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RESEARCH ARTICLE

MICROBIOLOGY

AEROBIC BACTERIAL RESISTANCE IN DIABETIC FOOT ULCER FROM CHENNAI

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ABSTRACT

Fifteen percent of all diabetics develop a foot ulcer at some point in their lives that is highly susceptible to infection and that spreads quite rapidly, leading to overwhelming tissue destruction and subsequent amputation. Wound specimens were collected and processed from patients attending diabetic foot clinic tertiary care Hospital isolates were characterized and antimicrobial susceptibility testing performed by standard protocols. Out of 123 patients analyzed the age group between 25-75 years with an average of 55.52 ± 11.64. 171 isolates were obtained wound specimens were grouped in to different Wagner grading, gram-positive organisms were 66/171 and grams negative were 97/171. The salient features of the present study are Gram-negative isolates are predominant from wound specimens. Linezolid and Vancomycin found to be highly sensitive to the group especially gram-positive isolates. In gram negative Ciprofloxacin and Tobramycin found better sensitivity and ceftazidime showed higher percentage of resistance by MIC.

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KEY WORDS

Diabetic foot ulcer, MRSA, antibiotic resistance, wagner grading, Enterobacteriaceae, and non-Enterobacteriaceae

INTRODUCTION

Diabetes mellitus is a metabolic disorder in which there is increased level of blood glucose because of insulin deficiency leading to significant morbidity and mortality [21]. The prevalence of diabetes is rapidly increasing worldwide and a real epidemic of the disease expected in this century. Global prevalence of diabetes is 6.3% in the general population and 8.7% among persons aged 20 years and older, which correspond to total number of 171 million of patients [36], this number more than double and would reach 366 million in 2030.

A substantial and increasing proportion of the elderly population is therefore at risk of micro- and macro vascular complications, including diabetic foot disease [26]. Approximately 15–20% of persons with diabetes will develop a DFU in their lifetimes [2, 7 & 20].

Foot disease is one of the most serious and costly complications of diabetes worldwide. In recent years, major progress has been in the recognition of the problem and in the understanding and management of the disease[27].

DFU 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, DFU can be classified using Wagner's Classification or University of Texas Diabetic Wound Classification and treatment instituted appropriately. Approximately 20% of diabetes admitted to the hospital was primarily for their foot problems [35]. 50-70% of all non-traumatic amputations performed on these diabetic patients. Patients with diabetes also can have a combined infection involving bone and soft tissue called fetid foot. In general, people with diabetes have infections that are more severe and take longer to cure than equivalent infections in other people

Pseudomonas spp., *Enterococcus* spp. & *Proteus* spp. carry a special role and are responsible for continuing and extensive tissue destruction with the poor blood circulation of the foot[3]. The infection leads to the early development of complication even after a trivial trauma, the disease progresses and becomes refractory to antibacterial therapy[28]. It is essential to assess the magnitude of bacterial infection of the lesions to avoid further complications and save the diabetic foot. Early diagnosis of microbial infections and appropriate antibacterial therapy leads to avoid further complications[4].

Many studies have reported on the bacteriology of DFU over 25yrs, but with varied results from different places. Earlier studies have found that *S. aureus* as the main causative pathogen[12, 24], but recent investigations reported a predominance of gram-negative aerobes, documented variety of gram-negative bacteria notably Pseudomonas and Enterobacteriaceae especially in south India[13, 15, 1, 10 & 33].

Role of fungi in the DFU not well documented, however, in a few cases *Candida spp.* has been reported[20].

Infection with multidrug- resistant organisms (MDROs) may increase the duration of hospital stay and cost of management and may cause additional morbidity and mortality. In India, recent report shows use of antibiotics without proper prescriptions leading to huge selection pressure[23].

Proper management of infections requires appropriate antibiotic selection based on culture and antimicrobial susceptibility results; however, initial management comprises empirical antimicrobial therapy, which based on susceptibility data extrapolated from studies performed on general clinical isolates[18]. The determination of antimicrobial susceptibility of a clinical isolate is often crucial for the optimal antimicrobial therapy of infection. Testing is required not only for therapy but also to monitor the spread of resistant organisms throughout the hospital and community.

MATERIALS AND METHOD

(i) Collection and processing of Diabetic foot wound swab:

Total 123 wound swabs collected from diabetic foot ulcers of both in-patients and outpatients attending diabetic foot clinic in tertiary care, Chennai between 2007 September to 2008 October. Clinical details of the patients also collected.

(ii) Characterization of Bacterial isolates:

Wound swabs collected using sterile cotton swab, by taking fresh pus. Direct microscopy performed to assess the load of microbes, 5% blood agar, In-house cooked meat medium, Mac Conkey and SDA agar plates and quadrant streaking performed for isolation of bacteria and fungi. The streaked plates incubated at 37^{o}C for 18-24hrs. After, 24 hours of incubation the plates examined for isolated colonies. Identification of isolates were done based on the colony morphology, Gram staining, Motility, Catalase test, Oxidase test, Coagulase test, Standard biochemical tests and Oxidation – Fermentation test and other tests[14].

Antimicrobial susceptibility test

All the isolates were tested for antimicrobial susceptibility by two methods i.e., disk diffusion method and MIC determination by micro broth dilution method.

(iii)Disc diffusion:

All the isolates tested for antimicrobial susceptibility against antimicrobial agents, which showed in table-4.1 and 4.2. Antibiotic discs purchased from Hi Media Ltd., Mumbai, India. The diameters of complete inhibition zone measured and interpreted according to the chart provided by the manufacturers. The organisms reported as either sensitive, intermediate sensitive or resistant to antimicrobial agents tested. *S. aureus* ATCC 25923, *Enterococcus faecalis* ATCC29212, *K. pneumoniae* ATCC70063 & *P. aeruginosa* ATCC 27853 used as control strains respectively[11].

(iv)Minimum inhibitory concentration

Inoculum of the test organisms were prepared from colonies grown on Nutrient agar, which had been incubated overnight (18-20 hours) at 37°C in incubator. Colonies suspended in Mueller Hinton broth (MHB) and adjusted to a turbidity of a 0.5 McFarland standard (1x 10^8 CFU/ml). Each well of microtitre plate contained 100µl of respective antimicrobial solution followed by bacterial suspension (5µl; 1x10^7 CFU/ml). The quality control strains, *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 25922 and *K. pneumoniaee* ATCC 700603, used in each run of daily testing. Each batch included a growth control well (no antimicrobial agent) and a negative control well (un-inoculated). After inoculation, each tray covered with a

lid to prevent evaporation during incubation. The micro dilution trays incubated at 37°C for 16-20 hours in ambient incubator prior to reading. The micro dilution trays should not be stacked more than four trays.

MIC/MBC was determined based on the growth observed in the lowest dilution of the well after streaking on MHA plate[11].

RESULT

1. **Collection and processing of Diabetic foot wound swab:**
   In the 123 patients with diabetic foot studied 75 (60.97%) were men and 48 (39.02%) were women, male-to-female ratio being 1.56:1. The DFU patients belonged to age group between 25-75 years with an average of 55.52 ± 11.64.
   Among the total patient population, 119 (96.75%) had type 2 diabetes mellitus, clinical details including Wagner grading 4 (3.25%) patients had type 1 diabetes mellitus. Out of 123 patients, 32 (26.02%) had neuropathy, 43 (34.96%) showed signs of Peripheral vascular diseases, 23(18.69%) had both complication.

2. **Characterization of Bacterial isolates:**
   Poly-microbial nature of DFU observed, 123 wound swabs in triplicate were analyzed, 10 specimens did not yield any isolates and were from Wagner grade I. almost all the patients had previous history of treatment. The organisms that were isolated from DFU are presented in table-1 Out of 171 isolates gram positive organism were 66 (38.59%) and gram negative were 97(56.72%)
   Comparison of the Wagner grading with organisms isolated. Grade II & III yield high number of bacterial isolates 82 and 41 respectively. *S. aureus* and *P. aeruginosa* were isolated predominately in Wagner grading II and III, whereas other commonly isolated pathogens includes *P. vulgaris, P. mirabilis* and *E. coli* & *Klebsiella spp.* grade of wounds.

Table - 1
Microorganisms isolated from Diabetic foot ulcer

Isolates	No. of Strain
***Aerobic bacteria*(163)**	
***Gram positive*(66)**	
***MSS*[#] (16)**	
CONS	3
S. aureus	13
***MRS*[*] (36)**	
CONS	5
S. aureus	31
Other gram positive (14)	
E. faecalis	8
E. faecium	4
E. hirae	2

***Gram negative*(97)**	
***Enterobacteriaceae* (56)**	
Lactose fermenting Enterobacteriaceae(27)	
K. pneumoniae	7
K.oxytoca	4
E. coli	11
C. freundii	2
C. koseri	1
E. aerogenes	2
Non- Lactose fermenting Enterobacteriaceae(29)	
P. vulgaris	21
P. mirabilis	8
Non-Enterobacteriaceae (41)	
P. aeruginosa	33
A. baumanii	8
Fungi(8)	
C. albicans	8
TOTAL	**171**
MSS[#]-Methicillin Sensitive Staphylococci, MRS[*]-Methicillin Resistant Staphylococci	

Antimicrobial Sensitivity Pattern:

3. Disc diffusion test:

The antibiotic sensitivity pattern of Staphylococcus spp. shown in Table-2, Antibiotic sensitivity pattern of *staphylococcus spp* (include *S. aureus* (44) and CONS (8)), showed that Oxacillin resistance, i.e., Methicillin resistant Staphylococcus (MRS), was 65.38%. Almost all the strains were sensitive to Linezolid and Vancomycin. Gentamycin and Chloramphenicol also showed good sensitivity. Enterococci were fully sensitive to Linezolid, Vancomycin and Tetracycline, were found sensitive to isolates of Enterococci routinely used drugs.

Table-3 shows the sensitivity pattern of Enterobacteriaceae. It shows a wide range of resistance to many of the drugs tested. Imipenem, Cephotaxime and Cefepime showed 25 % (14/56), 60.07% (34/56) and 55.36 % (31/56) of resistance respectively. Whereas aztreonam, and trimethoprim higher percentage of resistance 85.71%, and 75% respectively.

Antibiotic sensitivity pattern of Non-Enterobacteriaceae include *P. aeruginosa* (33) and *A. baumanii* (8) shown in Table-3 isolates of Non-Enterobacteriaceae tested of the following resistant pattern was observed Cephotaxime 39.02% (16/41), Ceftazidime 68.29% (28/41) and Cefepime 46.34% (19/41). Whereas other beta-lactams like Imipenem, Aztreonam, Ticarcillin and Piperacillin/Tazobactam showed resistant are of 36.58% (15/41),

95.12% (39/41), 80.48% (33/41), and 97.56% (40/41) respectively. Aminoglycosides like tobramycin and amikacin showed 8/33(24.2%) of resistance.

Table - 2

Antibiotic resistant pattern of gram-positive bacteria by disc diffusion n=66 (%)

Antibiotic	Staphylococcus spp.			Enterococci spp.		
	Resistant	Intermediate	Sensitivity	Resistant	Intermediate	Sensitivity
Ampicillin	-	-	-	2 (14.28)	7 (50)	5 (35.71)
Chloramphenicol	18 (34.61)	4 (7.69)	30 (57.69)	1 (7.142)	6 (42.85)	7 (50)
Ciprofloxacin	17 (32.69)	10 (19.23)	25 (48.07)	1 (7.142)	5 (35.71)	8 (57.14)
Clindamycin	20 (38.46)	6 (11.53)	26 (50)	-	-	-
Co-trimoxazole	22 (42.30)	11 (21.15)	19 (36.53)	-	-	-
Erythromycin	23 (44.23)	19 (36.53)	10 (19.23)	-	-	-
Gentamycin	25 (48.07)	2 (3.84)	25 (48.07)	2 (14.28)	6 (42.85)	6 (42.85)
Linezolid	0	0	52 (100)	0	2 (14.28)	12 (85.71)
Oxacillin	34 (65.38)	3 (5.76)	15 (28.84)	-	-	-
Penicillin	42 (80.76)	4 (7.69)	6 (11.53)	5 (35.71)	3 (21.42)	6 (42.85)
Streptomycin	-	-	-	2 (14.28)	5 (35.71)	7 (50)
Tetracycline	20 (38.46)	8 (15.38)	24 (46.15)	2 (14.28)	3 (21.42)	9 (64.28)
Vancomycin	0	0	52 (100)	0	0	14 (100)

Table - 3

Antibiotic resistant pattern of gram-negative by disc diffusion n=97(%)

Antibiotic	Enterobacteriaceae			Non-fermentative, non-Enterobacteriaceae		
	Resistant	Intermediate	Sensitivity	Resistant	Intermediate	Sensitivity
amikacin	-	-	-	9 (21.95)	5 (12.19)	27 (65.85)
amoxicillin	23 (41.07)	8 (14.28)	25 (44.64)	-	-	-
ampicillin	37 (66.07)	1 (1.78)	18 (32.14)	-	-	-
aztreonam	48 (85.71)	5 (8.92)	3 (5.35)	39 (95.12)	2 (4.87)	0
cefepime	31 (55.35)	15 (26.78)	10 (17.85)	19 (46.34)	7 (17.07)	15 (36.58)
ceftazidime	36 (64.28)	11 (19.64)	9 (16.071)	28 (68.29)	4 (9.75)	9 (21.95)
cephotaxime	34 (60.71)	9 (16.07)	13 (23.21)	16 (39.02)	19 (46.34)	6 (14.63)
ciprofloxacin	23 (41.071)	17 (30.35)	16 (28.57)	11 (26.82)	2 (4.87)	28 (68.29)
gentamicin	30 (53.57)	3 (5.35)	23 (41.07)	-	-	-
imipenem	14 (25)	15 (26.785)	27 (48.21)	15 (36.58)	6 (14.63)	20 (48.78)
piperacillin /tazobactam	-	-	-	40 (97.56)	1 (2.43)	0
tetracycline	40 (71.42)	5 (8.92)	11 (19.64)	-	-	-
ticarcillin	-	-	-	33 (80.48)	4 (9.75)	4 (9.75)
tobramycin	-	-	-	9 (21.95)	6 (14.63)	26 (63.41)
trimethoprim	42 (75)	9 (16.07)	5 (8.92)	-	-	-

Enterobacteriaceae^ -include *E. coli, P. mirabilis, P. vulgaris K. pneumoniaee, K. oxytoca, E. aerogenes, C. freundii, and C. koseri*

4. MIC by Micro-broth dilution method:

The micro-broth dilution method done for gram-positive (oxacillin) and gram negative (i.e., cephotaxime, ceftazidime and Imipenem) bacterial pathogen, the result were shown in table-4.

Dilution range of 1 to >32 µg/ml for Oxacillin and 1-256 µg/ml for Cephotaxime, Ceftazidime and Imipenem were used. According to micro-broth dilution method, 29.35% of MSSA and 70.45% are MRSA. This is comparatively much higher when compared with coagulase negative staphylococci; it showed 62.5% (5/8) resistant and 37.5% (3/8) sensitive to methicillin.

From 56 Enterobacteriaceae isolates, 32.14% (18/56), 21.42% (12/56) and 46.42% (26/56) were susceptible, intermediately resistant and resistant to cephotaxime, respectively. For ceftazidime, 23.21% (13/56), 21.42% (12/56) and 55.35% (31/56) were susceptible, intermediately resistant and resistant, respectively. For Imipenem, 55.35% (31/56), 23.21% (13/56) and 21.42% (12/56) were susceptible, intermediately resistant and resistant, respectively.

P. aeruginosa showed 54.54% (18/33) isolates tested were resistant, 24.24% (8/33) isolates were intermediately resistant and 21.21% (7/33) isolates were sensitive to Cephotaxime, 63.63% (21/33) isolates were resistant, 6.60% (2/33) were intermediately resistant while remaining isolates 30.30% (10/33) were sensitive to Ceftazidime. In case of Imipenem, 13/33 isolates tested were resistant, another 7/33 isolates were intermediately resistant While 13/33 isolates were found as sensitive.

A. baumanii showed 12.5% (1/8), 37.5% (3/8) and 50% (4/8) were susceptible, intermediately resistant and resistant to cephotaxime, respectively. For ceftazidime, 25% (2/8), 37.5% (3/8) and 37.5% (3/8) were susceptible, intermediately resistant and resistant, respectively. For Imipenem, 50% (4/8), 12.5% (1/8) and 37.5% (3/8) were susceptible, intermediately resistant and resistant, respectively.

Table - 4
MIC by Micro-broth dilution method

S.no	Antibiotic concentration (µg/mL)	*P. aeruginosa*			Enterobacteriaceae^			*A. baumanii*			*S. aureus*	CONS
		CTX*	CAZ**	I***	CTX*	CAZ**	I***	CTX*	CAZ**	I***	Oxa#	Oxa#
1	256	13	2	5	9	4	1	1	3	1	-	-
2	128	4	9	4	13	15	3	3	0	0	-	-
3	64	1	8	0	4	9	2	0	0	0	-	-
4	32	4	2	1	8	3	4	2	0	0	7	-
5	16	4	2	3	4	12	2	1	3	2	2	-
6	8	2	1	7	6	3	13	0	0	1	16	2
7	4	3	5	6	7	6	18	1	1	1	6	3
8	2	2	2	7	5	4	13	0	1	3	12	1
9	1	0	2	0	0	0	0	0	0	0	1	2
Total		**33**	**33**	**33**	**56**	**56**	**56**	**8**	**8**	**8**	**44**	**8**

*CTX-Cephotaxime, **CAZ-Ceftazidime, ***I-Imipenem, #Oxa- Oxacillin, **Enterobacteriaceae^** - include *E. coli, P. mirabilis, P. vulgaris K. pneumoniaee, K. oxytoca, E. aerogenes, C. freundii, and C. koseri* and shaded- Intermediately resistant

DISCUSSION

Diabetes mellitus recognized to be common in Indians of the Asian subcontinent. Currently, 50.8 million Indians have diabetes. The projections indicate that India will have the largest number of diabetic patients by the year 2030AD[32]. Diabetic foot infection is a common cause for the hospital admissions of the diabetic patients and caused by a number of sociocultural practices in India[34]. Such practices include bare foot walking, inadequate facilities for diabetic care, low levels of education, and poor socioeconomic conditions[32].

Out of 123 patients, 32 (26.02%) had neuropathy, 43 (34.96%) showed signs of peripheral vascular diseases, 23(18.69%) had both complications. Several studies conducted over the world have reported 50% of them primarily affected by diabetic neuropathy and vascular disease, and 30% afflicted with both conditions [6 & 30]. Gin (1993)[17] conducted a study in south Indian diabetic population documented predominance of diabetic polyneuropathy (56.8%) among all of the other associated co-morbid conditions of diabetes mellitus. In addition, this condition reported to be the prime factor involved in the initiation of an infection in the deeper tissues of the diabetic foot upon initial bacterial exposure[8].

For our study, showed comparatively less common i.e. out of 123 patients, 32 (26.02%) had neuropathy, 43 (34.96%) showed signs of Peripheral vascular diseases, 23(18.69%) had both complication foot ulcers on individuals with diabetes.

In the present study, 171 organisms were isolated from 123 patients and an average of 1.33 organisms per case found. This is slightly higher than the findings by Vishwanathan *et al.,* 2002. Where culture yielded an average of 1.21 per case, Poly-microbial nature of diabetic foot infections observed in various studies in the subcontinent and abroad [3, 16 & 30].

Among 171 isolates 66-gram positive isolates, 97-gram negative isolates, and remaining were candida albicans (8). Studies from western countries show that Gram-positive aerobes are the predominant organisms isolated from DFI [10, 12, 18 & 24]. In contrast, two recent Indian studies have shown a preponderance of Gram-negative aerobes. Gadepalli[15] *et al.,* 2006, in their study on 80 ulcer specimens, recovered 183 isolates, of which 28.7% were Gram-negative and only 13.8% Gram-positive.

According to Dhanasekaran[13] *et al.,* 2003, documented that 84% of diabetic foot ulcers are mono-microbial, in contrast to our findings. Studies by Viswanathan[33] *et al.,* 2002, from South India, reported 35% gram-positive pathogens isolated and 65% gram-negative ones, these finding emphasizing the high prevalence of gram-negative pathogens in Southern India. Three large diabetes research centers (India, Germany, and Tanzania) have obtained very similar results.

The difference observed in the prevalence of Gram-negative bacilli in DFI between diabetic patients from eastern and western countries remains largely unknown. However, environmental factors such as sanitary habits, e.g. use of water for peri-anal wash (ablution) after defecation leading to contamination of hands with fecal flora, are proposed to be responsible for increased Gram-negative infections in the developing world compared. Similar findings reported in continuous ambulatory peritoneal dialysis (CAPD) peritonitis by Prasad[29] *et al.,* 2004.

We have observed in our study that the Wagner grading II and III yielded higher isolation of microorganisms. This similar pattern of result observed with the studies

conducted by Gonzatez[19] *et al.,* 2003, from Spain, Sharma[31] *et al.,* 2006, from Katmandu wherein they had also seen predominant isolation in Wagner grading II and III of DFU.

Since wound swabs of the present study was collected from a tertiary care hospital and awareness programme is also being conducted by the hospital, we have not collected samples frequently from Wagner IV and V. Gram negative organisms were isolated from almost all the grades of ulcer.

The result of antibiotic disc diffusion showed that all the 52 isolates of *Staphylococcus* showed uniform sensitivity to Linezolid and Vancomycin. The other drugs showed resistance between 32-80%. Methicillin resistant *Staphylococcus aureus* (MRSA) found to be 70.45% (31/44), out of eight, 5 isolates (62.5%) of CONS found resistant to Methicillin Resistant.

The rate of MRSA in the present study was found to be slightly higher than the studies conducted by Gadepalli[15] *et al*, 2006 and Bansel[5] *et al*, 2008, where 56% and 55.56% of MRSA respectively were reported.

Though we have observed a higher percentage of MRSA in our study, the isolates were uniformly susceptible to vancomycin, the incidence of vancomycin-intermediate S. aureus (VISA) and vancomycin-resistant S. aureus (VRSA) has been increasing in various parts of the world. The first clinical isolate of VRSA reported from the United States in 2002[9].

E. faecalis was the common isolate than *E. faecium* as seen in all other clinical specimens. *Enterococci spp* showed uniform susceptibility to Vancomycin and Linezolid. The strains, which showed intermediate sensitivity to Linezolid and Vancomycin by disc diffusion, also found to be sensitive to the drugs by disc diffusion; the result is similar to that of studies reported by Abdulrazak[1] *et al.,* 2005.

Gram Negative organisms showed a varied pattern of resistance in disc diffusion method. Enterobacteriaceae showed wide range of resistance to many of the drugs tested. Imipenem, Cephotaxime and Cefepime showed 25 % (14/56), 60.07% (34/56) and 55.36 % (31/56) of resistance respectively. Whereas Aztreonam, and Trimethoprim higher percentage of resistance 85.71%, and 75% respectively (Table-3).
Among the gram-negative bacteria, *P. aeruginosa* showed resistance to more than two drugs tested (multidrug resistance). 41 isolates of Non-Enterobacteriaceae includes *P. aeruginosa* (33) and *A. baumanii* (8) were tested of the following resistant pattern was observed Cephotaxime 39.02% (16/41), Ceftazidime 68.29% (28/41) and Cefepime 46.34% (19/41). Whereas other beta-lactams like Imipenem, Aztreonam, Ticarcillin and Piperacillin/Tazobactam showed resistant are of 36.58% (15/41), 95.12% (39/41), 80.48% (33/41), and 97.56% (40/41) respectively. Aminoglycosides like Tobramycin and Amikacin showed 9/41(21.95%) of resistance (Table-4).

However Gadepalli *et al.*, 2006 showed different pattern *P. aeruginosa* where both Cephotaxime and Ceftazidime having same resistance of 61.1%, Piperacillin/Tazobactam (72.2%). Whereas our study found different resistant pattern for Cephotaxime as (30.3%) with Ceftazidime as (69.7%).The Piperacillin/Tazobactam showed 100% resistant pattern almost in concordance with that of showing resistant pattern of (92%). Apart from these various resistant patterns toward commonly used antibiotics a different observation was reported by Bansal[5] *et al*., 2008 showing sensitivity to Amikacin, Ceftazidime and Pipercillin.

Moreover, few author reported 100% sensitivity pattern to Imipenem[5 & 15], in our study showed 25 % (14/56) Enterobacteriaceae and

36.82%(15/41) for non-Enterobacteriaceae were resistant.

Comparison of MIC and disc diffusion method for antibiotic sensitivity test of Enterobacteriaceae against 3 drugs were cephotaxime, ceftazidime and imipenem. Out of 26/56 resistant to MIC and same for disc diffusion also, 12/56 isolates showed intermediately resistant to MIC among these 7/56 resistant and 5/56 were sensitive to disc diffusion method of cephotaxime. Second drug Ceftazidime MIC showed 31/56, 12/56 resistant, 13/56 intermediate was resistant, intermediately resistant, and sensitive; they compared with disc diffusion with same drug. When tested for non-Enterobacteriaceae gram negative isolates. For example, Intermediate, resistance, sensitive observed in disc diffusion for cephotaxime actually showed resistance in MIC increasing the number of isolates from 16 in disc diffusion in MIC it showed 18 in numbers, It showed varied results

129/171(75.44%) multidrug resistance isolates found out of 123 specimens in our study, it include gram-positive and gram-negative pathogen. The erosion of effective antimicrobials continues as we witness the increased frequency of resistance to all drugs—in particular, the fluoroquinolones, vancomycin and carbapenems, which are often the drugs of last choice. New drugs that can combat MDR Gram-positive bacteria have averted forthcoming crises. With the relative absence of new antimicrobials coming to market and with new threats arising from the Gram-negative bacteria, however, the number of drug options leaves us perilously close to none or only a single effective agent for some life-threatening infections. Hundreds of Beta-lactam-degrading enzymes are rapidly undermining the mainstay penicillins and late-generation cephalosporin agents. The increase in metallo-beta-lactamases, which are active against carbapenems and most other Beta-lactams, is an alarming new development[25].

CONCLUSION

The salient features of the present study are Gram-negative isolates are predominant from wound specimens. We have not found resistance to Linezolid and Vancomycin by disc diffusion and MIC, they are strict found to be highly sensitive to the group especially *S. aureus*. In gram negative Ciprofloxacin and Tobramycin found better sensitivity and ceftazidime showed higher percentage of resistance by MIC. The observed difference between disc diffusion and MIC studies re-emphasizes the importance of MIC determination in case of diagnosis and management of the important pathogens.

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