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BACTERIOLOGICAL PROFILE OF DIABETIC FOOT ULCER IN TERTIARY CARE HOSPITAL RIMS RANCHI

Microbiology

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ABSTRACT

INTRODUCTION- Diabetic foot ulcer is growing as a global concern,being one of the most common complication notoriously responsible for fatal sequelae like amputation,deformity and disseminated infection. **AIMS AND OBJECTIVES-**To compare bacteriological profile along antibiogram of different etiological agents of diabetic foot ulcer. **MATERIAL AND METHODS-**This study was conducted between November 2017 to April 2018 in surgical ward, RIMS, Ranchi.Two sterile cotton swabs per pus sample were collected aseptically and sent to Microbiology Department of RIMS,Ranchi for further investigations.First swab was made for Gram's staining followed by next swab to be inoculated on blood agar and Macconkey agar incubated at 37℃ overnight. Growth on culture plate was identified by its colony characteristic as well as their biochemical reactions.The antimicrobial susceptibility testing was performed as per CLSI guidelines. **RESULTS-**In study of 84 patients, 64 were positive on culture and 20 were sterile.The most common organism isolated from foot ulcer were Staphylococcus aureus (34.37%) followed by E.coli(25%) ,Pseudomonas aeruginosa (18.75% cases), ,Klebsiella(15.63%) and others(6.25%). The prevalence of Gram negative infection was higher in diabetic foot ulcer. **DISCUSSION AND CONCLUSION-**The prevalence of gram negative infection was higher in diabetic foot ulcer.It was most prone to elderly patients with DM Ty 2.In our study, males were more predominant than females. Appropriate and judicious use of antibiotics along proper dressing care can help,reducing the burden of diabetic foot ulcer.

KEYWORDS

INTRODUCTION

The diabetic foot ulcer is a global concern and develops in regions with improper foot care and increases the life time risk for developing foot ulcer.[1] This infection begins superficially , spreads to other subcutaneous tissue and extends to deeper structures leading to gangrene and amputations.[2] Diabetic foot ulcers (DFUs) are a prevalent complication of diabetes mellitus and account for significant morbidity, mortality, and healthcare expenditures. It is estimated that 19–34% of patients with diabetes are likely to be affected with a diabetic foot ulcer in their lifetimes, and the International Diabetes Federation reports that 9.1–26.1 million people will develop DFUs annually.[3] Patients with DFUs were also found to have a 2.5-fold increased risk of death compared with their diabetic counterparts without foot wounds.[4]

And among persons with diabetes (PWD), 12%–25% have a risk of developing a foot ulcer during their lifetime.(5-7) The most common cause of morbidity and mortality in DFU is infections, which are seen in 40%–80% of the cases.[8] Diabetic neuropathy and micro- or macroischemia are the two main risk factors that cause DFU.[9] Impaired microvascular circulation limits the access of phagocytic cells to infected area, and this results in poor concentration of antibiotics in infected tissue.[10] Hence, diabetic foot wounds are commonly infected, and hence infection leads to the formation of microthrombi causing further ischemia, necrosis, and progressive gangrene. Rate of amputation of a limb is estimated to be forty times greater in infected nonhealing ulcer in diabetics than the patients of trauma. Infections precede in >60% cases of foot amputations.[11] These types of situations necessitate limb amputation. Thus, accurate diagnosis of the causative organism is essential for the management of these cases. The burden of PWD in India is expected to increase to 57 million by the year 2025. [12]

The bacteriology of diabetic foot infection is highly complicated and mostly polymicrobial. It involves both aerobes and anaerobes. Many researchers have presented a picture of mixed infection with aerobic and anaerobic bacteria. [13]

AIMS AND OBJECTIVES

To study the bacteriological profile and antibiogram of different etiological agents isolated from diabetic foot ulcer cases.

MATERIALS AND METHODS

This study was conducted between November 2017 to April 2018 in DEPARTMENT OF MICROBIOLOGY,RIMS,Ranchi. Two sterile cotton swabs for each pus sample were collected aseptically from Diabetic foot ulcer patients admitted in surgery ward ,RIMS,Ranchi.

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Ulcer surfaces were rinsed with sterile normal saline, and swabs samples were collected from the base of the ulcer and deep tissue. Specimen from infected diabetic ulcer foot were obtained after debriding the superficial exudates.[14]

One swab was used for Gram's staining and the other swab was used for culture inoculation on Blood agar, Mac Conkey agar and Nutrient broth .The plates and broth were incubated at 37^{0}C overnight. Growth on culture plate was identified by its colony characteristic as well as by their biochemical reactions .The antimicrobial susceptibility testing was performed by Kirby-Bauer Disk diffusion methods.

For Staphylococcus isolates' methicillin resistance was tested by disc diffusion technique using Muller Hinton agar containing 4% NaCl with a 1 µg of oxacillin disc. Vancomycin resistance was preliminarily detected by 30 µg vancomycin disc and was further confirmed by HiComb MIC test from HiMedia. Extended spectrum beta lactamase (ESBL) testing was done using both the ceftazidime (30 µg) and ceftazidime clavulanic acid (30/10 µg) discs. Metallo beta lactamase (MBL) production was detected using 10 µg of imipenem disc along with a second disc containing 10 µl of 0.5 M ethylenediaminetetraacetic acid placed at a 15 mm distance.

Inclusion criteria :- Patients with Diabetes who had foot ulcers or foot infection were included.

Exclusion criteria : - The exclusion criteria were other foot ulcers and foot infection in persons without diabetes.

RESULTS AND DISCUSSIONS:-

In our study, out of 84 patients,68 were males and 16 were females, whose ranged from age 40 -65 years.

Eighty four pus samples were collected from patients with diabetic foot ulcers, from these samples, in 64 patients samples are culture

positive and 20 samples were culture sterile. The most common organism isolated from foot ulcer were *Staphylococcus aureus* (34.37% ,22 cases*)* followed by *Escherichia coli (25%)* than *pseudomonas aeruginosa (*18.75%) *klebsiella spp* (15.63%) and others (6.25%). prevalence of Gram negative infection was higher in diabetic foot ulcer. With reference to the gram negative organisms, 50% of organism were ESBL (Extended spectrum beta lactamase) producer, with the highest production by *Escherisia coli*. *Pseudomonas aeruginosa* was sensitive to Pipercillin-Tazobactam, Imipenam, ceftazidime-clavulanic acid and amikacin. Staphylococcus aureus was sensitive to linezolid, daptomycin, Amikacin,and Piperacillin-Tazobactam, clindamycin, vancomycin.

Nearly 63.6 % *Staphylococcus aureus* were methicillin-resistant staphylococcus aureus (MRSA). Among the combinations antibiotics, piperacillin-tazobactam , imipenem, linezolid and clindamycin was found to be the effective drug of choice for treating foot ulcers.

The ESBL was detected by Broth microdilution method by testing both cefotaxime and ceftazidime ,alone and in combination with clavulanic acid. The MRSA in Staphylococcus aureus was detected by the use of cefoxitin disc succeptibility test and oxacillin screen agar.

Table 1:

	No.of cases	percentage
Culture positive	64	76.19%
Culture negative	20	23.81%
Total	84	100

Table 2:- Number of microorganisms isolated

NO.	Name of microorganism	Number	Percentage
1.	Staphylococcus aureus	22	34.37
2.	Escherichia coli	16	25
3.	Pseudomonas aeruginosa	12	18.75
4.	Klebsiella spps.	10	15.63
5.	Others	04	6.25

Table 3:- Sensitivity pattern of gram negative bacteria

Sl No.	Antibiotics	Sensitive	%	Resistant	%
	Amikacin	30	79	8	21
	Meropenam	32	84	6	16
	Cefotaxime	10	26	28	74
	Cefoperazone-salbactam	19	50	19	50
	Colistin	38	100	0	0
	Ceftazidime	16	42	22	58
	Ceftriaxone	19	50	19	50
	Imipenem	38	100	0	0
	Levofloxacin	23	60	15	40
	Piperacillin-tazobactam	32	84	06	16

Table 4:- Sensitivity pattern of gram positive bacteria

Sl No.	Antibiotics	Sensitive	Percentage	Resistant	Percentage
1.	Amoxicillin	11	50	11	50
2.	Ciprofloxacin	12	55	10	45
3.	Chloramphenicol	12	55	10	45
4.	Cefoxitin	00	00	22	100
5.	Clindamycin	22	100	00	00
6.	Erythromycin	11	50	11	50
7.	Gentamicin	16	73	06	27
8.	Linezolid	22	100	0	0
9.	Tetracycline	17	77	05	23
10.	Vancomycin	22	100	00	00

DISCUSSION:-In our study, most of the patients of diabetic foot ulcer belongs to the age group of 51-65 years . Diabetic foot ulcer is more common in male than in female. This may be an indication of higher level of physical activities undertaken by aging PWD to run their family and increased prevalence of comorbidities such as neuropathy, peripheral vascular disease, and kidney diseases in this age group. Among the 84 DFU patients in the present study, 78.5% (66/84) of patients were males and 21.5% (18/84) of patients were females. Higher male prevalence has been reported by Harrison and Lederberg (19) .

In the present study, there was a preponderance of monomicrobial culture growth (69%). In this study, 69% of pus cultures shows monomicrobial growth, about 6.25% (4cases) polymicrobial growth isolated , 24% pus samples are culture sterile.In our study , out of 84 pus samples , 64 pus samples are culture positive . out of total culture positive cases , the gram negative bacteria(59%, 38cases) is most commonly isolated then gram positive bacteria(22cases, 34.37%).

In present study Gram-negative organisms were more prevalent, and the predominant organisms isolated were members of the *Enterobacteriaceae.* (18,19)

In gram negative bacteria , *Escherichia coli* (16 cases 25%) was the most common bacteria isolated followed by *Pseudomonas aeuroginosa* (12 cases 18.75%) and *Klebsiella spp* (10 cases 15.63%).

In gram positive bacteria , *Staphylococcus aureus* (22cases 34.37%) was isolated. This study is very similar to Asima Banu et al.(20) *S. aureus* was the single most frequent pathogen (26%, 46/179), followed by *E. coli* (20%, 37/179). Other studies have also found the same.(19,21,22) In contrast, another study carried out by Ako-Nai *et al.* (23) showed *Escherichia coli* as the frequent bacterial pathogen.

The highest prevalence of ESBL was observed in Escherichia coli (62.5%) followed by *P. aeruginosa* (33.3%), which was consistent with the study carried out by Shobha *et al.* (24) In our study, 63.6% of *S. aureus* isolates were methicillin resistant. This finding was similar with studies reported earlier.(22,25)

This could be due to the prolonged and indiscriminate use of antibiotic therapy and administration of broad-spectrum antibiotics that may increase the prevalence of antibiotic resistance organism such as MRSA in Diabetic Foot Infection.

In this study, most of the culture isolated Escherichia Coli was sensitive to amikacin (80%), imipenem (100%), meropenem (84%), and piperacillin-tazobactam (100%). And this study was very similar with the results of other studies.(26,27) However, the nonfermenting Gram-negative bacteria culture isolates showed the following sensitivity pattern - amikacin (90%), imipenem (72%), meropenem (70%), and piperacillin-tazobactam (74%). This was similar to the findings of Al Benwan *et al.* (18)

As for the *Staphylococcus aureus* culture isolates, linezolid (100%), vancomycin (100%), Clindamycin (100%) and Gentamycin (83%) were the most effective antibiotics. Other studies have shown different antibiotic susceptibility patterns, and in most, vancomycin and linezolid have shown good activity against the strains. (26) A study by Al Benwan *et al*. showed vancomycin, Linezolid and Clindamycin as the most effective antibiotic for Gram-positive bacteria. (18)

Conclusion:-

Treatment of diabetic foot ulcers frequently involves the use of an empirical antibiotics. The severity of wound infection and their antimicrobial susceptibility pattern determine the choice of empirical treatment.

In our study , the most common organisms isolated in PWD and with foot ulcer were gram negative bacteria.

Staphylococcus aureus was the most predominant single organism isolated from lesions. Monomicrobial infection was more common than polymicrobial infection in diabetic foot infection cases.

There is an increasing prevalence of Multidrug resistance organisms associated with diabetic foot ulcer patients. Linezolid, clindamycin, imipenem and PTZ can be used as a empirical treatment. Proper knowledge of foot care is essential to prevent foot ulcer in diabetic patients.

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