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A Prospective Observational Study of Microbiological Analysis and Antibiotic Sensitivity Patterns in Diabetic Foot Ulcer Patients

[1]Ashok Reddy Rapaka, [1]Srividya Veeramalla, [1]Mounika Gollamandala,
[2]Ravindhar Nayak Maloth, [2]Samuel Prabhumithra, [1]Nagesh Adla,
[1]Amulya Gade, [1]Sharvanabhava Bandaru and [1]Goverdhan Puchchakayala

[1]Department of Clinical Pharmacy, Vaagdevi College of Pharmacy,
Ramnagar, Hanamkonda, Warangal – 506002, Telangana, India
[2]Department of Surgery, Kakatiya Medical College,
MGM Hospital, Warangal – 506002, Telangana, India

Abstract: Foot infections are one of the foremost causes of mortality and morbidity in developing countries. Diabetes is the principal cause of nontraumatic lower extremity amputations and accounts for more than 85% of amputations. The determination of antimicrobial susceptibility of a clinical isolate is often essential for the most advantageous antimicrobial therapy of infection. Infection with multidrug resistant organisms (MDR) may enhance the length of hospital stay, cost of management and may cause further morbidity and mortality. Aims of this study were to determine the bacterial spectrum in diabetic foot ulcers and institute the appropriate antibiotic therapy to avoid further complications. Deep wound swab were cultured from 37 patients, who were receiving the treatment for the diabetic foot infections. Specimens were tested by Gram stain, culture & antibiotic sensitivity. Results showed that Gram negative aerobes were the most frequently isolated bacteria constituting 26 isolates (87%), followed by Gram positive aerobes 4 (13%) cases. Commonest pathogens isolated were *Klebsiella species* 12 (40%), followed by *Proteus species* 7 (23%), *E. coli* 5 (17%), *Citrobacter* 2 (7%), *S.aureus* 1(3%), *Coag. negative staph*. was found in 3 (10%) cases. Commonest antibiotics effective against Gram negative isolates were amikacin 69% followed by meropenem 62%, cefipime 35% and imipenem 23%. Gram positive organisms were sensitive to amikacin (100%) and vancomycin (100%). It can be concluded that Gram negative isolates were leading infectious agents from specimens. Gram negative bacteria were more sensitive to Amikacin followed by Cefipime, Meropenem, Imipenem and Ciprofloxacin and more resistant to Piperacillin/Tazobactam, Ofloxacin, Cefuroxime and Tetracycline. Our study also helps the physician for selection of the proper antibiotics to improve the wound healing and also leads a pathway for the formation of the institutional antibiotic committee to avoid the irrational antibiotic usage.

Key words: Diabetic Foot Infections · MDR · *E. coli* · Klebsiella · Amputations

INTRODUCTION

Diabetes is one of the endocrine disorders and is considered as a disease of developed countries [1]. Diabetes mellitus is a metabolic disorder, which resulted from insulin deficiency and is leading to significant morbidity and mortality [1]. A chronic hyperglycemic condition in diabetes precipitates long term damage, dysfunction and failure of various organs, such as eyes, kidneys, nerves, heartand blood vessels [2]. The remarkable increase in the prevalence of diabetes can be attributed to several factors [3]. World wide Diabetes has showed the spread of modern lifestyle and can be associated to an increasingly overweight and sedentary population [3]. Diabetes is a fairly common disease seen in India with a prevalence of almost 12-17% in the Indian urban population as per a study in 2001 with a prevalence of 2.5% in the rural population [4]. Diabetes hampers the life of nearly 50.8 million people in India and of equivalent enormity in other developing countries [4]. According to the Diabetes Atlas 2013 accessible by the International Diabetes Federation, the number of people with diabetes

Corresponding Author: P. Goverdhan, Department of Clinical Pharmacy Vaagdevi college of Pharmacy, Kakatiya University, MGM Hospital, Warangal – 506002 (T.S.) India.

in India currently is 65.1 million, which is anticipated to increase to 142.7 million by 2035 unless imperative defensive steps are taken [5]. About 1 million people died from diabetes in India in 2012 [6]. Diabetic foot ulcer is an infection, ulcer or destruction of deep tissue associated with neurological abnormalities, musculoskeletal deformities and various degrees of peripheral vascular disease of lower limb [7]. Foot infections are one of the foremost causes of mortality and morbidity in developing countries [8]. Diabetes is the principal cause of non traumatic lower extremity amputations and accounts for more than 85% of amputations [9]. Life time risk of a diabetic foot ulcers ranges from 15-25% diabetic population [9]. In general, infections in diabetic people are more severe and take longer to cure than equivalent infections in nondiabetics. The majority of the diabetic foot infections are mixed bacterial infections [10]. Theinfection leads to the early advancement of complication even after a miner trauma, the disease progresses and becomes noncompliant to antibacterial therapy [11]. Early detection of microbial infections and accurate antibacterial therapy leads to avoid further complications [12]. Preliminary management comprises empirical antimicrobial therapy, which based on susceptibility data [13]. The determination of antimicrobial susceptibility of a clinical isolate is often essential for the most advantageous antimicrobial therapy of infection. Infection with multidrug resistant organisms (MDR organism) may enhance the length of hospital stay, cost of management and may cause further morbidity and mortality [14].

This study was conducted to appraise the bacteriological profile of diabetic foot ulcer patients and antibiotic sensitivity and resistance patterns for proper treatment.

MATERIALS AND METHODS

This is a prospective observational study which was conducted at Department of surgery, Mahatma Gandhi Memorial Hospital, Warangal, between April 2015 to October 2015. This study was conducted on 41 patients with diabetic foot ulcer suspected of being infected who are thought by their clinicians to require antibiotic therapy. The study was approved by Hospital /Institutional Human Ethical Committee (IHEC) and informed consent was taken from all the patients. A Proforma was prepared according to the protocol designed for the study, which contains detailed information on each patient.

All the applicable data pertaining to demographic characteristics, duration of diabetes and past history of diabetic foot infections was taken from the patients who are admitted in the hospital as well as out patient department. We also recorded history of trauma, type and site of ulcer on the basis of Wagner's classification and whether any surgical intervention was done.

Pus or discharge were collected from the patient's wound and were treated in local hospital microbiological laboratory. Pathogens isolated along with its sensitivity pattern, antibiotic used and its response depending on complete resolution of symptoms of infection was done. Samples were collected before the empirical treatment. Wound debridement /disarticulation of toe or amputations were performed based on the extent and severity of the lesions.

Inclusion Criteria:

- The patient has a diagnosis of diabetes (Type 1 and type 2)
- Patients with recent and recurrent infected diabetic foot were included

Exclusion Criteria:

- Patients with non diabetic foot ulcer(Any skin disease,allergy,trauma)
- Patients with above knee ulcer were excluded.

RESULTS

Forty one patients admitted to surgery ward were analyzed of which 31 were males and 10 were female. The average age of patients was 57.25±11.24 years. Among the total patient population, 30 (73%) had type-2 diabetes mellitus and 11 (27%) patients had type-1 diabetes mellitus. The duration of diabetes mellitus for below 5 years was observed in 25 patients (61%) and 5-10 years were observed in 9 patients (22%) and above 10 years was observed in 6 patients (15%). The average duration of diabetes was 7.76±1.04 years. The majority of patients (32%) having the foot ulcer were in the age of 46-65years. Among the 41patients, 27patients (66%) from rural area were more predominant for developing the foot ulcer when compared with the patients 14 (34%) of urban area. The mean duration of ulcer is 2.6±0.36 months. Mostly worsening infection was seen in the patients with the ulcer duration above one month. Average of hospital stay was 20 days. And one patient was died of poor glycemic control (Hypoglycemia). Out of 41 patients, 14 patients had recurrent ulcers, 3 members followed disarticulation, 3 members under gone amputations.

Fourteen (34%) patients had hypertension, three (7%) patients had coronary artery disease, one patient had asthma, four (10%) patients had nephropathy, five (12%) patients had anemia, 2 (5%) patients had jaundice and one (3%) patient had tuberculosis apart from diabetes (Table 1).

Table. 1: Demographic variables among diabetic patients with recurrent ulcer and Single exposure to ulcer

Variables	Total No. (%)	Diabetic patients with recurrent ulcer (13)	Diabetic patients with single exposure (24)	Overall Mean±sd	P-value
Age intervals				56.19±11.46	0.13
35-45	9(22%)	2	7		
46-55	13(32%)	4	9		
56-65	13(32%)	7	6		
66-75	6(14%)	2	4		
Sex				NA	0.51
Male	31(76%)	12	19		
Female	10(24%)	3	7		
Residence				NA	0.23
Rural	27(66%)	10	17		
Urban	14(34%)	4	10		
Habits				NA	0.0194
Alcoholic	31(76%)	12	19		
Smokers	21(51%)	9	12		
Diabetes mellitus (years)				7.76±1.04	0.39
□5years	25(61%)	9	16		
5-10 years	9(22%)	3	6		
□10 years	6(14%)	2	4		
Duration of ulcer				2.608±0.36	0.68
<2 months	35(85%)	11	24		
>2 months	6(15%)	3	3		
Size of ulcer				NA	0.66
<5 cm^2	32(78%)	8	24		
>5 cm^2	9(22%)	6	3		
NA: not applicable					
Occupation				NA	0.86
Employee	3	-	3		
Farmer	18	11	7		
Housewife	9	-	9		
Daily labor	11	3	8		
History of ulcer				NA	0.22
Trauma	16	5	11		
Spontaneous	25	9	16		
Bare foot walking	37	14	23		
Type of comorbidity				NA	0.53
Hypertension	14(34%)	5	9		
CAD	3(7%)	1	2		
Osteomyelitis	3(7%)	2	1		
Asthma	1(2%)	-	1		
Nephropathy	4(11%)	1	3		
Anemia	5(14%)	1	4		
Jaundice	2(5%)	-	2		
Tuberculosis	1(3%)	-	1		
Cholelithiasis	1(3%)	-	1		
Treatment				NA	0.6
Consevative	31(77%)	6	25		
Amputations	9(22%)	8	1		
Hospital stay				20.57±5.01	0.3
<20 days	20(49%)	5	15		
20-40 days	18(44%)	6	12		
>40 days	3(7%)	2	1		
Status				NA	0.6
Discharge	29(71%)	11	18		
Dead	1(3%)	1	-		

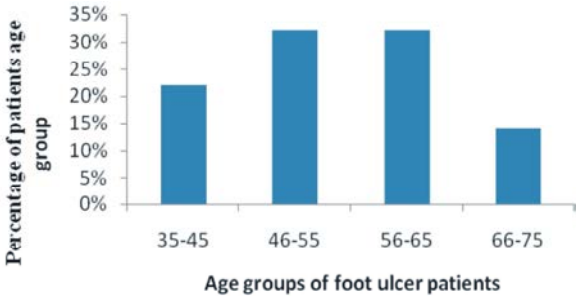

Fig. 1: Showing prevalence of diabetic foot ulcer among the various age groups

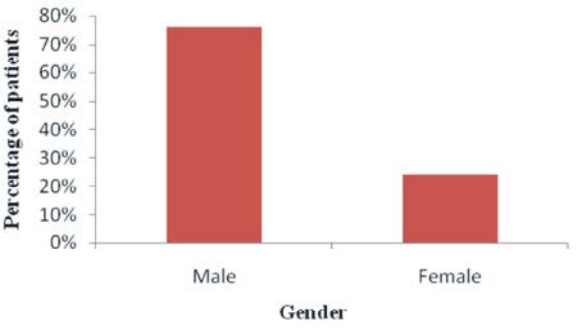

Fig. 2: Showing the Foot Ulcer Distribution in Male and Female Patients

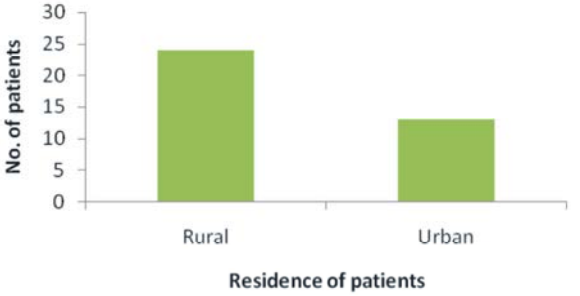

Fig. 3: Showing residence ofpatients with diabetic foot ulcer

Ten (24%) patients had ulcer on forefoot, 20 (49%) with midfoot ulcers (Table 2). 16 patients had history of trauma. Fourpatients had gangrene, 18 patients had cellulitis and 19 patients had diabetic foot according to Wagner's classification (Table 3).

Table 2: Diabetic Lesion Characteristics

S.No.	Characteristics	No	%
1.	Diabetic foot – Right	19	46%
	Left	22	54%
2.	Sole	11	27%
	Toes	10	24%
	Dorsum	12	29%
	Leg	8	20%

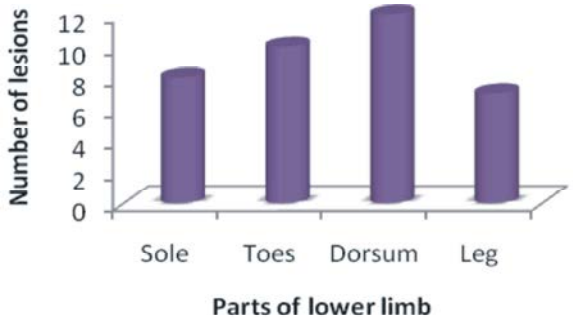

Fig. 4: Showing the Occurrence of foot ulcer on patients lower limb

Table 3: Bacterial load per culture specimen

Ulcer Type (Wagner classification)	No. of patients	Positive culture for bacteria	
		Single	Two
Gangrene	4	4	-
Diabetic foot			
Ischemic	10	10	-
Neuropathic	6	6	-
Neuro-ischemic	3	3	-
Cellulites	18	16	2
Total	41	39	2

In our study a total of 41 specimens were cultured and isolated 34 organisms, 9 specimens were culture sterile, 30 isolates were showed single organism growth, remaining were infected with polymicrobial growth. In our study 30 (88%) isolates were Gram negative in nature with predominant organisms being *Klebsiella* (12, 35%), *Proteus* (7, 21%), *E.coli* (5, 15%), *Pseudomonas* (4, 12%) and *Citrobacter* (2, 6%). Gram positive organisms were isolated in 4 cases from this *coagulase negative staph*. were 3 (9%) and *S.aureus* was 1 (3%) (Table 4).

Table 4: Type and percentage of Bacterial isolates

Bacteria category	Percentage
No. of isolates	34
Gram negative	30(88%)
Klebsiella species	12(35%)
E. coli	5(15%)
Proteus species	7(21%)
Pseudomonas species	4(12%)
Citrobacter species	2(6%)
Gram positive	4(12%)
Coag.neg.staph.	3(9%)
S.aureus	1(3%)

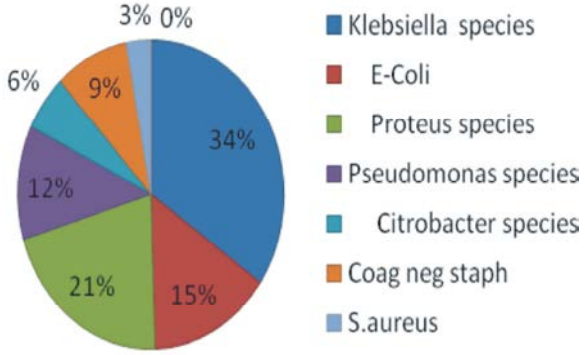

Fig. 5: Showing percentage of bacterial isolates from culture specimens

Antibiotic susceptibility is shown in Tables 5& 6. Gram negative isolates like *Klebsiella* species were susceptible to Amikacin (75%), Cefipime (67%), Meropenem (58%), Ciprofloxacin (42%), Imipenem (33%), *Proteus* species were susceptible to Meropenem (71%), Amikacin (43%), Piperacillin/tazobactam (29%). *E.coli* species were susceptible to Clotrimaxazole (100%), Gentamycin (100%), Amikacin (80%) and Meropenem (80%). *Pseudomonas* species were sensitive to Amikacin (50%), Meropenem (50%) and Imipenem (50%). *Citrobacter* were susceptible to Amikacin (100%) and Cefipime (50%). Whereas Gram positive bacteria were susceptible to Amikacin (100%), Vancomycin (100%) and Teicoplanin (100%).

Table 5: Percentage of antibiotic susceptibility in Gram negative aerobes

Antibiotics	*Klebsiella* (%) N=12	*E-Coli* (%) N=5	*Proteus* (%) N=7	*Pseudomonas* (%) N=4	*Citrobacter* (%) N=2
Amikacin	9 (75%)	4(80%)	3(43%)	2(50%)	2(100)
Ofloxacin	3(25%)	-	-	-	-
Meropenem	7(58%)	4(80%)	5(71%)	2(50%)	-
Ciprofloxacin	5(42%)	-	-	-	-
Cefipime	8(67%)	-	-	-	1(50%)
Imipenem	4(33%)	2(40%)	-	2(50%)	-
Clotrimaxazole	-	5(100%)	-	-	-
Gentamycin	-	-	-	-	5(100%)
Piperacillin/Tazobactam	1(8%)	-	2(29%)	-	-

Table 6: Percentage of antibiotic susceptibility in Gram positive aerobes

Antibiotic	*S.aureus*	*Coag. Neg. Staph.*
Teicoplanin	1(100%)	3(100%)
Vancomycin	1(100%)	3(100%)
Amikacin	1(100%)	3(100%)

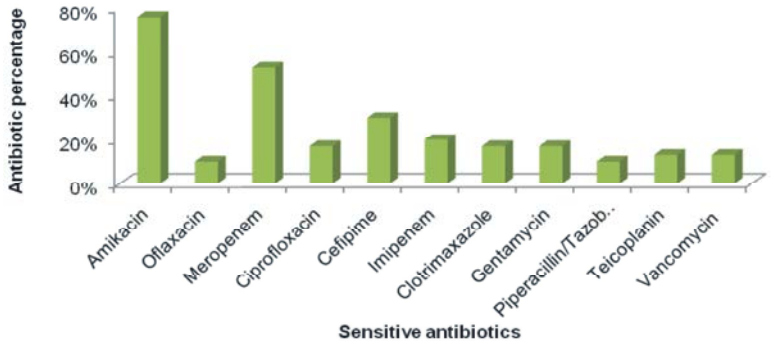

Fig. 6: Showing Percentage of antibiotic sensitivity in both Gram Positive and Gram negative bacterial isolates.

Antibiotic resistance is shown in Tables 7&8. *Klebsiella* were resistant to Ofloxacin (88%), Pipracillin/Tazobactam (50%). *Proteus* were resistant to Ofloxacin (71%), Piperacillin/Tazobactam (43%). *E.coli* was resistant to Piperacillin/Tazobactum (100%), Cefuroxime (100%), Tobramycin (80%), Tetracycline (80%), Cefipime (60%), Augmentin (60%), Ampicillin/Sulbactam (40%) and Amoxicillin (40%). *Pseudomonas* species showed equal sensitivity and resistance towards Amikacin. *Pseudomonas* was resistant by 100, 75 and 75% to Ofloxacin, Augmentin and Piperacillin/Tazobactam respectively. *Citrobacter* were resistant to Meropenem (100%), Clarithromycin (100%) and Ciprofloxacin (50%). Gram positive bacteria were resistant to Ampicillin/Sulbactam (100%), Cefipime (100%), Clarithromycin (100%) and Meropenem (100%).

Table 7: Antibiotic Resistance in Gram negative bacteria

Antibiotics	*Klebseilla* N-12(%)	*Proteus* N-7(%)	*E Coli* N-5(%)	*Pseudomonas* N-4(%)	*Citrobactar* N-2(%)
Amikacin	2(17%)	4(57%)	2(40%)	2(50%)	-
Ampicillin/sulbactam	1(8%)	-	2(40%)	-	-
Augmentin	-	-	3(60%)	3(75%)	-
Amoxicillin	1(8%)		3(60%)	-	-
Cefipime	2(17%)		3(60%)	-	
Cefuroxime		1(14%)	5(100%)	-	
Tetracycline	2(17%)		4(80%)	-	
Ciprofloxacin				-	1(50%)
Clarithromycin	2(17%)	1(14%)		2(50%)	1(50%)
Meropenem	1(14%)			-	2(100%)
Oflaxacin	7(58%)	5(71%)		4(100%)	-
Piperacillin/tazobactam	6(50%)	3(43%)	5(100%)	3(75%)	-
Tobramycin			4(80%)	-	-

Table 8: Antibiotic Resistance in Gram positive bacteria

Antibiotics	*Coagulase negative staphylococcus*	*Staphylococcus aureus*
Ampicillin/sulbactam	3(100%)	-
Cefipime	-	1(100%)
Clarithromycin	-	1(100%)
Meropenem	-	1(100%)

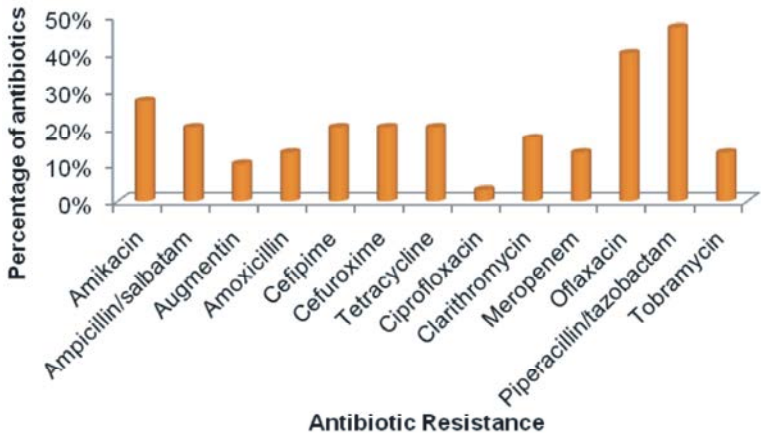

Fig. 7: Showing the antibiotic resistance in Gram positive and Gram negative isolates

DISCUSSION

India is the home of the largest number of diabetic individuals and their socio-economic conditions are poor [15]. Currently, 60.1 million Indians have diabetes. Diabetic foot infection is a common cause for the hospital admissions of the diabetic patients [14]. Our study showedthat 65% patients from rural area suffering from the diabetic foot infection are more as compared to the urban people. This is similar to a study by Deribe *et al*. [15]. Diekema *et al*. [16] showed that extended spectrum antimicrobial therapy may delay the improvement of the diabetic foot infection due to resistance to the antimicrobial therapy. This study reported that the small-spectrum antibiotic therapy is effective to avoid the development of the resistant organisms. In our study, a total of 34 organisms were isolated from 41 samples with an average of 1.23 organisms per case as similar to the Vishwanathan *et al*. [17] study. They yielded an average of 1.21 organisms per case. Our study is slightly lower than the findings from Suresh *et al*. [4] and Bansal *et al.* [18] studies where cultures yielded an average of 1.52and 1.33 per case respectively. Some studies reported that, mild diabetic foot infections are mostly monomicrobial whereas severe and moderate diabetic foot infections are polymicrobial. Some studies in other countries showed that most ofthe infections havethe poly microbial pathogens in their culture specimen [19, 20]. According to Tan *et al*. [21] and Sharma *et al*. [22] studies, 75.2 and 62.7%of infections yielded multiple organisms and single

organisms were found in 21.1 and 37.2% of each, respectively in contrast to our study. Among 34 isolates, 30 were Gram negative and 4 were Gram positive. Which is similar to two Indian studies found that 28.7% & 13.8% and 65% & 35% were Gram negative and Gram positive respectively [17,23]. Another study conducted by Alavi SM *et al*. [24] yielded that 54.8% of cases were Gram negative and 42.9% of cases were Gram positive. Raja *et al*. [25] study showed 52% Gram negative bacterial growth whereas 45% cases with Gram positive bacterial infection. According to Dhanasekaran *et al.* [26], 84% of diabetic foot ulcers are mono-microbial, similar to our study findings. We have observed in our study that the Gram negative bacteria were predominant than Gram positive. In this, *Klebsiella* (35%), *Proteus* (21%), *E.coli* (15%), *Pseudomonas* (12%), *Citrobacter* (7%) were observed. A study by Ramani *et al.* [27] made slightly similar observation and predominance of *Proteus* (20.73%), *Klebsiella* (12.35%) and *Pseudomonas* (11.73%) as the most common pathogens. Prabhakar *et al*. [28] also showed a predominant Gram negative (*Proteus, E.coli*) growth. Zubair *et al.* [29] also showed a similar observation to our study. They reported 56.6% monomicrobial growth and 56% of Gram negative bacterial infection.

Our study contrast to Hayath *et al*. [30] study, they reported that *Pseudomonas aureginosa* was the most common isolated pathogen accounting for 27.65% followed by *Proteus* species (12.93%), *E.coli* (11.76%) and *Klebsiella pneumonia* (8.23%).

Umadevi *et al*. [31] and Tiwari *et al*. [32] conducted a prospective study and they reported that 52.4% of cases were infected with Gram negative organism, 8.6% with Gram positive organisms and 68% were infected with Gram negative, 32% with Gram positive organisms, respectively. *Klebsiella* and *E.coli* were the most common isolated bacteria found in our study.

In our study *Klebsiella* species showed sensitivity to Amikacin (75%), Ciprofloxacin (42%) and Imipenem (33%) and Piperacillin/Tazobactam (8%). *Proteus* was sensitive to Meropenem (71%), Amikacin (43%) and Piperacillin/Tazobactam (29%). *E.coli* was sensitive to Clotrimaxazole (100%), Gentamycin (100%), Amikacin&Meropenem (80% of each) and Imipenem (40%). *Pseudomonas* was sensitive to Amikacin (50%), Meropenem (50%) and Imipenem (50%). *Citrobacter* was sensitive to Amikacin (100%) and Cefipime (50%).

According to Amsaveni *et al*., [33] *Proteous* species were highly sensitive to Amikacin, Imipenem, Ofloxacin and Piperacillin.

Gram positive bacteria showed sensitivity to Amikacin (100%), Vancomycin (100%) and Teicoplanin (100%). Amikacin was effective against Gram positive and Gram negative isolates [23, 34]. A study conducted by Umadevi *et al*. [31] reported that Amikacin, Piperacillin/Tazobactam and Imipenem were effective against Gram negative bacteria.

Our study can be distinguished from Murugan *et al*. [35] study; they reported that *E.coli* showed 100% sensitivity to imipenem and meropenem and resistance to Gentamycin. Our study reported that *E.coli* exhibited 100% sensitivity to Gentamycin and Clotrimaxazole and 80% to meropenem and 40% to Imipenem. In our study *Klebsiella* species were resistant to Piperacillin/Tazobactam (50%), Ofloxacin (58%), Clarithromycin (17%) and Cefuroxime (17%). *Proteus* was resistant to Ofloxacin (71%), Piperacillin/Tazobactam (43%) and Amikacin (43%). *E.coli* was resistant to Piperacillin/tazobactam (100%), Cefuroxime (100%), Tobramycin (80%) and Tetracycline (80%). Amikacin showed equal sensitivity and resistance towards patients infected with *Pseudomonas* species. And *Pseudomonas* species was 100% resistant to Ofloxacin and 75% resistant to augmentin and Piperacillin/Tazobactam. Meropenem (100%), Ciprofloxacin (50%), Clarithromycin (50%) showed effectiveness towards *Citrobacter*. Gram positive are resistant to Ampicillin/Sulbactam.

Inappropriate usage of antibiotics may lead to treatment failure. Multidrug resistance occurs as a result of the treatment failure, or failed to control the infection using a combination of the different antimicrobials. Infection with multi drug resistant organisms limit the choice of antibiotic treatment may lead to a worse outcome. Our findings are important to ensure proper treatment. Here is a key role for the clinical pharmacist to improve these outcomes for the proper management of diabetic foot ulcer and to prevent or avoid the amputations.

CONCLUSIONS

Our study concluded that Gram negative isolates are leading infectious agents from specimens. Amikacin was effective against Gram negative as well as Gram positive bacteria. Gram negative bacteria are more sensitive to Amikacin followed by Cefipime, Meropenem, Imipenem and Ciprofloxacin and more resistant to Piperacillin/Tazobactam, Ofloxacin, Cefuroxime and Tetracycline. Our study also helps the physician for selection of the proper antibiotics to improve the wound

healing and also leads a pathway for the formation of the institutional antibiotic committee to avoid the irrational antibiotic usage. From our findings, we found that the decrease in the multi-drug resistance improves the wound healing; thereby improve the patient quality of life. Our study found that rural diabetic patients having the bare foot walking history were more prone to develop injuries that may lead to diabetic foot ulcer.

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