

ORIGINAL RESEARCH

Evaluation of Bacterial Profile and Their Antibiotic Susceptibility Among Gram Negative Isolates from Diabetic Foot

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ABSTRACT

Background and Aims: This investigation was carried out on individuals suffering from diabetic foot ulcers (DFUs) with the purpose of assessing the clinical characteristics, spectrum of gram negative microbial populations, antibiotic susceptibility, and establishing a pragmatic approach for administering antimicrobial therapy.

Materials and Methods:Clinical data, along with specimens of tissue and pus, were procured from a total cohort of 50 patients who had received a diagnosis of diabetic foot ulcers. These samples were handled in strict adherence to the protocols outlined by the Clinical and Laboratory Standards Institute. Both the clinical and microbiological data sets underwent meticulous analysis.

Results: The present study, encompassing 50 patients afflicted by diabetic foot ulcers, unveiled a predominance of male subjects. The majority of patients fell within the age range of 50 to 60 years. In the collected samples, Gram-negative microorganisms constituted 70.52% of the total. Notably, Gram negative organisms showcased the most notable receptivity towards Colistin, Imipenem, and Meropenem..

Conclusion: Within the realm of DFUs, Gram-negative bacteria exhibited varying degrees of resilience against frequently employed antibiotics, concurrently demonstrating receptivity to aminoglycosides. These findings suggest that Colistin, Imipenem, and Meropenem.hold promise as potential choices for empirical antibiotic intervention in cases of DFU infections.

Key words: Anti-Bacterial Agents, Imipenem, and Meropenem., Gram-Negative Bacteria, Diabetic Foot.

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Introduction

Diabetes is emerging as a potential epidemic within the Indian context, encompassing a diabetic populace exceeding 62 million individuals. The prevalence of diabetes displays regional variations across distinct geographic areas of the nation, ranging from 5.3% in central India to 13.6% in the northern regions. Nonetheless, owing to the non-notifiable nature of diabetes, the veritable magnitude of its impact within India remains obscured, potentially resulting in an

underestimation of its authentic pervasiveness and repercussions within the country's framework (1-3).

Notably, the complication of foot ulceration stands as a significant manifestation of diabetes, afflicting an estimated 15% of the diabetic population over their lifetime. The prevalence of diabetic foot ulcers (DFUs) exhibits a range of 4% to 10%. Among the intricate spectrum of complications associated with DFUs, infections emerge as the foremost contributor

to both morbidity and mortality, manifesting in 40% to 80% of instances (4).

In the realm of DFU infection management, the initiation of empirical antimicrobial therapy represents a standard practice. However, the limited innovation in novel antimicrobial agents alongside the excessive employment of extant antibiotics have jointly engendered a widespread apprehension concerning antibiotic resistance within the ambit of healthcare establishments (5). Adding to the complexity of this scenario is the presence of diabetic nephropathy, impacting approximately one-third of the diabetic populace, coupled with the escalating incidence of multi-drug resistant infections within DFUs. This confluence of factors intricately compounds the challenges confronting clinicians in their endeavor to efficaciously manage this patient cohort (6).

The fundamental objective of this inquiry was to meticulously explore the clinical and microbiological attributes characteristic of DFU patients, discern the susceptibility patterns of Gram-negative microorganisms relevant to DFUs vis-à-vis antibiotics, and formulate a protocol for empiric antibiotic intervention. Given the paucity of analogous investigations within the geographical domain that have articulated empiric therapeutic strategies for diabetic foot infections, comprehending the intricate nuances of antibiotic responsiveness assumes pivotal significance in devising an empiric antibiotic treatment regimen that is bespoke to this locale. Such an initiative holds the potential to redound to the benefit of primary care practitioners and specialized clinicians alike, enabling them to embark upon empiric antibiotic regimens that are marked by enhanced efficiency. This, in turn, may engender a prospective abatement in antibiotic resistance dynamics, concomitant with potential alleviation of treatment-associated financial encumbrances borne by patients.

Materials & Methods

This prospective and observational investigation was conducted within the purview of the Department of Microbiology at Sri Aurobindo Institute of Medical Sciences, situated in Indore, India. This institution serves as a tertiary care teaching hospital. Over a span of two years, the study engaged a cohort of 50 patients afflicted with Diabetic Foot Ulcers (DFUs), who were enrolled from the diabetic foot clinic. The ethical tenets of the research adhered to the precepts delineated in the Declaration of Helsinki (7) and were ratified by the Institutional Ethics Committee of the aforementioned institution. It is noteworthy that the participants of the study furnished their written informed consent before their inclusion. The diagnostic criterion employed for DFUs aligned with

the specifications delineated by the International Working Group on the Diabetic Foot (IWGDF), wherein a DFU was characterized as a wound extending through the dermis that attains full-thickness and is situated below the ankle, specifically within individuals bearing a diagnosis of diabetes (8).

The ambit of inclusion criteria encompassed patients aged 18 years or older, who had been diagnosed with DFUs and were availing the services of the diabetic foot clinic, provided they had accorded their consent for participation. Conversely, exclusion criteria dictated the exclusion of children, individuals with non-diabetic foot ulcers, and instances involving repetitive isolates from the same patient.

Upon securing informed consent, pertinent demographic details of the participants were collated and systematically recorded in an Excel spreadsheet. Concurrently, the outcomes of blood investigations were documented within the same electronic repository. Elevated fasting blood glucose levels equal to or exceeding 126 mg/dL and glycosylated hemoglobin (HbA1c) levels equal to or surpassing 7% were classified as anomalous, respectively.

The acquisition of specimens for pus and tissue cultures transpired within a minor operation room, conforming to a specific protocol. The procedural sequence involved the meticulous cleansing of the wound followed by thorough irrigation with a saline solution. Subsequent to these measures, any superficial exudate and necrotic tissue were meticulously excised to mitigate the potential for capturing surface colonization flora. The specimens were meticulously collected utilizing sterile culture bottles preloaded with normal saline. The act of collection encompassed scraping the ulcer base or procuring samples from the deeper aspects of the wound margin through the instrumentality of a sterile curette. Additionally, incised tissue samples measuring approximately 0.5 centimeters in diameter were excised from diverse locations within the wound. This approach to procuring deep tissue samples via curetting was elected due to its heightened reliability and fidelity in yielding microbiological cultures when juxtaposed with conventional swab-based sampling methods (9).

Canonical techniques were deployed for sample processing, isolation, and subsequent identification of aerobic bacteria. The tissue samples were homogenized and subsequently inoculated onto both Blood agar and MacConkey agar substrates. Incubation of these samples was conducted under aerobic conditions at a temperature of 37 degrees Celsius, sustained for a period ranging between 24 to 48 hours. Following this, the resultant colonies underwent identification, and their susceptibility to antibiotics was gauged employing the Kirby-Bauer's

disc diffusion methodology, consonant with the stipulations set forth in the Clinical Laboratory Standard Institute guideline of 2012 (10).

The analytical facet of the data encompassed both descriptive and inferential statistics. For the latter, the Chi-square test was enlisted. The software instrumentalities harnessed for this analytical undertaking comprised SPSS (Statistical Product and Service Solutions) version 18.0 and GraphPad Prism version 5.0. A predetermined level of significance

was established at $P < 0.05$ to adjudge statistical significance within the outcomes.

Results

In this investigation, the primary demographic stratum of the participants comprised individuals aged 50 to 60 years. Within the study cohort, a notable majority was constituted by male participants. The prevailing majority of instances were characterized by the presence of Type II Diabetes, which exhibited a chronicity ranging from 10 to 15 years, as illustrated in Table 1.

Table1:Demographics and clinical features of study population.

Variable	Number	%
Age (years)		
40-50	11	22
50-60	25	50
60-70	6	12
70-80	7	14
>80	1	2
Gender		
Male	35	70
Female	15	30
Type of Diabetes		
Type I	2	4
Type II	48	96
Duration of Diabetes (years)		
>5	1	2
5-10	15	30
10-15	30	60
>15	4	8
Drugs		
Insulin	35	70
Oral	15	30
Personal Habits		
Drinking	11	22
Smoking	15	30
Both	16	32
Nil	8	16
Specimen Collected		
Pus	37	74
Tissue	13	26

As delineated in Table 2, an enumeration of microorganisms extracted from Diabetic Foot Ulcers (DFU) within the studied populace is furnished. A substantial proportion of the collected

specimens displayed a polymicrobial nature, wherein Gram-negative organisms constituted an approximate majority of 70% in relation to the aggregate isolates.

Table2:Type of organisms isolated from DFU in study population

Type of Isolate	Number	%
Monomicrobial	21	42
Polymicrobial	29	58
Isolated Organism		
Gram Negative		
Pseudomonas aeruginosa	11	14.10
Klebsiellapneumoniae	15	19.23
Klebsiellaoxytoca	2	2.56

Escherichia coli	12	15.38
Proteus vulgaris	3	3.85
Proteus mirabilis	1	1.28
Providencia species	4	5.13
Acinetobacter	6	7.69
Citrobacterfreundii	1	1.28
Total	55	70.51
Gram Positive	23	29.49

The antibiotic susceptibility profiles manifested by the Gram-negative microorganisms, isolated from Diabetic Foot Ulcers (DFU), are presented in Table

3. The findings underscore that these entities showcased the most notable receptivity towards Colistin, Imipenem, and Meropenem.

Table3:Antibiotic sensitivity among Gram Negative organisms isolated from DFU.

Antibiotic	Enterobacteriace (n=38)		Pseudomonas (n=11)		Acinetobacter (n=6)	
	Sensitive Isolates	%	Sensitive Isolates	%	Sensitive Isolates	%
Amikacin	22	64.71	6	54.55	-	-
Amox/Clav	16	47.06	-	-	-	-
Ampi/Sulbactam	15	44.12	-	-	-	-
Aztreonam	6	17.65	5	45.45	-	-
Cefipime	14	41.18	6	54.55	-	-
Cefazolin	20	58.82	2	18.18	-	-
Ceftazidime	5	14.71	2	18.18	-	-
Cephoxitin	11	32.35	5	45.45	-	-
Cephotaxime	5	14.71	2	18.18	-	-
Chloramphenicol	9	26.47	-	-	-	-
Levofloxacin	14	41.18	7	63.64	1	16.67
Colistin	33	97.06	11	100.00	6	100.00
Gentamicin	18	52.94	7	63.64	2	33.33
Imipenem	32	94.12	8	72.73	4	66.67
Meropenem	30	88.24	8	72.73	4	66.67
Doripenam	28	82.35	7	63.64	4	66.67
Piperacillin	26	76.47	5	45.45	-	-
Piperacillin/Tazobactam	22	64.71	7	63.64	-	-
Tetracycline	18	52.94	-	-	1	16.67
Tobramycin	-	-	6	54.55	2	33.33
Cotrimoxazole	14	41.18	-	-	-	-
Ampicillin	13	38.24	-	-	-	-
Cefuroxime	0	0.00	-	-	-	-
Ciprofloxacin	13	38.24	6	54.55	-	-
Ticarcillin	-	-	7	63.64	-	-

Discussion

The occurrence of foot ulcers represents a considerable burden on individuals afflicted with diabetes, often culminating in severe ramifications. Notably, nearly fifty percent of lower extremity amputations can be attributed to complications stemming from diabetes [4]. Among the primary catalysts for amputation of the diabetic lower limb, foot infections rank as the foremost cause, second only to gangrene [4]. In the context of managing infections in diabetic foot ulcers (DFUs), initial antibiotic treatment typically commences empirically,

grounded in the presumed etiologic agent. Subsequent therapeutic adjustments are predicated on bacterial culture and sensitivity results. The duration of treatment varies in accordance with infection severity, ranging from one to two weeks up to a span exceeding four weeks [11]. Clinicians face the added complexity of addressing diabetic patients afflicted by nephropathy, a condition affecting approximately one-third of individuals with diabetes. Moreover, the escalating prevalence of multidrug-resistant infections in DFUs exacerbates the challenges confronted by healthcare practitioners in delivering efficacious treatment [6].

The mean age of the patients incorporated in this investigation stood at 58.55 years. A predominant portion of patients fell within the age bracket of 50 to 60 years, with a male-to-female ratio of 2.33. These outcomes closely mirror those documented in a study conducted by Yerat et al. [4]. Situated within a rural locale, the institution in question attracted predominantly rural patients, with 80% originating from rural areas and the remaining 20% from urban localities. This distribution concurs with findings reported by Shahi et al. [12]. Noteworthy is the considerable proportion of DFU patients exhibiting a diabetes duration exceeding 10 years, which corroborates prior conclusions outlined in studies conducted by various researchers [13-15].

From the cohort of 50 enrolled patients, a total of 101 isolates were procured, culminating in an average of 2.02 bacteria per lesion. By way of comparison, Kaur et al. [16] and Bansal et al. [17] reported wound bioburdens of 1.38 and 1.52 bacteria per culture-positive patient, respectively. The relatively higher isolation rates observed in our investigation could potentially be attributed to the non-isolation of anaerobic organisms.

Within this study, a comprehensive assessment of 50 samples was undertaken, with the isolates indicating that 42% evidenced growth of a singular organism, while 20% manifested growth of two organisms, and 38% displayed polymicrobial growth. Similar patterns were noted in the study by Bansal et al. [17], wherein 61.8% of cases exhibited monomicrobial growth, 37.08% displayed polymicrobial growth, and 7.2% of cases returned sterile cultures. Among the total bacterial isolates, Gram-negative bacteria constituted a majority (73.7%), with Gram-positive bacteria accounting for the remaining 27.3%. Analogous trends have been documented in other investigations by Manikandan et al. [18] and Kaur et al. [16], aligning with the higher prevalence of Gram-negative pathogens documented in low-income countries, as reported by Perez-Fevila et al. [19].

In India, Gram-negative bacilli have emerged as a prevalent causative agent of diabetic foot infections [20], in contrast to Western countries where Gram-positive organisms have predominated, as seen in the research conducted by Mendes et al. [21]. The underlying factors accounting for the divergence in Gram-negative prevalence in the East and Gram-positive prevalence in the West remain largely unexplored, necessitating further inquiry.

Within the ambit of our present study, Gram-negative organisms exhibited heightened susceptibility to Colistin, Imipenem, and Meropenem. This attenuated effectiveness may stem from the widespread utilization of these antibiotics within the community [23].

Rational empiric therapy is considered an indispensable facet in the management of DFU infections. Drawing from the findings of our study and analogous investigations conducted across

diverse geographical domains, it was deduced that aminoglycosides evinced the most pronounced efficacy against both Gram-positive and Gram-negative bacteria in the context of DFU infections. This observation could potentially be linked to the relatively restricted use of aminoglycoside antibiotics within the community [23].

Notwithstanding its contributions, this study harbors certain limitations warranting consideration. Chief among these is the relatively modest sample size, which may curtail the extrapolation of findings to a broader population. Moreover, due to logistical and resource constraints, the presence of anaerobic flora was not ascertained, potentially leading to an incomplete comprehension of microbial composition within the studied populace.

Conclusion

The implications of this study underscore that DFUs predominantly affect males during their fifth and sixth decades of life, with a significant proportion of patients exhibiting inadequate glycemic control. Gram-negative bacteria prevail as the predominant causative agents in DFUs, while *Klebsiella Pneumoniae* stands as the primary Gram-negative strain. Intriguingly, aminoglycosides showcase sensitivity towards both Gram-negative and Gram-positive bacteria, attributed to their limited application in community settings. Conversely, resistance patterns have surfaced within the community against commonly prescribed extended-spectrum penicillins and cephalosporins. Given the insights gleaned from this study, a reevaluation of empiric antibiotic strategies for treating diabetic foot infections is warranted.

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