University of Basra

College of pharmacy

(Diabetic foot infections: A review of antibiotic prescribing in a diabetic foot clinic)

A project submitted to the department of clinical lab sciences of the pharmacy college in Basra university as partial completion of requirement sore graduation

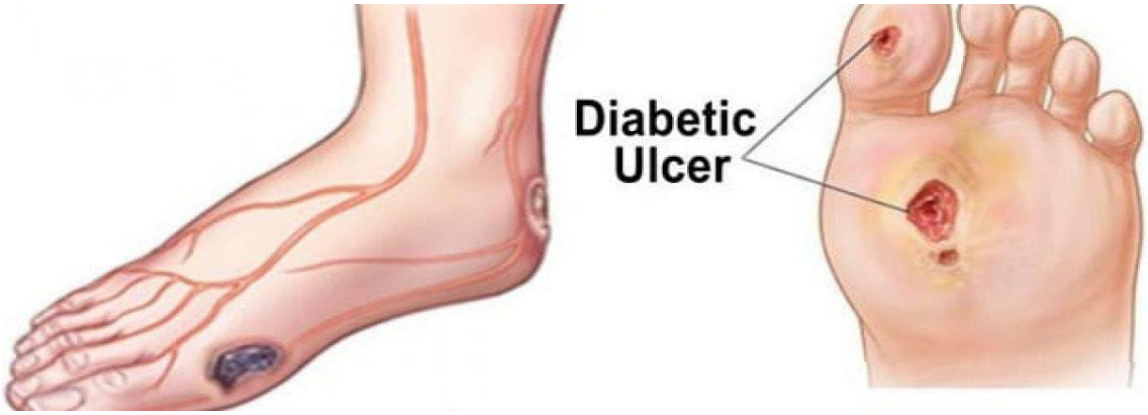
Prepared by:

Mhmoud twfeeq mhmoud

Silwan Uday Jaber

Esraa Abdullah Youssef

Supervisor: D. Dawood chalob Hillel



ABSTRACT:

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder affecting a large segment of population and also a major public health problem.

Two major factors are considered important in development of the ‘diabetic foot’

1. Peripheral neuropathy
2. Macro and microangiopathy

Diabetic ulcers are caused by a lack of blood supply as a result of diabetes.

Diabetic foot disease is one of the most severe diabetic consequences. It causes the patient significant pain and financial cost, as well as a significant strain on the patient's family, healthcare personnel and institutions, and society in general.

Patients and methods

A prospective study of 75 patients with diabetic foot infections admitted to Al-Azhar university hospitals was undertaken. Bacteriological specimens were obtained and processed using standard hospital procedure for microbiological culture and sensitivity testing.

Results

Overall, 40 (54%) patients had subcutaneous infections, 22 (29%) had infected superficial ulcers, seven (9%) had infected deep ulcers involving muscle tissue, and six (8%) patients had osteomyelitis. A total of 99 pathogens were isolated. Forty percent of patients had polymicrobial infection, 39 (52%) had single organism infections, and six (8%) had no growth. Gram-negative bacteria (67%) were more commonly isolated compared with Gram-positive bacteria (30%). The three most frequently found Gram-positive organisms were Staphylococcus aureus (10.2%), Streptococcus pyogenes (7.1%) and methicillin-resistant S. aureus (7.1%), and the most common Gram-negative organisms were Pseudomonas aeruginosa (19.4%), Klebsiella pneumoniae (15.3%), and Acinetobacter spp. (10.2%). Vancomycin was found to be the most effective against Gram-positive bacteria, whereas imipenem and amikacin were most effective against Gram-negative bacteria on antibiotic testing.

Conclusion

Forty percent of diabetic foot infections were polymicrobial. S. aureus and P. aeruginosa were the most common Gram-positive and Gram-negative organisms, respectively. This study helps us to choose empirical antibiotics for patients with diabetic foot infections.

INTRODUCTION:

Diabetes mellitus is a chronic metabolic disorder affecting a large segment of population and also a major public health problem[1].
Diabetes affects 425 million people globally, according to 2017 data. When compared to the numbers reported in 2013 and 1980, which were 382 million and 108 million, respectively.[2]The number of people with diabetes is increasing due to population growth, ageing, urbanization, increasing prevalence of obesity and physical inactivity.
Diabetic foot ulcers are the source of major suffering and very large costs for both the patient and the health-care system, and every 30 s, a leg is lost somewhere in the world. Investing in a diabetic foot care guideline can therefore be one of the most cost-effective forms of health-care expenditure.[3]

Two major factors are considered important in development of the 'diabetic foot'[4]
1. Peripheral neuropathy causing sensory impairment and weakness of intrinsic muscles of the foot and joint that leads to foot deformities.
2. Macro and microangiopathy occurring frequently and leading to ischemia of foot tissues.

Wounds become infected five times more often in diabetics than in non-diabetic patients. Selecting appropriate antimicrobial therapy for diabetic foot infections requires knowledge of likely etiologic agents[5]. The most important characteristic of diabetic foot infection is its polymicrobial nature, and frequent involvement of anaerobes synergistically with aerobes[1].The Incidence of aerobic infection is more in lower grades of Wagner's classification. As the grade increases anaerobic infections are encountered frequently[6].

Diabetic ulcers are caused by a lack of blood supply as a result of diabetes. The foot is the most prevalent location for diabetic ulcers. Although other parts of the body are susceptible to such ulcers, the toes are particularly susceptible for a variety of reasons, including neuropathy as the primary cause, neglecting this part of the body, the shape of the arch and toes, and the colonization of bacteria and fungi between the toes due to sweating of the foot in the socks.[7] Diabetic foot ulcers are often chronic, small, midpunctured wounds that occur on the plantar surface of deformed metatarsals and Charcot's joints.[8]

Diabetic foot as defined by the World Health Organization is, “The foot of a diabetic patient that has the potential risk of pathologic consequences, including infection, ulceration, and/or destruction of deep tissues associated with neurologic abnormalities, various degrees of peripheral vascular disease, and/or metabolic complications of diabetes in the lower limb”.[9]

Diabetic foot disease is one of the most severe diabetic consequences. It causes the patient significant pain and financial cost, as well as a significant strain on the patient's family, healthcare personnel and institutions, and society in general.[10]

Diabetic foot ulcers (DFU) affect one out of every four persons with diabetes.[11] The chance of developing a DFU, as well as the factors linked to consequences including hospitalization, lower-extremity amputation (LEA), and death, maybe patient, limb, or ulcer related. Individual variables will have different effects on DFU outcomes in different communities and countries.

In areas where antibiotics are few, infection, for example, will have a higher impact on outcomes, whereas ischemia will have a bigger impact in nations where peripheral artery disease is more common. It's worth noting that 80 percent of diabetics living in low- and middle-income economies,[12] where many diagnostic tools are unavailable.

Aims :

The National Institute for Clinical Excellence recommends that antibiotics should be prescribed if infection is present.[13]

The aim of this audit was to evaluate whether antibiotic usage was appropriate for the type of wound managed in the foot clinic. Our set standards of care were classified according to Wagner’s Classification and the University of Texas Wound Classification System. [14]

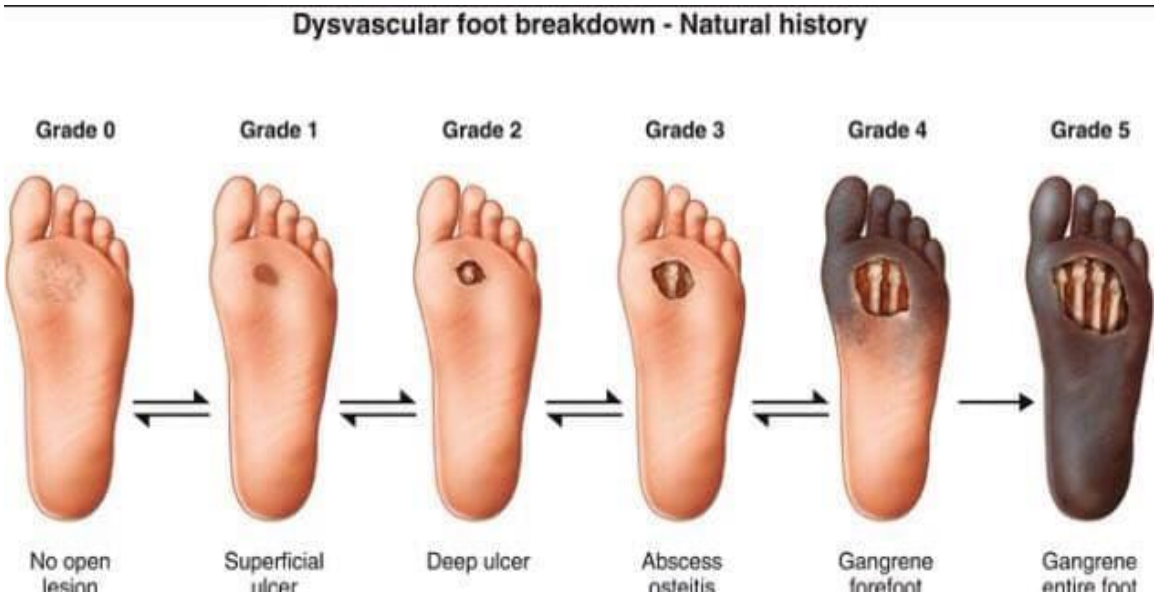
-All patients with wound infections should be on antibiotics.

-All patients with uninfected wounds should not be on antibiotics.

Methods:

A prospective study was conducted on 75 diabetic foot infection patients admitted to Al-Azhar university hospitals. Bacteriological specimens were collected and processed according to standard hospital microbiological culture and sensitivity testing procedures. Participants' demographics, such as age, sex, diabetes diagnosis, and complications, were recorded. All patients' clinical data, as well as details on the examination requested, microorganism identity, and antimicrobial therapy, were extracted from their files.[15] Diabetic foot ulcers were classified according to Wagner's Classification and the University of Texas Wound Classification System.[16]

Wagner's Classification of Diabetic Foot Ulcers[17]



Grade 0: no ulcer in a high-risk foot.
Grade 1: superficial ulcer involving the full skin thickness but not underlying tissues.
Grade 2: deep ulcer, penetrating down to ligaments and muscles, but no bone involvement or abscess formation.
Grade 3: deep ulcer with cellulitis or abscess formation, often with osteomyelitis.
Grade 4: localized gangrene.
Grade 5: extensive gangrene involving the whole foot.

University of Texas Wound Classification System of Diabetic Foot Ulcers[17]

The University of Texas Wound Classification System

	Grade 0	Grade I	Grade II	Grade III
Stage A	Preulcerative or postulcerative lesion completely epithelialized	Superficial wound, not involving tendon, capsule, or bone	Wound penetrating to tendon or capsule	Wound penetrating to bone or joint
Stage B	Infection	Infection	Infection	Infection
Stage C	Ischemia	Ischemia	Ischemia	Ischemia
Stage D	Infection and ischemia	Infection and ischemia	Infection and ischemia	Infection and ischemia

Depth is signified by grade number, hortzontally. Infection and ischemia are signified by stage letter, vertically.

Grade IA: noninfected, nonischemic superficial ulceration.
Grade IB: infected, nonischemic superficial ulceration.
Grade IC: ischemic, noninfected superficial ulceration.
Grade ID: ischemic, infected superficial ulceration.
Grade IIA: noninfected, nonischemic ulcer that penetrates to capsule or bone.
Grade IIB: infected, nonischemic ulcer that penetrates to capsule or bone.
Grade IIC: ischemic, noninfected ulcer that penetrates to capsule or bone
Grade IID: ischemic and infected ulcer that penetrates to capsule or bone.
Grade IIIA: noninfected, nonischemic ulcer that penetrates to bone or a deep abscess.
Grade IIIB: infected, nonischemic ulcer that penetrates to bone or a deep abscess.
Grade IIIC: ischemic, noninfected ulcer that penetrates to bone or a deep abscess.
Grade IIID: ischemic and infected ulcer that penetrates to bone or a deep abscess.
During the initial admission to the hospital, pus samples were collected (provided that no antibiotics were taken within the past 2 days). They were collected by swabbing directly at the base of the infected wound, and similarly, for those who required surgical intervention, pus swabs were taken intraoperatively at the deepest part of the wound. The samples were collected using sterile, commercially purchased swabs and immediately sent to the microbiology lab. For direct examination, all pus samples were Gram stained. They were grown on blood agar plates, MacConkey medium, and enriched broth culture tubes. The media were incubated at 371 overnight. The broth culture was further sub cultured onto the same above-mentioned solid media after overnight incubation, and the plates were incubated aerobically.

The API technique was used to identify the Gram-negative colonies (Biomerieux, Paris, France). Staphylococcal isolates were additionally tested for coagulase enzyme production to confirm the presence of S. aureus.
The slide latex agglutination test for fast identification of PBP2 proven MRSA (MRSA screen; Denka Seiken Co., Ltd, Tokyo, Japan).Isolated streptococci were further divided into groups based on their sera (A, B, C, D, and G).
Antibiotic sensitivity testing was performed on all isolates using the Kirby–Bauer disc diffusion method with commercially available antibiotic discs, and results were interpreted according to Clinical and Laboratory Standard recommendations. All patients were given the appropriate antibiotics based on the culture and sensitivity results, as well as metronidazole for anaerobic organisms.

Result:

Patient characteristics:

The current study included 75 patients, 37 of those were males and 38 of those were females, with a nearly equal male to female ratio. The individuals' ages ranged from 27 to 72, and the mean age was 48 years. The age group 51–60 years had the highest rate of diabetic foot infections, followed by the 41–50 year age group (Table 1).

Table 1 Demographics of patients

	Age group (years)						Total	
	21–30	31–40	41–50	51–60	61–70	Above 70	Frequency	%
Male	1	4	11	14	6	1	37	49
Female	2	8	8	14	4	2	38	51
Total	3	12	19	28	10	3	75	100

Table 2 Diabetic complications in 75 patients infected with diabetic foot ulcers

Diabetic complication	Value [*n* (%)]
Retinopathy	58 (77.3)
Cardiopathy	53 (70.6)
Nephropathy	48 (64)
Neuropathy	40 (53.3)
Gastropathy	20 (26.6)
Vasculopathy	55 (73.3)
Poor glycemic control[a]	42 (56)

[a]HbA1c ≥ 8.0%.

Diabetic complications were searched for by consulting different specialties (Table 2).

Wound characteristics:

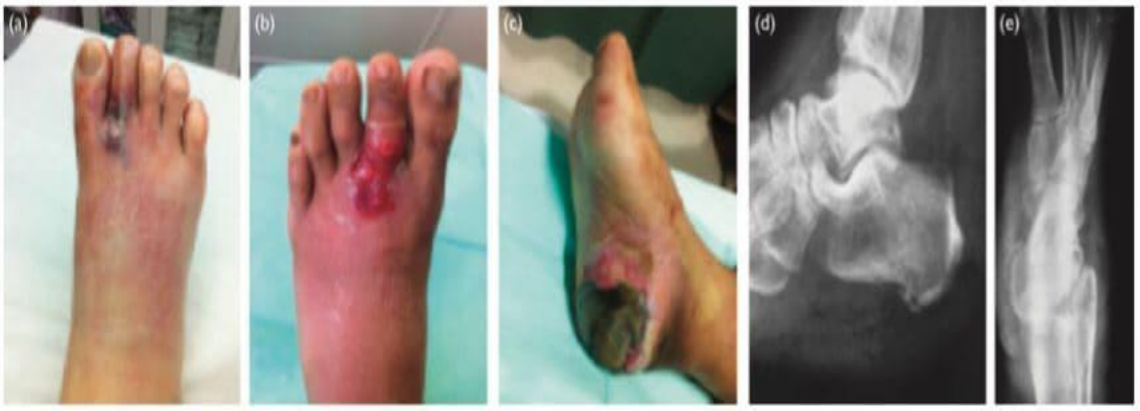
The degree and extension of diabetic foot wound were classified in all patients according to Wagner and the University of Texas Wound Classification Systems (Table 3).

Table 3 Distribution of patients (%) according to the Wagner and the University of Texas Wound Classification Systems

Classification system	Patients [*n* (%)]
Wagner Classification of Diabetic Foot Ulcers	
Grade 0	0 (0)
Grade 1	10 (13.3)
Grade 2	20 (26.7)
Grade 3	18 (24)
Grade 4	16 (21.3)
Grade 5	11 (14.7)
University of Texas Wound Classification System of Diabetic Foot Ulcers	
Stage A	
Grade I	2 (2.6)
Grade II	6 (8)
Grade III	6 (8)
Stage B	
Grade I	3 (4)
Grade II	7 (9.3)
Grade III	6 (8)
Stage C	
Grade I	4 (5.3)
Grade II	6 (8)
Grade III	8 (10.7)
Stage D	
Grade I	5 (6.7)
Grade II	9 (12)
Grade III	13 (17.4)

Overall, 52% (n=39) of the cultures showed the presence of a single organism, 40% (n=30) of the cultures exhibited mixed infections, and 8% (n=6) of the cultures showed no growth. In terms of clinical severity, 54% (n=40) of the infections involved the subcutaneous level, 29 % (n=22) of the infections involved superficial ulcers, 9 %(n=7) of the infections involved deep ulcers, and 8 % (n=6) of the infections involved osteomyelitis (Fig. 2).The data in Table 4 show the profile of the pathogens isolated. A total of 98 pathogens were discovered, with 1.31 organisms per patient on average. Gram-negative bacteria (n=66, 67.3 %) were found to be more commonly isolated than Gram-positive bacteria (n=29, 29.6 %) among aerobic microbes.

Figure 2



Progression of diabetic foot infections from superficial to subcutaneous infections, osteomyelitis and eventually amputation: (a) infection of the second toe with cellulites, (b) progression to deeper cutaneous infection with exudation of blood-stained serious fluid, (c) deep subcutaneous infection with necrotic slough of the heel and (d) radiographs showing osteomyelitis changes with subcutaneous gas collection (lateral view), and (e) superior inferior view.

Table 4 Types and profiles of organisms isolated in patients with diabetic ulcers

Organisms	Frequency (%)	Organisms	Frequency (%)
Gram-positive aerobes	29 (29.6)	Gram-negative aerobes	66 (67.3)
Staphylococcus aureus	10 (10.2)	*Pseudomonas aeruginosa*	19 (19.4)
Streptococcus pyogenes	7 (7.1)	*Klebsiella pneumoniae*	15 (15.3)
MRSA	7 (7.1)	*Acinetobacter* spp.	10 (10.2)
Group D streptococcus	4 (4.1)	*Proteus mirabilis*	6 (6.1)
Streptococcus pneumoniae	1 (1.0)	*Escherichia coli*	4 (4.1)
Fungus		*Enterobacter cloacae*	3 (3.1)
Candida albicans	1 (1.0)	*Chryseomonas luteola*	3 (3.1)
		Proteus vulgaris	2 (2.0)
		Citrobacter spp.	2 (2.0)
		Alcaligenes faecalis	1 (1.0)
		Pseudomonas cepacia	1 (1.0)

MRSA, methicillin-resistant *Staphylococcus aureus*.

Table 5 Combinations of organisms isolated from patients with mixed infections

Combination of organisms	Frequency (%)
Gram-positive and gram-positive	2 (7)
Gram-positive and gram-negative	12 (40)
Gram-negative and gram-negative	16 (53)
Total	30 (100)

The data in Table 5 show the combination of organisms in mixed infections. The results of the sensitivity patterns of the five commonly detected Gram-positive and Gram-negative pathogens are shown in Tables 6 and 7.

Table 6 Sensitivity patterns of all isolated Gram-positive organisms

Antibiotics	*Staphylococcus aureus* (*n*=10)	*Streptococcus pyogenes* (*n*=7)	MRSA (*n*=7)	Group D streptococcus (*n*=4)	*Streptococcus pneumonia* (*n*=1)
Vancomycin	100	100	100	100	100
Linzolid	100	–	100	–	–
Penicillin	10	100	0	100	100
Oxacillin	100	100	0	50	100
Erythromycin	70	60	0	50	100
Fusidic acid	80	80	0	50	–
Amoxy/clavulanic acid	100	99	0	100	100
Ampicillin/ Sulbactam	90	100	0	100	100
Gentamicin	90	90	0	100	100
Netilmicin	90	80	0	100	100
Amikacin	100	100	0	100	100
Cephalexin	100	95	0	25	-
Cefuroxime	100	99	0	50	100
Ceftriaxone	90	97	0	100	100
Chloramphenicol	90	100	40	50	100
Imipenem	100	100	20	100	100
Meropenem	80	100	20	100	100
Tetracycline	70	43	10	25	0
Levofloxacin	90	80	90	50	100
Sulfa/trimethoprim	99	70	80	0	100

MRSA, methicillin-resistant *Staphylococcus aureus*.

Table 7 Sensitivity patterns of all isolated Gram-negative organisms

Antibiotics	*Klebsiella* spp. (*n*=19)	*Pseudomonas* aeruginosa (*n*=15)	*Acinetobacter* (*n*=10)	*Proteus* (*n*=8)	*Escherichia coli* (*n*=4)	*Enterobacter* (*n*=3)	*Chryseomonas* spp. (*n*=3)
Ampicillin	100	10	5	65	75	5	5
Ampicillin/ sulbactam	90	30	40	100	75	45	10
Amoxy/ clavulanic acid	100	20	15	100	100	55	20
Cephalexin	90	10	50	90	100	25	10
Cefuroxime	90	20	40	100	100	56	10
Ceftazidime	100	95	50	100	100	83	50
Chloramphenicol	80	30	50	90	100	85	10
Gentamicin	95	100	70	90	100	90	90
Netilmicin	100	99	70	95	100	91	90
Amikacin	100	100	70	97	100	92	95
Tetracycline	50	30	30	50	100	60	5
Imipenem	100	100	55	95	100	97	98
Meropenem	100	100	55	89	100	98	98
Levofloxacin	100	90	50	85	100	90	95
Piperacillin	100	90	20	87	–	70	–
Sulfa/ trimethoprim	80	5	45	93	60	80	50
Polymyxin	–	98	100	–	–	–	–

Discussion:

According to this study, polymicrobial infections were found in 40% of diabetic foot infections. Gram-negative pathogens were isolated in greater numbers than Gram-positive bacteria, with a ratio of almost 2 to 1, and they were mostly responsive to vancomycin and amikacin, respectively.

Our data show that a smaller percentage of patients (40%) were infected by two or more infections, compared to 52 % of patients with a single pathogen.Raja[18]reported that 42% of patients developed mixed growth. Similarly, Renina et al.[19] reported that 58.9% of cases were polymicrobial in nature. According to other research from Jamaica and France, the frequency of polymicrobial infection could range from 80–87.2 %[20],[21]. Clinically mild

and superficial subcutaneous infections could be one reason for the low incidence of polymicrobial infection in our study.

Table 8 Sensitivity patterns of the all the isolated Gram-negative microorganisms

References	Gram-negative organisms (%)	Gram-positive organisms (%)
Raja [13]	*Proteus* spp. (28) *Pseudomonas aeruginosa* (25) *Klebsiella pneumoniae* (15) *Escherichia* coli (9)	*Staphylococcus aureus* (44) Group B streptococcus (25) *Enterococcus* spp. (9)
Lea *et al.* [18]	*Proteus* spp. (24) *Enterobacter* spp. (21) *Citrobacter* spp. (9) *E. coli* (12)	*S. aureus* (29) *Staphylococcus epidermidis* (3)
Bansal *et al.* [17]	*P. aeruginosa* (22) *K. pneumoniae* (17) *E. coli* (18) *Proteus* spp. (11)	*S. aureus* (19)

Overall, Gram-negative microbes were the most commonly isolated pathogens, which has been confirmed in Indian studies by Bansal et al.[22], by Shankar et al.[23], and by Gadepalli et al.[24] (76 vs. 24%, 57.6 vs. 42.3%, and 51.4 vs. 33.3%, respectively). Raja[18] and Renina et al.[19]also documented more Gram-negative bacteria than Gram-positive bacteria (52 vs. 45% and 67 vs. 33%, respectively). As a result, it's essential choose the antibiotics that are more active against Gram-negative bacteria rather than Gram-positive bacteria, which clinicians are more likely to prescribe when a deep tissue infection or infected gangrene is observed. The majority of causative Gram-negative microbes were P. aeruginosa (19.4 %), Klebsiella pneumoniae (15.3%), and Acinetobacter spp. (10.2%). S. aureus (10.2 %), S. pyogenes (7.1 %), and MRSA (7.1 %) were the most commonly isolated Gram-positive bacteria. These pathogens were believed to have colonized the superficial foot ulcers. These results are comparable with those of Raja[18], of Renina et al.[19], and of Bansal et al.[22] (Table 8).

Vancomycin and amikacin appeared to be the best antibiotics for therapy against Gram-positive and Gram-negative bacteria, respectively, based on susceptibility patterns. Vancomycin is normally only used to treat MRSA, whereas amikacin has been linked to nephrotoxicity, which can worsen diabetic nephropathy in those who already have it.

Based on the results shown in Tables 6 and 7, we could also assume that monotherapy may not be the best management for causal microbes As a result, there are a variety of factors to consider when prescribing empiric antibiotic therapy for diabetic foot infections:

(a) the severity of infection,
(b) the depth and extent of involvement of infection, and
(c) the antibiogram and local pattern of bacterial etiology

The severity of infection was proportional to the depth of infection in the current study, and the majority of infections were classified as superficial or subcutaneous. Mild infection is usually caused by a single bacterium, and the most common causal organism is S. aureus23, which is completely resistant to flucloxacillin (oxacillin) and amoxy/clavulanic acid (Table 6).

If the infection spreads to deeper tissues, it may be polymicrobial in nature, with Gram-negative germs in various combinations being more likely. As a result, amoxy/clavulanic acid, ampicillin/sulbactam, and cefuroxime can be used to treat the infection. Ceftazidime, imipenem, meropenem, and levofloxacin are more appropriate if the infection is severe and affects deep tissue and bone, with sensitivities of 98–100%.

There are various limitations in this study that need to be taken into account when interpreting its results. First, the sample size was notably small ,with only 75 patients (as the capacity of the surgical department beds is limited), which may limit the power of the study. Second, the procedure for collecting specimens was based on current practice and may not be standardized. All of the specimens evaluated here were collected from pus swabs. However, there are reports that have shown that sampling of bone and soft-tissues is more sensitive compared with sampling from pus swabs alone[25],[26]. Another limitation is the study's prospective nature, which is always a big problem in regular patient follow-up. However, given that the follow-up data are not regular, this study still provides important information and serves as a basis for future studies.

Several research have looked into the link between the method of collecting specimens and the number and types of organisms recovered from infected wounds. Tissue specimens, according to some research, are more sensitive and specific than swab cultures, including less visible impurities and more pathogens[27],[28]. In contrast, others studies have reported that with adequate preliminary debridement, the use of a wound swab is as reliable as the use of a tissue specimen[28],[29]. In our study, swab specimens were collected only after thorough cleaning with sterile normal saline, after debridement of the wound and before application of an antiseptic agent. Only culture material from deeper tissues was sent for microbiological analysis. Sample collection

protocols, on the other hand, must be carefully established and monitored, as skin contaminants may alter microbial profiles, possibly leading to misunderstanding of culture reports and affecting clinical decisions.

It's difficult to make a decision about how to treat a diabetic foot infection, and it's still subject to debate. Although optimal therapy is yet to be established, most authors agree that the management of these infections requires isolation and identification of the microbial flora; appropriate antibiotic therapy, according to the sensitivity patterns; precise selection and identification of the chronic complications and proper surgical intervention for these complications. Most diabetic foot infections are polymicrobial in nature, and mixed organisms are frequently encountered [30]. The range of microorganisms, on the other hand, is mostly determined by the microbial flora of the lower limb, metabolic factors, foot care, and antibiotic use[31].

Emergence of resistance among organisms against the commonly used antibiotics has been clearly outlined in various studies as being largely due to their indiscriminate use [32]. The total amount of a particular antibiotic used in a certain hospital over a given period of time has a direct association with the number of resistant strains that develop[33].

Conclusion:

According to our findings ,that 40% of diabetic foot infections were polymicrobial. P. aeruginosa and S. aureus were the most commonly identified Gram-negative and Gram-positive microorganisms, respectively. Amikacin and vancomycin were the most effective antimicrobial therapy against Gram-negative and Gram-positive microorganisms, respectively. Levofloxacin and imipenem are also very effective in empiric treatment but are very expensive. Due to these medicines' limited suitability, empiric antibiotic therapy should be based on the clinical features of the infections as well as the local pattern of bacterial etiology and antibiogram.

Reference

1. Anandi, C. *et al.* Bacteriology of diabetic foot lesions. *Indian J. Med. Microbiol.* **22**, 175–178 (2004).

2. Atlas, I. D. F. D. Brussels, Belgium: international diabetes federation; 2013. *Int. Diabetes Fed.* 147 (2017).

3. Prompers, L. *et al.* Optimal organization of health care in diabetic foot disease: introduction to the Eurodiale study. *Int. J. Low. Extrem. Wounds* **6**, 11–17 (2007).

4. Pickup, J. C. & Williams, G. Epidemiology of diabetes mellitus. *Textb. Diabetes* **1**, 1–3 (1997).

5. Lipsky, B. A. *et al.* Diagnosis and treatment of diabetic foot infections. *Clin. Infect. Dis.* 885–910 (2004).

6. Ramani, A., Ramani, R., Shivananda, P. G. & Kundaje, G. N. Bacteriology of diabetic foot ulcers. *Indian J. Pathol. Microbiol.* **34**, 81–87 (1991).

7. Zhang, P. *et al.* Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis (†). *Ann. Med.* **49**, 106–116 (2017).

8. Jeffcoate, W. J. & Lipsky, B. A. Controversies in diagnosing and managing osteomyelitis of the foot in diabetes. *Clin. Infect. Dis. an Off. Publ. Infect. Dis. Soc. Am.* **39 Suppl 2**, S115-22 (2004).

9. Pinzur, M. S. The diabetic foot. *Compr. Ther.* **28**, 232–237 (2002).

10. Jakosz, N. Book review – IWGDF Guidelines on the Prevention and Management of Diabetic Foot Disease. *Wound Pract. Res.* **27**, 144 (2019).

11. Armstrong, D. G., Boulton, A. J. M. & Bus, S. A. Diabetic foot ulcers and their recurrence. *N. Engl. J. Med.* **376**, 2367–2375 (2017).

12. Mahfouz, N., Shakweer, T. T. & Abd-Elaziz, M. Effect of Diabetic Foot Program on High Risk Patient's Health Status.

13. Home, P., Mant, J., Diaz, J. & Turner, C. Management of type 2 diabetes: summary of updated NICE guidance. *Bmj* **336**, 1306–1308

(2008).

14. Lavery, L. A., Armstrong, D. G. & Harkless, L. B. Classification of diabetic foot wounds. *J. Foot Ankle Surg.* **35**, 528–531 (1996).

15. Al-Hamead Hefni, A. *et al.* Bacteriological study of diabetic foot infection in Egypt. *Arab Soc. Med. Res.* **8**, 1687–4293 (2013).

16. Katsilambros, N., Dounis, E., Makrilakis, K., Tentolouris, N. & Tsapogas, P. *Atlas of the diabetic foot*. (John Wiley & Sons, 2010).

17. Monteiro-Soares, M. *et al.* Guidelines on the classification of diabetic foot ulcers (IWGDF 2019). *Diabetes. Metab. Res. Rev.* **36**, 1–8 (2020).

18. Raja, N. S. Microbiology of diabetic foot infections in a teaching hospital in Malaysia: a retrospective study of 194 cases. *J. Microbiol. Immunol. Infect.* **40**, 39 (2007).

19. Renina, L., Llanes, I., Pena, A. C. & Cauton-Valera, R. Clinical microbiological profile and outcome of diabetic patients with foot ulcers admitted at the Quirino Memorial Medical Center. *Phil J Microbiol Infect Dis* **30**, 101–107 (2001).

20. Wright-Pascoe, R., Roye-Green, K. & Bodonaik, N. The medical management of diabetes mellitus with particular reference to the lower extremity: the Jamaican experience. *West Indian Med J* **50**, 46–49 (2001).

21. Loan, C. A. *et al.* Severe Streptococcus agalactiae infection of the diabetic foot. A deleterious role of Streptococcus agalactiae? *Press. medicale (Paris, Fr. 1983)* **34**, 491–494 (2005).

22. Bansal, E., Garg, A., Bhatia, S., Attri, A. K. & Chander, J. Spectrum of microbial flora in diabetic foot ulcers. *Indian J. Pathol. Microbiol.* **51**, 204 (2008).

23. Shankar, E. M., Mohan, V., Premalatha, G., Srinivasan, R. S. & Usha, A. R. Bacterial etiology of diabetic foot infections in South India. *Eur. J. Intern. Med.* **16**, 567–570 (2005).

24. Gadepalli, R. *et al.* A clinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. *Diabetes Care* **29**, 1727–1732 (2006).

25. Ng, L. S. Y., Kwang, L. L., Yeow, S. C. S. & Tan, T. Y. Anaerobic culture of diabetic foot infections: organisms and antimicrobial susceptibilities. *Ann. Acad. Med. Singapore* **37**, 936 (2008).

26. Eckhard, M., Lengler, A., Liersch, J., Bretzel, R. G. & Mayser, P. Fungal foot infections in patients with diabetes mellitus–results of two independent investigations. *Mycoses* **50**, 14–19 (2007).

27. Senneville, E. *et al.* Culture of percutaneous bone biopsy specimens for diagnosis of diabetic foot osteomyelitis: concordance with ulcer swab cultures. *Clin. Infect. Dis.* **42**, 57–62 (2006).

28. Pellizzer, G. *et al.* Deep tissue biopsy vs. superficial swab culture monitoring in the microbiological assessment of limb-threatening diabetic foot infection. *Diabet. Med.* **18**, 822–827 (2001).

29. Slater, R. A. *et al.* Swab cultures accurately identify bacterial pathogens in diabetic foot wounds not involving bone. *Diabet. Med.* **21**, 705–709 (2004).

30. Viswanathan, V., Jasmine, J. J., Snehalatha, C. & Ramachandran, A. Prevalence of pathogens in diabetic foot infection in South Indian type 2 diabetic patients. *J. Assoc. Physicians India* **50**, 1013–1016 (2002).

31. Lavigne, J.-P., Richard, J.-L. & Sotto, A. Nouvelles avancées dans les infections des plaies du pied chez le patient diabétique. *Rev. Francoph. des Lab.* **2011**, 57–64 (2011).

32. Bozkurt, F., Gülsün, S., Tekin, R., Hoşoğlu, S. & Acemoğlu, H. Comparison of microbiological results of deep tissue biopsy and superficial swab in diabetic foot infections. *J. Microbiol. Infect. Dis.* **1**, 122–127 (2011).

33. Shakil, S. & Khan, A. U. Infected foot ulcers in male and female diabetic patients: a clinico-bioinformative study. *Ann. Clin. Microbiol. Antimicrob.* **9**, 1–10 (2010).