suggested CLSI 24.

## Results

A sum of 77 patients (46 guys and 31 females) with diabetes mellitus (5 type 1 and 72 type 2) and diabetic foot ulcers were admitted to the medical clinic. Their mean age was 63 a long time and their mean Hb A1C was 6.3%. The related comorbid conditions recorded from the 77 patients are displayed in Table 1. The restriction of wounds was

generally on the toes, with 40% on the huge toe and 31% on alternate toes, 27% on the underside, and 2% on different parts of the foot. Twenty-three percent of the patients (n =18) were named Wagner organize 2, 38.9% (n =30) as stage 3, 20.7% (n =16) as stage 4, and 6.4% (n =5) as stage 5. The dimension of removal was resolved with the assistance of Doppler ultrasound thinks about. The kinds of careful removal performed were as per the following: (I) underneath the knee, n =1; (ii) Symes task (through the lower leg), n =1; (iii) through the knee, n =2; (iv) transmetatarsal, n =1; and extraordinary toe, n =3. The middle clinic stay was 8 days. Bacterial confines experienced in the investigation are appeared.

**Table 1:** One hundred and eighteen bacterial disconnects were Bacteriology of 77 different Wagner grade diabetic foot ulcers

Micro-organisms	W2	W3	W4	W5	Total	%
Gram-negative aerobes	39	21	5		65	84.4
<i>Pseudomonas aeruginosa</i>	14	6	3		23	29.8
<i>Escherichia coli</i>	12	4	1		17	22.0
<i>Klebsiella pneumoniae</i>	5	4			9	11.6
<i>Proteus mirabilis</i>	5	2	1		8	10.3
<i>Proteus vulgaris</i>		1			1	1.2
<i>Citrobacter koseri</i>	1	1			2	1.2
<i>Citrobacter freundii</i>		1			1	1.2
<i>Klebsiella oxytoca</i>		1			1	1.2
<i>Edwardsiella tarda</i>	1				1	1.2
<i>Klebsiella ozaenae</i>	1				1	1.2
<i>Enterobacter aerogenes</i>		1			1	1.2
Gram-positive aerobes	28	9	6	5	48	62.3
Methicillin-sensitive <i>Staphylococcus aureus</i>	6	2	1	2	11	14.2
Methicillin-resistant <i>Staphylococcus aureus</i>	2		3	3	8	10.3
Coagulase-negative staphylococci (CONS)	16	3	1		20	25.9
<i>Enterococcus faecalis</i>		2	1		3	3.8
<i>Corynebacterium jeikeium</i>	2	1			3	3.8
<i>Bacillus subtilis</i>	2	1			3	3.8
Gram-negative anaerobes		3			3	3.8
<i>Bacteroides fragilis</i>		3			3	3.8
Gram-positive anaerobes		1	1		2	2.5
<i>Peptostreptococcus spp.</i>		1	1		2	2.5
Sterile culture	1	1	1	2	5	6.4
Polymicrobial etiology					49	68.0

In the table refers to different Wagner grade diabetic foot ulcers.

Among 77 societies of which 5 were sterile. Transcendent vegetation was *Pseudomonas aeruginosa* (29.8%), trailed by *Staphylococcus aureus* (24.6%), coagulase-negative staphylococci (CONS) (25.9%), *Escherichia coli* (22%) and different Enterobacteriaceae. In 49 positive societies (68%), polymicrobial etiology was experienced. There were three events on which *Corynebacterium jeikeium* was secluded. Five anaerobic separates were gotten viz. *Bacteroides fragilis* (n =3) from Wagner review 3 ulcers, though *Peptostreptococcus spp.* (n =2) had a place with one each from evaluations 3 and 4. An antibiogram of the oxygen consuming gram-negative detaches uncovered that ciprofloxacin, amikacin and, to some degree, cefotaxime and gentamicin were successful, while a large portion of the gram-positives were delicate to gentamicin. Moreover, among the 19 disconnects of *S. aureus*, 8 (10.3%) were methicillin-safe that were delicate to vancomycin (30 Ag/circle). Ten segregates of *P. aeruginosa* (43.5%) were multidrug-safe with at least two of the ordinary antipseudomonal anti-infection agents like amikacin, gentamicin, cefotaxime or ciprofloxacin.

## Discussion

With an expanding diabetic populace worldwide and with primary significance to the world's diabetic capital, there is a critical ascent in the predominance of foot contaminations additionally in Bhubaneswar, India, the Indian diabetic capital. Eastern India has the biggest number of diabetic people and their financial conditions are poor. Therefore, an investigation of the parts of vigorous and anaerobic microscopic organisms and their antibiogram profile accept incredible centrality. Similarly as with past information appeared by Gin *et al.* [18], the present South Indian examination recorded the power of diabetic polyneuropathy (56.8%) among the majority of the other related co-dreary states of diabetes mellitus. It is accounted for to be the prime figure included the commencement of a contamination in the more profound tissues of the diabetic foot upon beginning bacterial introduction [19]. It is amazing that our examination uncovered an obvious, overwhelming contribution of gram-negative secludes (84.4% aerobes and 3.8% anaerobes) contrasted with gram positive microscopic organisms (62.3% aerobes and 2.5% anaerobes). Besides, dissimilar to contemplates by others [20, 21], we found a 29.8% (n =23) recuperation rate of *P. aeruginosa* in the

cases screened. In 65.21% of the cases (15 of the 23 secludes), *P. aeruginosa* demonstrated a blended contribution with different living beings, for example, *E. coli*, *Enterococci spp.*, *S. aureus*, CONS, and *Proteus spp.* An ongoing clinical investigation by Dhanasekaran *et al.* [22] reported that 84% of diabetic foot ulcers are as often as possible monomicrobial, rather than our discoveries. Concentrates by Viswanathan *et al.* [23] from another middle in South India, revealed 35% gram-positive pathogens secluded and 65% gram-negative ones. These discoveries are like our own, underlining the high pervasiveness of gram-negative pathogens in Eastern India. Three extensive diabetes investigate focuses have gotten fundamentally the same as results. Moreover, 43.5% (n =10) of the *P. aeruginosa* segregates were multi-tranquilize impervious to at least two of the ordinary antipseudomonal anti-infection agents, for example, amikacin, gentamicin, cefotaxime, or ciprofloxacin. Wheat *et al.* have recommended that *P. aeruginosa* may cause extreme tissue harm in diabetics and ought to never be slighted as unimportant in diabetic foot ulcers [24]. They ought to never be consider as contaminants or ordinary vegetation except if they are unmistakably separated as pathogens. The outcome of considering these microorganisms as contaminants or commensals may result in sepsis and removal [25]. Drawn out or expansive range anti-microbial treatment may incline patients to contaminations with anti-infection safe life forms like MRSA or vancomycin-safe enterococci (VRE) [26], yet our examination was not able give information relating to past anti-toxin treatment of the investigation cases. However, information introduced on the polymicrobial inclusion may, to some degree, clarify a conceivable earlier treatment history of the patients examined, as Lipsky *et al.* [20] and others [27] have detailed that polymicrobial etiology in diabetic foot ulcers may frequently be because of past treatment history. MRSA has turned out to be progressively predominant in diabetic foot wounds [28]. Among the 19 disconnects of *S. aureus*, 8 (10.3%) were methicillin-safe. Notwithstanding, the majority of the separates were touchy to vancomycin (30 Ag/plate), which expect significance as vancomycin-safe *S. aureus* (VRSA) is by and large progressively announced by others [29]. Our detachment of *C. jeikeium* (diphtheroid) from 3 patients (3.8%) recommends that they may hold some pathogenicity in the diabetic host as there dependably is by all accounts a relationship between *C. jeikeium* and diabetes mellitus. *C. jeikeium* is viewed as a deft pathogen in diabetic patients 30 that picks up section into necrotic delicate tissue or bone and expect a pathogenic job, paying little mind to its being a generally low-destructive microorganisms. In a past report, *Corynebacterium spp.* was accounted for in 12% of diabetic patients with positive societies [30]. Bessmann *et al.* have reported that *B. fragilis* and *Peptostreptococcus spp.* can be recouped in high numbers from extreme contaminations. Our confinement of *B. fragilis* with *E. faecalis* on two events affirms its synergistic job, as bolstered by Bessmann *et al.* [31] Reports on polymicrobial etiology were comparable, while a few creators have announced a higher level of anaerobic confines [32]. This distinction could be identified with the example accumulation and transport framework, which never incorporated a particular conditions for anaerobic greenery, despite the fact that Cary and Blair and RCM media bolster the tirelessness of anaerobic microbes [31]. We have to think about increasingly

dependable techniques, similar to curettage of the base of the ulcers and profound tissue societies [33]. Gram-negative microscopic organisms that are viewed as typical vegetation of the skin, as *Citrobacter spp.* what's more, *P. aeruginosa*, may cause extreme tissue harm in diabetics and ought to never be naturally neglected as unimportant in diabetic foot ulcers [28]. The Wagner grades with the most secludes were 2 and 3, as in past examinations [34]. We couldn't build up any relationship between's injury profundity and microbiological discoveries, albeit the majority of the anaerobes were recouped from review 3 and 4 wounds. In rundown, our investigation underpins the perspective set forth by past South Indian creators that the conveyance of gram-negative microorganisms (57.6%) is more typical than that of gram-positive ones (42.3%), however it doesn't bolster the see that diabetic foot diseases are much of the time monomicrobial. Besides, recuperation of multi-medicate safe *P. aeruginosa* disengages is a genuine worry, as nobody has detailed the equivalent from the South Indian milieu. Future endeavors must be focused at understanding the job of bacterial pathogens in diabetic foot ulcers, and starting treatment ought to be coordinated at both oxygen consuming and anaerobic microscopic organisms. The approval of a basic, cost effective calculation for the determination of diabetic foot diseases will be of principal use. Notwithstanding, as proposed by Lipsky *et al.* [18] location of neuropathy before its entanglements create is the most ideal approach to forestall diabetic foot diseases.

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