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ASSESSMENT OF ANTIBIOTIC SENSITIVITY PATTERN IN DIABETIC FOOT ULCER PATIENTS AT A TERTIARY CARE TEACHING HOSPITAL IN KERALA

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ABSTRACT

Diabetic foot infections (DFUs) are among the most common bacterial infections encountered in patients with diabetes mellitus (DM) typically begin in a wound most often a neuropathic ulceration, most commonly seen on weight bearing surfaces. Current knowledge of antimicrobial susceptibility pattern is essential for appropriate therapy. The purpose of this study was to assess the antibiotic sensitivity pattern in DFU patients and their antibiogram. A prospective observational study was conducted on clinical specimens which were taken from 110 patients with diabetic foot infections, over a six months period. The commonly isolated organisms were E.coli, S.aureus, Streptococcus, Klebsiella, Pseudomonas, Acinetobacter, Citrobacter and Proteus. We analysed the trends in antibiotic susceptibility pattern in DFU using chi- square analysis. Overall the prevalence of DFU among the diabetic population for a period of 6 months is 15.9%.The highest diabetic foot infections were among patients with the age group of 41–60 years. 113 bacterial isolates were obtained from 110 specimens**.** Gram negative isolates were more predominant (61%); in contrast 38.9% of isolates revealed Gram positive isolates. E.coli (40.9%) was the most common pathogen followed by Staphylococcus (23.6%). The results revealed that, aminoglycoside (amikacin> gentamycin) and ciprofloxacin were the most effective antibiotics against Gram negative isolates, on the other hand; the Gram positive isolates were more susceptible toward aminoglycoside (amikacin> gentamycin) followed by linezolid. The absence of an updated antibiogram is a major contribution to the antibiotic resistance. The

datas gathered will be beneficial for future determination of empirical therapy policies for the management of DFIs.

KEYWORDS: Diabetic foot ulcer, Diabetes mellitus, Antibiotic sensitivity, Antibiogram.

INTRODUCTION

Antibiotics are substance produced by a microorganism or a similar product produced wholly or partially by chemical synthesis and in low concentrations inhibits the growth of or kills microorganisms such as infectious bacteria and fung.[1]

The emerging resistance to antibiotics and the poor response of new antibiotics is creating a major health issue world wide. In the current situations of escalating antibiotic resistance it is essential to identify and report sensitivity pattern of these MDR bacteria in order to tailor empirical therapy and hygienic measures. The greater the volume of antibiotics used, the greater the chances that antibiotic- resistant populations of bacteria will prevail in the contest for survival of the fittest at the bacterial level.[2]

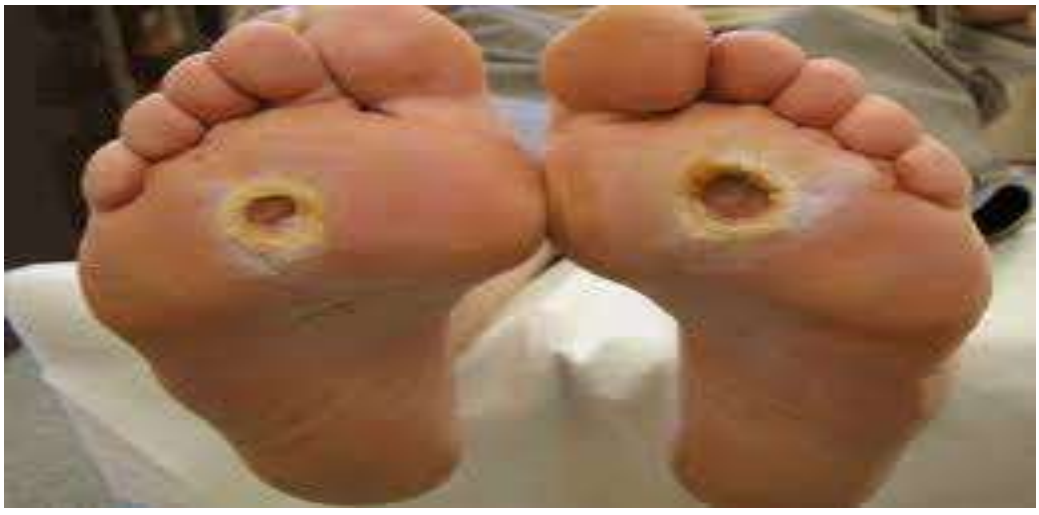
Misuse and overuse of antibiotics leads to resistance, which when potentially harmful bacteria reform, in a way that decrease or eliminates the effectiveness of antibiotics. When a person is infected with antibiotic resistant bacteria, it makes not only in treatment difficulty but also chances for spreading of their resistant organism.[3] The main reason for reduced effectiveness of bacteria is:-

- More complicated illness
- Early use of stronger and more expensive antibiotics
- Longer illness
- More doctor visit
- Use of antibiotic without prescription
- Wrong choice of antibiotic
- Irrational prescription.[4]

Diabetes mellitus is a group of metabolic disorder characterized by hyperglycemia, is associated with abnormalities in carbohydrate, fat and protein metabolism. Diabetic patients, both type 1 and type 2, are at significant risk for a number of health complications associated with the eyes, feet, heart, blood vessels and the kidneys. Complications arises due to derangement in the regulatory system for storage and metabolism of metabolic fuels,

including the catabolism and anabolism of carbohydrates, lipids and protiens emanating from defective insulin secretion insulin or both. Generally the injurious effects of hyperglycemia are separated into macrovascular complications (coronary artery disease, peripheral arterial disease and stroke) and microvascular complications (diabetic nephropathy, neuropathy, retinopathy and diabetic foot ulcer as well).[5,6,7]

One of the debilitating complications faced by people with diabetes is diabetic foot ulcer. More than 15% of people with diabetes will develop a diabetic foot ulcer in their lifetime. Diabetic foot characterized by several pathological complications such as neuropathy, peripheral vascular disease, foot ulceration and infection with or without osteomyelitis, which leads to the development of gangrene and which even necessitates limb amputation. The individuals with diabetes have atleast a 10 fold risk of being hospitalization for soft tissue and bone infections of the foot than individuals without diabetes. The selection of antibiotic therapy for diabetic foot infection involves decisions about choice of empiric and definitive antibiotic agent, route of administration, and duration of treatment. Empiric antibiotic regimen should include an agent active against E.Coli, Staphylococci, MRSA (methicillin resistant staphylococcus), Streptococci, Pseudomonas, Klebsiella, Acinetobacter, Citrobacter, Proteus etc.[8,9,10]



DFUs result from a complex interaction of a number of risk factors. Once the protective layer of skin is broken, deep tissues are exposed to bacterial infection that progresses rapidly and risk of lower extremity amputation. Treatment should be followed by culture-guided definitive therapy.[11]

Diabetic foot characterized by several pathological complications such as neuropathy, peripheral vascular disease, foot ulceration and infection with or without osteomyelitis, which leads to the development of gangrene and which even necessitates limb amputation. The Indian diabetic population is expected to increase to 57 million by the year. The individuals with diabetes have atleast a 10 fold risk of being hospitalization for soft tissue and bone infections of the foot than individuals without diabetes.[12]

Antibiotic therapy should not be used for foot ulcers without signs of infection because it does not enhance wound healing or prevent infection. Clinical failure of appropriate antibiotic therapy might be because of patient non-adherence, antibiotic resistance, superinfection, undiagnosed deep abscess or osteomyelitis, or severe tissue ischemia. Most amputations can be prevented with proper care of diabetic foot infections, suggest new guidelines released by the Infectious Diseases Society of America (IDSA).[9,10,13,14]

MATERIALS AND METHODS

Study site: General surgery department in a 500 bedded tertiary care teaching hospital.

Study design: Prospective Observational study

Study duration: Study was conducted within a time period of 6 months

Study criteria

Inclusion criteria

- Patients with DM
- Age 20-80 years

Exclusion criteria

- Malignant ulcers
- Pregnant and lactating women
- Mentally retarded patients

Study Procedure

Phase 1: Before conducting the study a protocol was prepared for which the permission was obtained from IEC held on 13th January 2016.Then a detailed literature review was done regarding the concerned topic.

Phase 2 After obtaining permission from IEC, the study began with data collection. About 141 DFU patients had visited the General surgery during the study period from January till

June. The patients were scrutinized based on the inclusion criteria. Data regarding culture and sensitivity testing of the microorganism isolated from pus were collected from the records of Microbiology department other details are collected from the General surgery ward and Medical Records Department (MRD). Collected data is transcribed on a data entry form which comprises of demographic details (age, sex, department), past medical history (co-morbidities), HbA1c, Urine examination results, type of organism, list of sensitive drugs, list of resistance drugs, treatment details.

Phase 3 All data were tabulated and analysed. Data analysis: Statistical analysis was done by using Microsoft Excel 2010 and SPSS version 20 (statistical software package).

Phase 4 The analysed datas were evaluated in detail and finally a hospital antibiogram for DFU is formulated.

RESULTS AND DISCUSSION

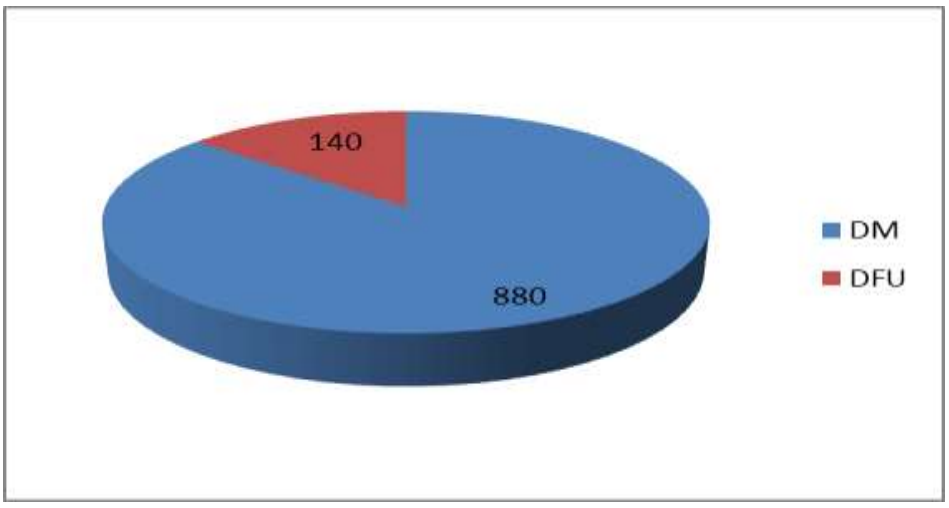
A non-invasive prospective observational study was conducted in the diabetic foot ulcer (DFU) patients of general surgery ward for a period of 6 months. A total of 141 patients were enrolled in the study. Of these 141 patients complete data was available for only 110 patients whose pus sample was cultured for analysis. Remaining 31 patients could not followed up because whose pus was not cultured and other various reasons as well.

1. PREVALENCE OF DIABETIC FOOT ULCER AMONG DIABETIC PATIENTS

Total number of diabetic (DM) patients admitted during the study period :- 880

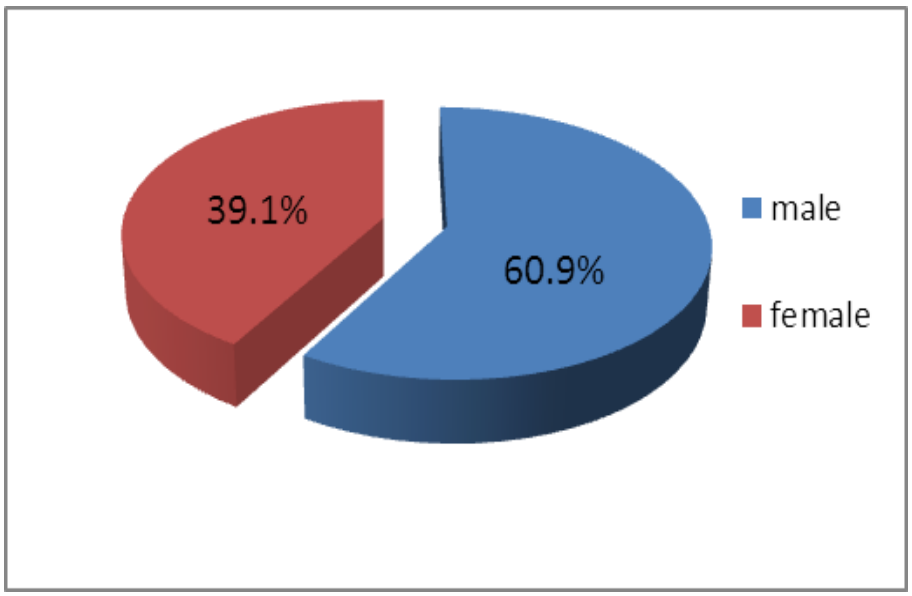
Total number of diabetic foot ulcer (DFU) patients admitted during the study period:- 140

So, prevalence =(140/880) * 100 = 15.9%



Among the total diabetic population prevalence of DFU is 15.9% in our area for a period of 6 months. According to a study done in North Indiaprevalence of DFUs among diabetic patients was 14.30% in 2012 reported by **Shailesh K and Ashok Kumar**.[15]

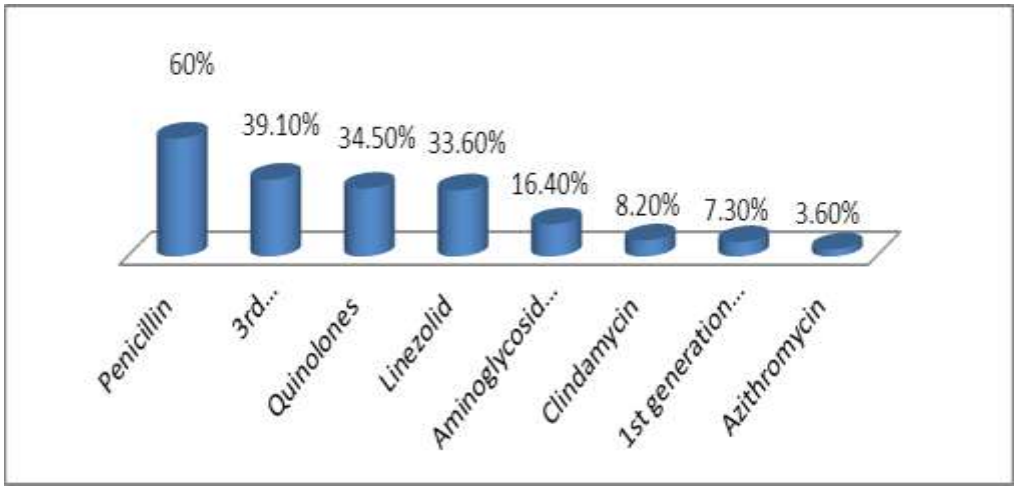
In our study, males (60.9%) are mostly affected by DFU compared to females (39.1%). The studies conducted by, **Khalid Al-Rubeaan et.al** (2015)**, Obulesu G Madan, Madan Mohan Rao** (2014) **and J. Vimalin Hena, Lali Growther** (2010) as well found that males are more prevalent to DFU than females.[16,17,18]



2. PRESCRIBING PATTERN OF ANTIBIOTICS IN DFU

Usual prescription pattern in IP

Antibiotic	No.	Percentage
Penicillin	66	60%
3rd generation cephalosporins	43	39.1%
Quinolones	38	34.5%
Linezolid	37	33.6%
Aminoglycosides	18	16.4%
Clindamycin	9	8.2%
1st generation cephalosporins	8	7.3%
Azithromycin	4	3.6%



According to study data, 60% of prescriptions were prescribed with penicillins, 39.1% of cases with 3rd generation cephalosporins, quinolones, linezolid, aminoglycosides; clindamycin and 1st generation cephalosporins were prescribed in 34.5%, 33.6%, 16.4%, 8.2% and 7.3% respectively. Placing penicillin class of drugs as a first choice (60%) in the antibiotic therapy carries no logic because theses has low activity against reported higher incidence of gram negative organisms (61%). More than half of the prescriptions had GI protectants, pain relievers, antihypertensive agents, insulin, OHA's, combination of both, antiplatelet agents and anti-inflammatory agents. Blood flow improvers, antiprotozoal agents and anxiolytic agents were 25.50%, 20% and 11.8% respectively. More than half of the cases had both insulin and OHA's for effective control of blood sugar level and there by treat foot infections faster.

A retrospective study conducted by **Ali N et al** analysed possible irrationalities in the prescribing pattern of antibiotics for management of hospitalized diabetic foot cases. Primary anti-diabetic therapy included insulin, oral anti-diabetic, or combination of both. Supportive therapy included antibiotics for diabetic foot cases and other physical measures like routine wound dressings and washing. Antibiotic therapy was analysed based on the culture sensitivity reports. The antibiotic therapy for management of diabetic foot (n=410) was in the order of ceftriaxone (83.3%) > co-amoxiclav (36.66%) > clindamycin and ciprofloxacin (26.66%) > cefuroxime and levofloxacin (10.0%) > clarithromycin. Placing ceftriaxone as a first choice (83.3%) in the antibiotic therapy carries no logic as ceftriaxone has low activity against reported higher incidence (85 %) of gram-positive organisms. Prescribing irrationality of antibiotics is a global phenomenon that shall be addressed right from the medical/pharmacy schools levels.[19]

Out of 110 prescriptions included for the study, 25 (22.72%) prescriptions had antibiotic monotherapy, 53 (48.18%) prescriptions had two antibiotic drug combinations, 19 (17.2%) prescriptions had three antibiotic drug combinations, 13 (11.8%) prescriptions were more than three antbiotic drug combinations. This was same as Given by **Zachariah Thomas** et al from Thamilnadu in 2015.[20]

3 PRESCRIBING PATTERN OF DRUGS OTHER THAN ANTIBIOTICS IN DFU

Drug category	No.	Percentage
GI Protectants	107	97.30%
Pain relievers	76	69.09%
Antihypertensive agents	75	68.20%
OHAs	67	60.90%
Antiplatelet agents	65	59%
Antiinflammatory agents	59	53.60%
Insulin	58	52.70%
Vasodilators/Blood flow improvers	28	25.50%
Antiprotozoal agents	22	20%
Anxiolytic agents	13	11.80%

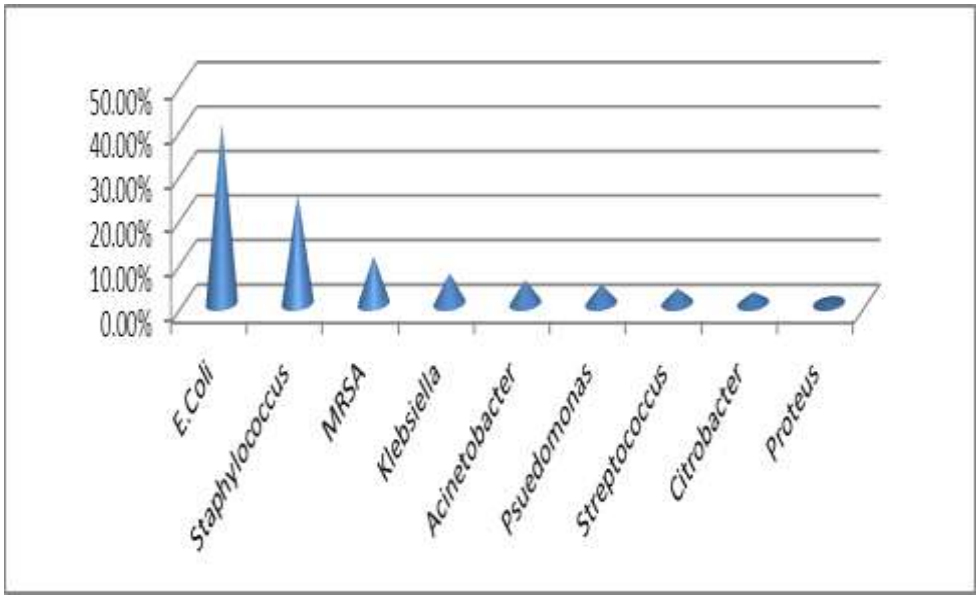
Out of 110 prescriptions enrolled for the study, more than half of the prescriptions had GI protectants, pain relievers, antihypertensive agents, insulin, OHA's, combination of both, antiplatelet agents and anti-inflammatory agents. Blood flow improvers, antiprotozoal agents and anxiolytic agents were 25.50%, 20%, and 11.8% respectively.

4 DISTRIBUTION OF ORGANISM CAUSING DFU

A total of 113 bacterial isolates were identified from 110 cases, which contain nine different organisms causing foot infections. They were gram neagative organisms (e.coli, klebsiella, acinetobacter, pseudomonas, citrobacter, proteus) and gram positive organisms (staphylococcus, MRSA and streptococcus).

Distribution of organism causing DFU in total population

Organism	Total	Percentage
E.Coli	46	40.70
Staphylococcus	28	24.77
MRSA	12	10.75
Klebsiella	8	7
Acinetobacter	6	5.30
Psuedomonas	5	4.42
Streptococcus	4	3.53
Citrobacter	3	2.65
Proteus	1	0.88



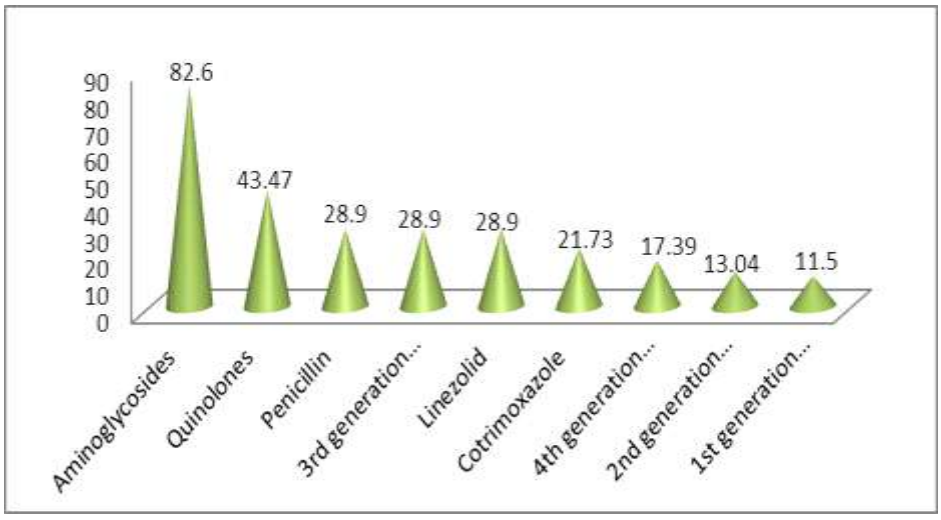
Distribution of organism causing DFU in total population

Among 110 cases, 107 (97.27%) cases were monomicrobial infections and 3 (2.72%) were identified as polymicrobial infections. The major bacterial isolates identified were Gram negatives, 69 (61%) and the other 44 (38.9%) were identified as gram positives. And organisms isolated in combinations are, E.Coli + Staphylococcus, Staphylococcus + Psuedomonas and MRSA + Citrobacter.

We observed that Gram-negative infections were more common in the studied population. E.coli was the most prevalent cause of DFU in our study (40.70%) followed by staphylococcus (24.77%). Among 113 bacteria isolated, 61% were Gram negative and 44% were Gram positive. A study reported by **Shalbha Tiwari et al** shows among 82 bacteria isolated, 68% were Gram negative and 32% were Gram positive. And also a study conducted by **Pugazhendhi Sugandhi, Durairaj Arvind Prasanth** in 2014 also shown the same. Gram-negative bacterias were most prevalent in diabetic foot infection. Among 110 cases, 97.2% had mono-microbial infection, 2.72% had poly-microbial infections. So, predominantly mono-microbial infections and our finding was in accordance with those of another similar study by **Y. Kavitha, Khaja Mohiddin**.[16,21,22]

5 OVERALL ANTIBIOTIC SENSITIVITY PATTERN OF GRAM NEGATIVE BACTERIAS IN DFU THERAPY

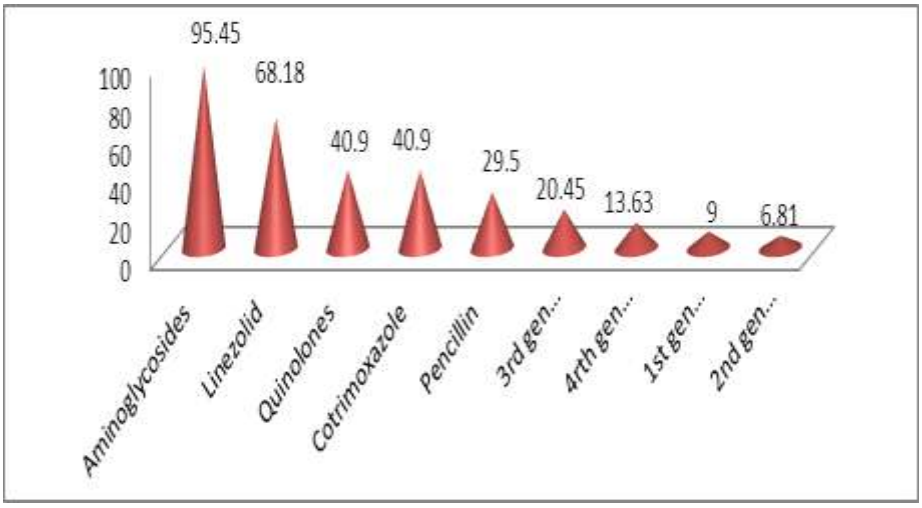
Classes of antibiotics	% sensitivity
Aminoglycosides	82.6
Quinolones	43.47
Penicillin	28.9
3rd generation cephalosporins	28.9
Linezolid	28.9
Cotrimoxazole	21.73
4th generation cephalosporins	17.39
2nd generation cephalosporins	13.04
1st generation cephalosporins	11.5



Aminoglycosides, (82.6%) are the most sensitive class of antibiotic (amikacin 57.97% & gentamycin 24.6%) for gram negative infections, followed by quinolones. Beta lactams, linezolid and cotrimoxazole shows lower sensitivity towards gram negative infestations.($p < 0.001$).

6 OVERALL ANTIBIOTIC SENSITIVITY PATTERN OF GRAM POSITIVE BACTERIAS IN DFU THERAPY

Classes of antibiotics	% sensitivity
Aminoglycosides	95.45
Linezolid	68.18
Quinolones	40.9
Cotrimoxazole	40.9
Pencillin	29.5
3rd gen cephaloprorins	20.45
4rth gen cephalosporins	13.63
1st gen cephalosporins	9
2nd gen cephalosporins	6.81



Aminoglycosides, (95.45%) are the most sensitive class of antibiotic for gram positive infections, followed by linezolid. Beta lactams shows least sensitivity (6-20%) towards gram negative infestations. Quinolones and cotrimoxazole (both have 40.9%) seems to be moderately sensitive towards gram positive infestations. ($p < 0.001$).

According to various single centered studies, the antibiotic resistance and sensitivity pattern vary among patients in different areas. According to this study, most of isolated organism were shown significant sensitivity to aminoglycosides (amikacin> gentamycin), which is in accordance with the study done by **Abd Al-Hamead Hefni et al**in 2012.In present study, E.Coli, Psuedomonas and Klebsiella shows sensitivities 96%, 100%, 100% respectively to amikacin. Pseudomonas and klebsiella are lakhs sensitivity to linezolid. Quinolones show significant sensitivity to all gram negatives. Cefazolin, Cefuroxime and cotrimoxazole seems to be least sensitive to all gram negative organisms. MRSA, staphylococcus shows 100% and Streptococcus shows 50% sensitivities to aminoglycosides. And linezolid also mostly sensitive to these three organisms (64-75%).E.Coli and Klebsiella shows significant resistance to beta lactams. Linezolid shows least resistance towards all gram negative isolates.[23]

7 ANTIBIOGRAM OF DIABETIC FOOT ULCER

ANTIBIOGRAM OF GRAM NEGATIVE ORGANISMS

- Out of the nine classes of antibiotics tested gram negative isolates are showing highest sensitivity to aminoglycosides 82.6% (amikacin 57.97% & gentamycin 24.6%) followed by quinolones 43.47% (ciprofloxacin).

- All the gram negative isolates are showing lowered sensitivity to beta lactam antibiotics, linezolid and cotrimoxazole.
- 44 E.Coli isolates were sensitive to aminoglycosides {amikacin (30) gentamycin (14)} followed by ciprofloxacin (24).
- 5 isolates of pseudomonas were sensitive to amikacin followed by penicillin class of antibiotics (amoxicillin + clavulonate, ampicillin, piperacillin/tazobactum). Ciprofloxacin and cefepime antibiotics also more than 50% sensitive to pseudomonas species.
- 8 isolates of klebsiella were sensitive to aminoglycosides {amikacin(6), gentamycin(2)} followed penicillin class of antibiotics, 50% (amoxicillin + clavulonate, ampicillin, piperacillin/tazobactum)

ANTIBIOGRAM OF GRAM POSITIVE ORGANISMS

- Out of the nine classes of antibiotics tested gram positive isolates are showing highest sensitivity to aminoglycosides 95.45% (amikacin 50% & gentamycin 45.45%) followed by linezolid 68.18%.
- All the gram positive isolates are showing least sensitivity to beta lactam antibiotics, quinolones except streptococcus.
- 12 MRSA isolates were sensitive to aminoglycosides {amikacin (7) gentamycin (5)} followed by linezolid and cotrimoxazole (9).
- 28 isolates of staphylococcus were sensitive to aminoglycosides (both amikacin & gentamycin equally sensitive) followed by linezolid (18). Ciprofloxacin is 46.42% (13) sensitive to staphylococcus.
- 4 isolates of streptococcus were sensitive to beta lactam antibiotics 100%. The beta lactam antibiotics include all penicillin class of drugs (amoxicillin + clavulonate, ampicillin, piperacillin/tazobactum), 3rd generation cephalosorins like cefixime, ceftriaxone, cefoperazone+ sulbactam, cefotaxime, ceftazidime and 4rth generation cephalosporin, cefepime. Followed by linezolid 75% (3). Aminoglycoside shows 50% (2) sensitivity to streptococcus species.

ANTIBIOGRAM OF POLYMICROBIAL INFECTIONS

- Out of 110 cases, only 3 cases were identified as polymicrobial infection (2.72%). Organisms isolated in combinations are, E.Coli + Staphylococcus, Staphylococcus + Psuedomonas and MRSA + Citrobacter.

- In case of polymicrobial infections coexistence of gram negative and gram positive microorganisms were more common. So empirical antibiotic therapy is necessary to get an effective treatment outcome.
- From analyzing all datas, it is evident that the most effective choice of antibiotic for either gram negative or gram positive or the combination of both isolated in DFU is aminoglycosides (amikacin > gentamycin) followed by ciprofloxacin and linezolid.

Empirical antibiotic selection based on resistance and sensitivity pattern

Choice of drug before get culture report	Choice of drug after get culture report
For gram negative isolates:- 1st Choice : Aminoglycosides (Amikacin > Gentamycin) 2nd Choice : Ciprofloxacin	Give most appropriate sensitive drug based on culture report
For gram positive isolates:- 1st Choice: : Aminoglycosides (Amikacin > Gentamycin) 2nd Choice: Linezolid	
For polymicrobial infections :- 1st choice: Aminoglycosides (Amikacin > Gentamycin) 2nd choice: Ciprofloxacin	

This choice of therapy is based on the population that we observed and hospital settings and hence the relevance of the topic.

CONCLUSION

Most of the antibiotic, which is commonly used, is now resistant. Antibiotic resistant pattern is changing continuously with time.

In DFI, antibiotic therapy should start before the returning of culture. So antibiograms are made to set the guidelines for empiric antibiotic therapy. These antibiograms are made according to analyzed report of recent microbiological laboratory report. Most hospital acquired DFI are mostly resistant to many drugs. So they must be treated according to culture report.

According to the data collected, we recommend an antibiotic guideline based on our results to implement in the hospital and thus improve the rational use of antibiotics and reduce resistance. This choice of therapy is based on the population that we observed and hospital settings and hence the relevance of the topic.

According to this study, it is concluded that antibiotic resistance is an already existed major problem in DFU. According to assessment of antibiotic sensitivity pattern most of isolated organism were sensitive to aminoglycosides, quinolones and linezolid, Where as beta lactam antibiotics showed high resistance to organisms. But, from prescription analysis, most of patients were treated with penicillin class of antibiotics and 3rd generation cephalosporins. Thus the most of prescribed antibiotics showed emergence of resistance to organisms isolated and this brings to light the need for timely and proper diagnosis of the major microbial causes of DFIs, in order to administer the appropriate therapy based on antibiotic susceptibility test of the causative agent.

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