Bacteriological Profile and Prevalence of ESBL and MRSA in Different Risk Categories in Diabetic Foot Infections (DFI) in a Teaching Hospital, Visakhapatnam, A.P.

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ABSTRACT

Introduction: Diabetes Mellitus (DM) is the 6th leading cause of death by 2015 and one of the leading causes of blindness, amputations and kidney failure. Our objective was to establish the variation of bacteriological profile and drug resistance prevalence, specifically extended spectrum beta lactamase (ESBL) producing gram negative bacilli (GNB) and methicillin resistant staph aureus (MRSA) among various risk categories of DFI patients to guide empirical antibiotic policy.

Material and methods: In this observational study done in our teaching hospital i.e. NRIIMS, Sangivalasa village, Visakhapatnam, A.P, for 2 months (June to July 2015), we got sample size of 48 DFI Patients. Specimens were collected prior to antibiotic therapy. Culture and antibiotic sensitivity was done. Screening for MRSA and ESBL producing GNB were done.

Results: Among total isolates (68), GNBs 46(68%) were predominant with a Ratio of 1:2 between GPC (22) and GNB (46). MDROs were 51(75%). In category I among 15 isolates, GNB were 10 (67%) with 7 ESBL+ve among GNB (70%). GPC were 5 (33%) with1MRSA (20%). In category II among 20 isolates 13(65%) were GNBwith10ESBL +ve (77%).

Conclusion: Risk Stratification of DFI patients is the crucial step in assessing MDRO risk and selection of Right choice of empirical Antibiotic before AST report arrives.

Keywords: MRSA, ESBL Producing GNB, MDRO, Observational Study, Diabetes Mellitus, Risk Stratification, Empirical Antibiotic Therapy.

INTRODUCTION

Diabetes Mellitus (DM) is the 6th leading cause of death worldwide by 2015 and one of the leading causes of blindness, amputations and kidney failure. DM is an emerging global epidemic, responsible for 422 million diabetics in 2017 and expected to reach a total of million by 2025.According to WHO's ten facts about diabetes, it is an emerging global epidemic, responsible for 1.6 million deaths in 2015.[1] About 2/3rds of diabetics live in developing countries, where the epidemic is most intense with an increasing proportion in younger age groups[2].

Among the various chronic complications associated with DM, Diabetic Foot Infection (DFI) is particularly considered as the main cause of hospital admission and also a cause of prolonged hospitalization. This is often a challenging clinical problem, in some cases the initial presentation of undiagnosed DM.

About 82,000 people have diabetes related amputations of feet and lower extremities each year as indicated by world population data.[3] 14-20% of diabetic patients with DFI undergo an amputation, while 85% of amputations are preceded by DFIs.[4]

The risk factors for severe and complicated DFIs are past history of DFI, grade of the ulcer, overall glycaemic control, previous hospitalizations, and presence of infection with more virulent microorganisms like Multi-Drug Resistant Organisms (MDRO).[5, 6]

The major problem in the management and study of DFI is that there is no unifying standard in diagnosing the infection, in scoring the DFIs and no universal guidelines for therapy. There is a lack of evidence in developing guidelines for therapy of DFI. Though culture supported standard specific targeted therapy is the gold standard, there must be a role for initial empirical therapy.[7]

Since most of the people from rural areas are bare footed and agricultural field workers, where studies on DFIs are scarce, we in this prospective study (from rural Visakhapatnam, Andhra Pradesh, India) have tried to elaborate the bacteriological profile of DFIs with special reference to MDROs. We also tried to establish a Risk Stratification (RS) to easily categorize DFI patients into three categories depending on the presence of different risk factors. These categories can guide the clinician to make a right choice of empirical antimicrobial therapy.

Our objective was to study the variation of bacteriological profile and prevalence of drug resistance among 3 different risk categories for DFI patients. This can guide the clinician in selecting the "Right Empirical Antibiotic", before the AST report arrives.

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MATERIAL AND METHODS

This was a hospital based observational study done in our teaching hospital i.e. NRI Institute of Medical Sciences (NRIIMS), Sangivalasa village, Visakhapatnam district, A.P, India. Study was conducted for 2 months from June 2015 to July 2015with a sample size of 48 DFI Patients after obtaining Institutional ethical committee clearance.

Inclusion and exclusion criteria:

Patients with Wagner's grade 2 to 5 DFIs are included in this study.

General workup: Samples were collected from DFI patients prior to the antibiotic therapy. An Informed consent was taken from all DFI Patients after explaining the patient in detail in his own language about the study and his role in the study. Required information as medical history and examination findings are collected and entered into Questionnaire form. Sample collection is done by the surgeons under strict aseptic techniques and followed from the time of collection till they reach the microbiology culture laboratory. Sample processing techniques like direct smear examination, culture and sensitivity are followed regularly and final reports are collected onto the Case Report Form. Wherever necessary p value for significance was calculated with Chi-Square Test.

Specimen collection: Chronic wounds can be colonized on the surface by a varied group of organisms, including aerobic Gram-positive cocci, Enterobacteriaceae, non-fermenter GNB and anaerobic bacteria. Isolates from superficial swab cultures may not represent the underlying infecting pathogen.[8] Therefore, after the debridement of superficial eschar, cultures are obtained which is best to guide targeted antibiotic therapy.[9]

Microbiological workup: The tissue was placed in 2 ml of sterile physiologic saline and then homogenized in a tissue grinder and divided into 3 portions.[10] 1st portion was used for preparing direct smears.2nd portion was plated on Blood agar plates with 5% sheep blood and Mac Conkey medium. 3rd portion was inoculated into Brain Heart Infusion (BHI) biphasic medium. All media were incubated at 37C for 18-24 hours and Identification of isolates is done by using specific Bio-chemical Reactions like Catalase, Coagulase, Indole, Methyl Red, Citrate, Urease, Triple Sugar Iron agar, Oxidase etc. A single, separated colony of the test organism was picked and emulsified in 0.9% normal saline to match the turbidity with 0.5% McFarland's standard. AST is done by standard Kirby- Bauer disc diffusion method (followed CLSI recommendations).[11]

Screening for MRSA: Screening for MRSA and MRCONS was done by testing sensitivity of all Coagulase positive and Coagulase negative Staphylococcal isolates against 30 µg Cefoxitin disk.

Screening for ESBL producing GNB: Strains were screened using double disc diffusion technique. An antibiotic disc containing amoxicillin-clavulanate (20/10µg) as inhibitor of beta lactamase was placed in the centre of the plate. Cefotaxime (30µg), ceftazidime (30µg), ceftriaxone (30µg) and aztreonam (30µg) discs were placed at a distance of 30 mm from the central disc as well as from each other. Zones of inhibition around the 3rd generation cephalosporin discs and aztreonam were observed after overnight incubation at 37°C. If the inhibition zone around one or more cephalosporin discs and aztreonam was extended on the side nearest to Amoxicillin- clavulanate, the organism showing this synergism was identified as an ESBL producer. Escherichia coli ATCC 25922 and Klebsiella pneumonia ATCC 700603 were used as controls.

ESBL confirmation: is with Double Disc Synergy test using ceftriaxone- Clavulanic and 20 + 10µg and ceftriaxone 30µg, considered positive when there is more than 5mm difference between the sensitivity zones of the two discs.

This is a hospital based observational study in which we tabulated our findings and compared our results with other studies and also with respect to risk category of DFI.

RESULTS

A Total of 54 DFI Patients were admitted in our teaching Hospital (NRI Institute of medical sciences, Sangivalasa, Visakhapatnam district, Andhra Pradesh, India), from which 06 patients were excluded (Wagner's Grade 1 ulcer) and only 48 DFI Patients were included in our study. From these 48 DFI Patient samples, 68 bacteria were isolated (Table-1). There were 51 MDROs from 68 isolates (i.e.75%MDROs) from our study. Prevalence of MRSA among 68 isolates - 4 (6%).Prevalence of ESBL+ve GNB among 68 isolates- 35(51.5%).

All DFI Patients were categorized into 03 categories depending on "Risk Stratification Criteria" (Table-2) in which 5 Risk factors which have a significant influence on the DFIs with MDRO were considered as the criteria to assess each DFI patient's MDRO Risk. They were:

1. Age of the Patient (years)
2. Duration of Diabetes (in years)
3. Previous hospitalizations
4. Multiple Antibiotic usages (in the past 1 year)
5. Associated co-morbid conditions like Chronic infectious diseases (Hepatitis B, Tuberculosis); Chronic complications of Diabetes (Neuropathy, Nephropathy, Retinopathy, Peripheral vascular disease).

All the 68 bacterial isolates were grouped into GPCs and GNBs and their prevalence rates and MDRO rates were calculated and showed in Table-3.Among the 68 Bacterial isolates from our study, 22(32%) were GPC, 46(68%) were GNB. Among the 22 GPC,8 were Staphylococcus aureus and 4(50%) of them were MRSA,8 were Coagulase negative Staphylococci (CONS) and 5(63%) of them were MRCONS,6 were Enterococci and 5 (83%) of them were MDR Enterococci.

Among the total MDROs (51), 14 were GPC (27%) where as GNB were 37 (73%). Out of these 35 were ESBL producing GNBs.

Among the 46GNB, 15 were Escherichia coli of which12(86%)were ESBL+ve.15 were Proteus species of

Number of samples	Number of isolates	Number of MDRO	MDRO (%)	ESBL (%)	MRSA (%)
48	68	51	75%	51.5%	**6%**

Table-1: Prevalence of Multi-Drug Resistant Organisms

Factors	CAT-1	CAT-2	CAT-3
Age	<40	40-65	>65 Years
Duration of Diabetes	0-10yrs	10-20yrs	>20 Years
Previous Hosp. exposures	No exposure	Minimal Exposure	Frequent exposure
Multiple antibiotic usages	No' usage in the near past	Minimal usage	Frequent usage
Co-morbidities	No Co-Morbidities / DM complications /Chronic Infection	Minimal co-morbidities Chronic infections like- Hepatitis B and Tuberculosis	Complications of DM - neuropathy/ nephropathy/ PVD/ Retinopathy/ Terminal illness like cancers.

Table-2: Risk Stratification Criteria for DFI Patients

Bacteria type	Number of isolates n=68	Multi drug resistant bacteria (MDRO) n=51
Gram positive COCCI:	22 - (32%)	14 - (64%)
Staphylococcus aureus	8 - (12%)	4 -(50%)-MRSA
CONS	8 - (12%)	5 -(63%)-MRCONS
Enterococcus species	6 - (9%)	5 -(83%) -MDR ENTERO
Gram negative bacilli	46 - (68%)	35-(76%)ESBL +ve
Escherichia coli	15 - (22%)	12-(80%) ESBL+ve,
Proteus species	15 - (22%)	9-(60%)ESBL+ve
Klebsiella species	6 - (9%)	6-(100%)ESBL+ve
Pseudomonas species	5 - (7%)	3-(60%) ESBL +ve
Enterobacter species	2 - (2%)	2-(100%)ESBL+ve
Acinetobacter species	3 - (4%)	3-(100%) ESBL+ve

Table-3: Bacteriological profile and their MDRO status among the isolates of DFI patients

Risk stratification category	CAT-1	CAT-2	CAT-3
1. Number of population in each category (n=48)	14	17	17
2. Number of isolates from each category (n=68)	15(22%)	20(29%)	33(49%)
3. Number of GPC (N=22)	5(23%)	7(32%)	10(45%)
4. Number of GNB (N=46)	10(22%)	13(28%)	23(50%)
5. Number of MDR GPC (N=14)	1(7%)	4(29%)	9(64%)
6. Number of MRSA (N=4)	1(25%)	1(25%)	2(50%)
7. Number of MRCONS(N=5)	0	2(40%)	3(60%)
8. Number of MDR enterococcus (N=5)	0	1(20%)	4(80%)
9. Number of MDR GNBs (N=37)	7(19%)	11(30%)	19(51%)
10. Number of ESBL+VE GNB (N=35)	7(20%)	10(29%)	18(51%)

Table-4: RS category wise - no. of patients, isolates and MDROs.

which 9(60%) were ESBL+ve.06 were Klebsiella species; all6 (100%) were ESBL+ve.05 were Pseudomonas species of which 3(60%) were ESBL +ve.03were Acinetobacter species of them all 3 (100%) were ESBL+ve.02 were Enterobacter species, all 2 (100%) were ESBL+ve

Table-4: From 48 patients, after analysing their RS criteria. 14 DFI Patients (with 15 isolates) were considered in Category-1. 17 DFI Patients (with 20 isolates) were considered in Category-2.17 DFI Patients (with 33isolates) were considered in Category-3.

From a total of 04 MRSA – 02MRSA (50%) were from Cat-3Patients.From a total of 05 MRCONS – 03 MRCONS (60%) were from Cat-3 patients.From a total of 05 MDR ENTERCOCCUS-04 MDRENTEROCOCCUS (80%) were from Cat-3 patients. From a total of 37 MDR GNBs – 16MDR GNBs (43%) were from Cat-3 patients.

DISCUSSION

In our present study from rural Visakhapatnam of Andhra Pradesh, from DFI patients GNBs (66%) were the predominant bacteria, the ratio between GPC (22) and GNB (45) was1:2.Prevalence of MDRO (51) from the total isolates (68) is 75%.Among the 46 GNBs, 35 were ESBL+ve comprising 76%. Among total MDROs (51) isolated in

our study, 35 were ESBL+ve, coming to 69%.Among 8 Staphylococcus aureus, 4 were MRSA i.e. 50%, prevalence among 68 total isolates was 6%.

In our present study we grouped the study population according to the Risk Stratification into three different categories to assess the MDRO risk depending on the factors Age, Co-morbidities, Duration of Diabetes, Previous hospitalizations and multiple antibiotic usages in the near past.[12] This is the first thing to be done by the clinician to select the right antibiotic against the likely pathogens for that RS category of DFI patients.[13] We grouped the study population (48) and isolates (68) into these 03 RS categories and MDROs rates were calculated.[14] Number of isolates per patient in our study was 1.42; whereas in a study by Mohammad Zubair[15](2010) it was 1.2; in a study by Gadepalli[16] (2006) it was 2.3.

In RS Categories, there was a predominance of MDR GNBs, MDR GPCs (MRSA, MRCONS, and MDR Enterococci) towards category 3.[14]

There were plenty of studies on bacterial profile of DFIs; some of them were compared with our Results. %GPC in our study was 22% while that in GADEPALLI study[16] (2006) it was 33.3% and in RICHARD[17] study (2008) it was 59%.%GNB in our study was 68% while that in GADEPALLI[16] study (2006) it was 51% and in RICHARD[17] study (2008) it was 34%.% MDRO among GPC in our study was 64% while that in GADEPALLI[16] study (2006) it was 56% and in RICHARD[17] study (2008) it was 63%.% MDRO among GNB in our study was 83% while that in GADEPALLI [16] study (2006) it was 45% and in RICHARD[17] study (2008) it was 34%.

In our study GPCs (32%) were less in comparison with other 02 studies (J.L.Richard[17], Gadepalli et al[16]); whereas GNBs (66%) were high in number. MDROs among GPCs from our study were the least when compared with other studies but MDROs among GNBs were very high (~ 2 times).

There was an overall predominance of GNBs in our study. GPC: GNB= 1:2. This is in correlation with results of an Indian study by Gadepalli[16] (2006) from Delhi, AIIMS. They found a ratio of 1:1.5(GPC: GNB).This differs from the results of a French study by J.L.Richard[17] (2008) which showed a ratio of 1:0.5(GPC: GNB), where we can notice the predominance of GPCs.

Highest numbers of isolates (33 out of 68 i.e.49%) were reported from category-3 ulcers. There is increasing trend noticed in Isolate/ Patient ratio from category 1 to 3 i.e.1.1: 1, 1.2:1 and 1.9:1 respectively. Also increasing trend in GPC and GNB. Increasing trend is noticed from Category 1 to Category 3 in number of Multi drug resistant (MDR) GPC and GNB

CONCLUSION

Risk Stratification of the patient into different Categories depending on the Factors associated with is the crucial step, from which the risk of harbouring MDROs in DFI Patients can be assessed and the "Right Choice" of Antibiotic can be made. The strict and vigilant Policy guided Antibiotic usage is the only measure that can curtail the pandemic of Drug Resistance.

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