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MICROBIOLOGICAL STUDY OF DIABETIC FOOT ULCERS IN AN INDIAN TERTIARY CARE HOSPITAL

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ABSTRACT: Diabetes mellitus is one of the most critical health conditions around the world, not only in terms of the number of affected people, disability, and premature mortality but also in regards to the health care costs involved in controlling and treating its complications. Among the most constant ailments, the diabetic patient suffers is the diabetic foot, defined as any infection, ulceration, and necrosis of deep tissues associated with neurological abnormalities and various degrees of peripheral vascular disease of the lower limbs. This study determines the microbiological profile and antibiotic susceptibility patterns of organisms isolated from diabetic foot ulcers. A record based study was conducted among 169 diabetic foot ulcer patients admitted in the hospital; Details regarding the Culture and antibiotic susceptibility of specimens (pus samples from foot ulcers) from these 104 patients were collected. Commonest microorganisms isolated in this study were *Pseudomonas, Staphylococcus, Klebsiella, Proteus, and E. coli.* Gram-positive organisms were highly sensitive to Vancomycin, Linezolide, and gram-negative were sensitive to Amikacin and Meropenam.

Keywords: Microbiological profile, Gram positive, Gram negative, Antibiotic susceptibility

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INTRODUCTION: Diabetes mellitus (DM) is a common, chronic, debilitating, and sometimes fatal endocrine disease with constantly growing global prevalence. In 2011, about 366 million people suffered from DM, and in 2030, the number would rise to 552 million [1].

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DM encompasses Type 1 DM, which can be associated with autoimmune damage of the pancreatic β cells, Type 2 DM, resulting from insulin resistance and disorder of insulin secretion and gestational DM, which can progress to Type 2 DM in the years afterward [2].

Type 2 DM encompasses most (usually 90–95%) of all diabetic patients. It is worrying that 26.9% of the people aged >65 years and 11.3% of those aged >20 years had diagnosed or non-diagnosed DM in the USA in 2011.[3] Worldwide, diabetic foot lesions are a major medical, social, and economic problem

and are the leading cause of hospitalization for patients with diabetes. Infectious agents are associated with amputation of the infected foot if not treated promptly. Proper management of these infections requires appropriate antibiotic selection based on culture and antimicrobial susceptibility results; however, initial management comprises empirical antimicrobial therapy, which is often based on susceptibility data extrapolated from studies performed on general clinical isolates [4]. Several studies found methicillin-resistant *Staphylococcus aureus* (MRSA) in as many as 15-30% of diabetic wounds [5, 6, 7].

Infection with multidrug-resistant organisms (MDROs) may increase the duration of hospital stay and cost of management and may cause additional morbidity and mortality. Among diabetic individuals, foot infections are the most frequent complication in the patient, accounting for 20 % of diabetic related hospital admissions [8]. Diabetic Foot Infection [DFI] or Diabetic Foot Ulcer [DFU] is defined as the infection caused by the introduction of an infectious agent into the otherwise sterile soft tissue of the foot through minor skin break. Infectious agents are usually associated with the worst outcome, which might lead to amputation of the infected foot unless prompt treatment strategies ensue. Though many studies have reported on the bacteriology of diabetic foot infections (DFIs) over the past 25 years, the results have varied and have often been contradictory [9, 10].

So, conducting surveillance study at equal intervals is a must to assess & update the condition prevailing. Drug resistance is a global problem affecting both developed and undeveloped countries. Antimicrobial resistance is a natural consequence of antimicrobial use, which kills the sensitive organisms leaving the resistant ones to survive and multiply (selection of resistance). Overuse and misuse of antimicrobials do not help patients; they merely add to the problem of resistance and waste resources. Antimicrobial resistance is on the increase – threatening our ability to treat some of the infectious diseases that cause most deaths. Infectious diseases still account for 45% of deaths in low-income countries and almost one in two premature deaths worldwide [11]. Today antibiotics remain the first line therapy for conquering bacterial infections. However, their indiscriminate use is no longer viewed as benign. Treatment with these drugs is acknowledged to be a two-edged sword. As antimicrobial agents have been misused or overused, bacteria have fought back with a selection process by which certain strains are no longer susceptible to one or more agents. Each new use of these drugs, in fact, contributes to the evolution of resistant microorganisms [12, 13]. Empirical antibiotic recommendations [14, 15, 16] by type of infection, are given in **Table 1**.

METHODOLOGY: A record based study was conducted among 169 diabetic foot ulcer patients admitted to the hospital in Pune. Patients with diabetic foot infections were identified whose microbiology request forms sent along with the clinical specimens to the Department of Microbiology. Prescription data were collected from the medical records that contain all the prescriptions for each patient, providing information about the demographic details, drugs prescribed empirically, culture and sensitivity reports of the patients were collected for analysis. Culture and sensitivity reports of patients were screened to determine the prevalence of sensitivity and resistance pattern of a particular organism to different antibiotics were determined. The data obtained were tabulated, and results are interpreted using cross tabulation for SPSS software has been used. The data collected from all the participants were recorded in a spreadsheet format and analyzed using the software JMP 8® ™ academic license from SAS® Inc, and cross-tabulation for SPSS software has been used for any statistical significance. Significance was reported by 95% Confidence Interval.

RESULTS: Males were predominant (76.33%) in the study subjects. The majority of subjects had type 2diabetes (96.44%). Most of the participants were in 40 to 60 years old range, **Fig. 1**. 36.09% had the condition for >10 years.81.6% of cases were using insulin treatment at the time of admission. 37% of participants did not have any complications, 23.07% had Gangrene, and only 2% had Septicaemia. Osteomyelitis was present in 50 (62.5%) subjects **Table 1**. A total of 186 isolates were detected from the 169 ulcer specimens, averaging 1.1species per patient.

TABLE 1: SUGGESTED ANTIBIOTIC REGIMENS FOR TREATMENT OF DIABETIC FOOT INFECTIONS

Severity of infection	Route of administration	Recommended agents (choose one or more)	Alternative agents
Mild/moderate	Oral	Cephalexin (500 mg q.i.d.) OR Dicloxacillin (250 mg q.i.d.) OR Clindamycin (300 mg t.i.d.) OR Amoxicillin/ clavulanate (875/125 mg b.i.d.)	Levofloxacin (750 mg q.d.) ± Clindamycin (300 mg t.i.d.) OR Trimethoprim-sulfamethoxazole (2 double-strength b.i.d.)
Moderate/severe	Intravenous until stable, then transition to an oral equivalent (or tailor based on culture results)	Ampicillin/sulbactam (3.0 gm q.i.d.) OR Clindamycin (450 mg q.i.d.) + ciprofloxacin (750 mg b.i.d.)	Piperacillin/tazobactam (3.3 gm q.i.d.) OR Clindamycin (600 mg q.i.d.) + ceftazidime (2 gm t.i.d.) *OR* Ertapenem (1 gm q.d.)
Life-threatening	Prolonged intravenous	Imipenem/cilastin (500 mg q.i.d.) OR Clindamycin (900 mg q.i.d.) + tobramycin (5.1 mg/kg/d) + ampicillin (50 mg/kg q.i.d.)	Vancomycin (15 mg/kg b.i.d.) + aztreonam (2.0 gm t.i.d.) + metronidazole (7.5 mg/kg q.i.d.)

TABLE 2: GENERAL CHARACTERISTICS OF STUDY SUBJECTS

Characteristics		Frequency	Percentage
Gender	Male	129	76.33
	Female	40	23.77
Type of Diabetes mellitus	Type I	6	3.55
	Type II	162	96.44
Duration	Newly detected	8	4.73
	< 1 year	12	7.100
	1-5 years	29	17.15
	6-10 years	59	34.91
	>10 years	61	36.09
Complications other than foot ulcer among Type II Diabetic patients	Peripheral occlusive vascular disease	16	9.46
	Septicaemia	4	2.36
	Neuropathy	24	14.20
	Retinopathy	3	1.77
	Osteomyelitis	36	21.3
	Gangrene	39	23.07
	None	63	37.27
Type of treatment at the time of admission	Insulin	138	81.65
	Oral hypoglycaemic drugs	31	18.34

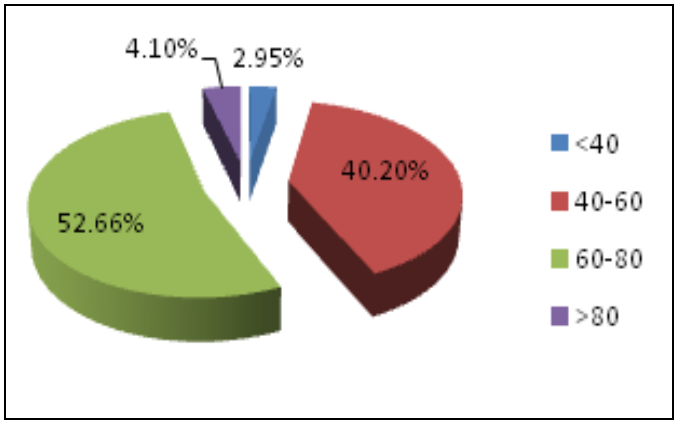

FIG. 1: AGE DISTRIBUTION OF PARTICIPANT

Gram-positive organisms isolated were 71 out of a total of 186 organisms (38%), and gram-negative organism isolated was 115 out of 186 organisms (61%). The profile of the gram-positive organisms isolated is detailed in **Fig 2**. Of the total 31.87% gram-positive isolated, Staphylococcus is mostly detected (19.89%) while coagulase-negative *Staphylococcus* were in less number as compare to another gram-positive organism (3.22%). Among gram-negative organism **Fig. 3** the most common isolates were *Proteus sp.* (18.27%), Pseudomonas (13.97%), *E. coli* (13.44%) The other gram-negative isolates found to be associated were *Klebsiella* and *Citrobacter*.The results of susceptibility studies to Gram-positive organism are summarized in **Fig 4**. Most of the Gram-positive were found to be highly resistant to penicillin (92%), gentamicin (77%), and erythromycin (88%). But they showed good sensitivity to a cephalosporin (69%), amikacin (59%) and rifampicin (67%). They have not yet shown resistance to vancomycin or linezolid. The results of susceptibility studies to Gram-negative organism are summarized in **Fig 5**. In the case of gram-negative isolated, we observed good sensitivity of these organisms to amikacin (63%) and meropenem (59%).

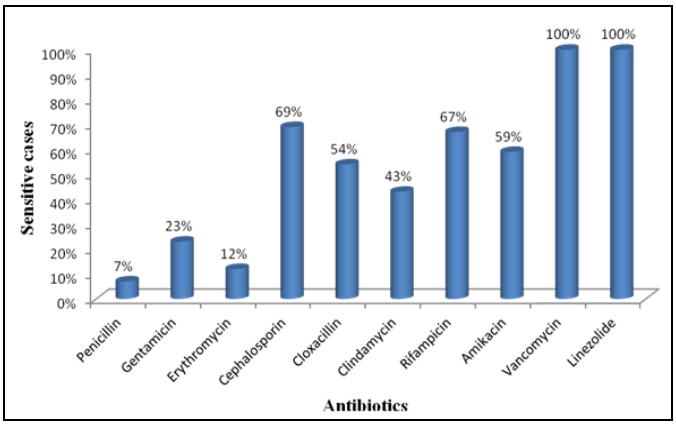

FIG. 2: COMPARATIVE FREQUENCY OF THE ORGANISMS ISOLATED (GRAM POSITIVE). No of specimens: 169. Total no. of the organism isolated: 186. Total no. of gram-positive isolated: 71/186 (38.17%).

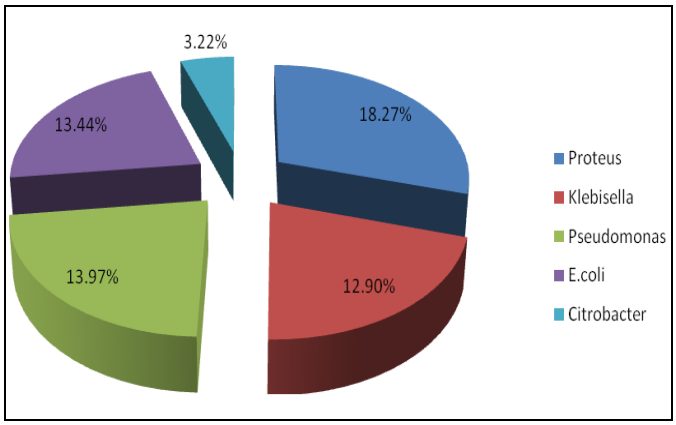

FIG. 3: COMPARATIVE FREQUENCY OF THE ORGANISMS ISOLATED (GRAM NEGATIVE). No. of specimens: 169. Total no. of the organism isolated: 186. Total no. of gram-positive isolated: 115/186 (61.82%).

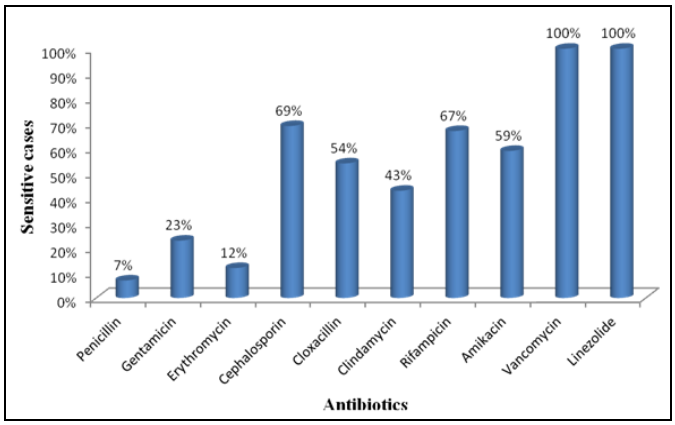

FIG. 4: ANTIBIOTIC SUSCEPTIBILITY OF GRAM-POSITIVE ORGANISM

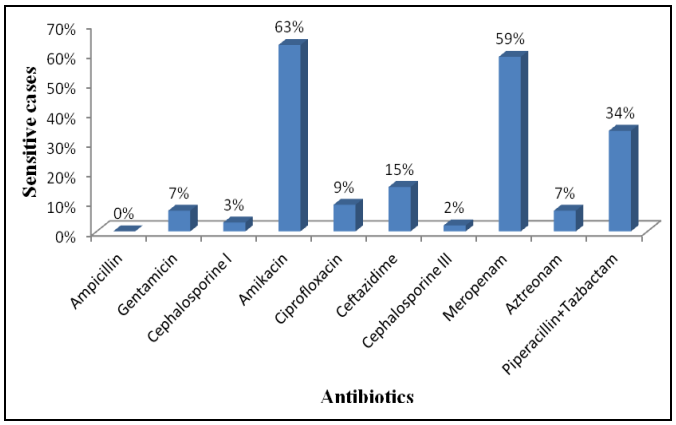

FIG. 5: ANTIBIOTIC SUSCEPTIBILITY OF GRAM-NEGATIVE ORGANISM

Thirty-four percent of organism showed sensitivity to Piperacillin and Tazobactam combination. On the other hand, no sensitivity detected in treatment with Ampicillin. Most of the gram-negative were found to be highly resistant to Cephalosporine l (3%) and Cephalosporine lll (2%) followed by Gentamicin and Aztreonam (7% for both).

DISCUSSION: Our study revealed that no. Males attending the foot infection were more than the no. of females and the main age group involved was patients above the age of 60 years. The majority of the study population had Type 2 diabetes mellitus, and most of the participants had a history of diabetes for more than 6 years. Our results showed that the 186 specimens yielded about 38% Gram positive sp. and 61% gram negative spp. The frequency of gram-negative organism was higher to the frequency of gram-positive; this result was by some study [18, 19]. Foot infections in diabetes are rarely due to a single organism [20, 21]. Aerobic bacteria (*Staphylococcus spp., Streptococcus spp.,* & *Enterobacteraciae*), anaerobic flora (*Bacteroides sp., Clostridium sp.,* and *Peptostreptococci sp.*), and fungi are the organisms that are isolated mot often.[22] In this study a total of 186 isolates were detected from the 169 ulcer specimensns, averaging 1.1 species per patient. Only 9.8% of the subjects had infections due to more than one organism. In this study, 61% of organisms isolated from diabetic foot ulcers were gram-negative organisms, and 38% of organisms were gram-positive organisms.

Gram-positive aerobic bacteria were found to be the predominant organisms causing diabetic foot infections in many studies [23, 24]. But the more recent studies report gram-negative aerobes to be the commonest organisms in diabetic foot ulcers [25, 26]. The predominant gram-positive *Cocci* isolated were *Staphylococcus aureus*, this was in accordance to study conducted in Indian tertiary care hospital and a US hospital [27]. The isolation rate of *Enterococci* spp. was 11.29 %, which was lesser than the isolation rate reported by a study

conducted in US [28]. In the case of the gram-negative organism, *Proteus* was the organism isolated in 18 % samples. Pseudomonas and *E.coli* were second and third commonest. They are followed by *Klebsiella* and *Citrobacter*. *Staphylococcus aureus, Pseudomonas aeruginosa*, *Proteus mirabilis,* and *Bacteroides fragilis* are the most common causes of diabetic foot infections [29, 30, 31]. Non-anaerobes were observed in this study.

Most of the study elucidates that gram-positive organism exhibited resistance to Penicillin and Erythromycin [32, 33] this result was completely the same to our results where our strains isolated exhibited resistance pattern to the mentioned antibiotics. A study from Nigeria also reports that the resistance percentage of the Gram-positive isolates from diabetic foot ulcers was Gentamicin 89%, Clindamycin 88%. This was higher when compared to the results of our study [34, 35]. Most Gram-negative is highly resistant to antibiotics such as ampicillin, Gentamicin, Cephalosporins, Ciprofloxacin, and Aztreonam. They show good sensitivity to Amikacin, Meropenem, and Piperacillin - Tazobactam. Likewise, most of the studies show high resistant of a gram-negative organism to Ampicillin, Cephalosporine, Aztreonam [31, 10].

CONCLUSION: Commonest microorganisms isolated in this study were *Pseudomonas*, *Staphylococcus*, *Klebsiella*, *Proteus*, and *E. coli*. Gram-positive organisms were highly sensitive to Vancomycin, Linezolid, and highly resistant to Penicillin. Gram-negative was sensitive to Amikacin and Meropenem and highly resistance to Ampicillin and Cephalosporine. These findings suggest that prospective multicenter studies are required to assess the appropriate empirical antibiotic regimen in diabetic foot ulcers taking into consideration the etiology of ulcers. Also, the results alert us that proper management of antibiotics must be implemented to decrease the incidence of resistant in the population. In many cases, empirical therapy is necessary, especially in therapeutic centers that have no microbiology laboratories and limited resources.

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CONFLICT OF INTEREST: Nil

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