

Surveillance on Multi Drug Resistant Organism (MDRO) associated with Diabetic Foot Ulcers in Pondicherry

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Citation

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Abstract

Aims: The primary objective of the study was to establish the association of Multi Drug Resistant Organism (MDRO) in Diabetic Foot Ulcers (DFU) in the patients attending Aarupadai Veedu Medical College & Hospital, Pondicherry. **Material & Methods:** The study period was 2 years- where about 560 pus & debrided tissue samples from patients with diabetic foot infections were collected and processed. **Results:** Out of 560 samples (329 males & 231 females) evaluated, 68 (85 %) specimen showed presence of Multi Drug Resistant Organism (MDRO) isolates and it also includes mixed infection in 63 specimens (10.58%). Of these 560 specimens gram positive isolates constituted about 224 (47.1%) and gram negative isolates constituted about 371 (77.94%). Among the 224 gram positive isolates 126 (21.17%) isolates were *Staphylococcus aureus* of which 28 (22.2%) were Methicillin resistant *Staphylococcus aureus* (MRSA) and among the 371 gram negative isolates *Proteus* spp was the predominant isolate - 126 (21.17%). The others were *Klebsiella* spp- 84 (14.11%), *Pseudomonas* spp - 84 (14.11%), *Escherichia coli* - 63 (10.5 %), *Enterococci* spp.-56(9.41%) *Coagulase Negative Staphylococcus* - 28 (4.70%) *Streptococci* spp & *Citrobacter* spp 14 (2.35%) each respectively. All these isolates were found to be Multi Drug resistant Strains; among the gram positive isolates, all the isolates showed resistance to Erythromycin (100 %). Among the gram positive isolates *Staphylococcus aureus* showed high level of resistance to most of the antibiotics - Penicillin (88.9 %), Cotrimoxazole (77.8 %) and among the gram negative isolates *Pseudomonas* showed highest resistance - Ampicillin (83.3%), Cefuroxime - 58.3 %, Cotrimoxazole-44.4%. **Conclusion:** The commonest isolates were *Staphylococcus aureus* & *Pseudomonas* spp. The drug resistance rate was comparatively higher among the gram positive organism than the gram negative organism.

INTRODUCTION

In the developing countries like India, one of the most important health problem is Diabetes mellitus with significant population being affected across the country. Among the diabetic individuals, foot infections are the most frequent complication in the patient, accounting for 20 % of diabetic related hospital admissions¹. Diabetic Foot Infection [DFI] or Diabetic Foot Ulcer [DFU] is defined as the infection caused by the introduction of infectious agent into other wise sterile soft tissue of the foot through minor skin break. Infectious agents are usually associated with worst out come, which might lead to amputation of the infected foot unless prompt treatment strategies are ensued. Though many studies have reported on the bacteriology of diabetic foot infections (DFIs) over the past 25 years, the results have varied and have often been contradictory. So, conducting surveillance study at equal intervals is a must to assess & update the condition prevailing. This study was designed to evaluate the current status of deep tissue microbiology of Diabetic Foot Ulcers and also to assess the

drug resistance pattern of the Multi Drug Resistance Organism [MDRO] associated with DFI.

MATERIAL AND METHODS

The study was planned for a period of 2 years, patients with diabetic foot infections were assessed for bacteriological spectrum and their drug resistance pattern to various antibiotics was also recorded. 560 pus specimens as well as debrided tissues from 329 male (58.75%) & 231 female (41.25%) patients belonging to Pondicherry were collected & processed as per the standard routine procedures. Simultaneously, during sample collection, the wounds were graded based on Wagner's grading of foot wounds² (Photo 1, 2,3)

Figure 1

Photo 1: Diabetic Foot Ulcer – Wagner's grade 3



Figure 2

Photo 2: Diabetic Foot Ulcer – Wagner's Grade 4 (post amputation)



Figure 3

Photo 3: Diabetic Foot Ulcer – Wagner's Grade 5



As a part of the study a complete history of the patient was collected from the patients, which is enlisted and compared in Table 1

Figure 4

Table 1: Profile of the study characters of the patient.

Characteristics	Male		Female		Total	
	No	%	No	%	No	%
No. of patients	329	58.75	231	41.25	560	100
No. of patients infected	301	91.48	175	75.75	476/560	85
Age of patients screened						
Less than 50 years	63/329	19.14	42/231	18.18	105/560	18.75
Between 51 – 59	98/329	29.78	77/231	33.33	175/560	31.25
More than	168/329	51.06	112/231	48.48	280/560	50
Age of infected patients						
Less than 50 years	42/301	13.95	14/175	8	56/476	11.76
Between 51 – 59	91/301	30.23	56/175	32	147/476	30.88
More than	168/301	55.81	105/175	60	273/476	57.35
No. Diabetic patients screened						
a. Type 1	49/329	14.89	14/231	6.06	63/560	11.25
b. Type 2	280/329	85.10	217/231	93.93	497/560	88.75
No. of diabetic patients infected						
a. Type 1	42/301	13.95	7/175	4	49/476	10.29
b. Type 2	259/301	86.04	168/175	96	427/476	89.70
No. Patients screened with ulcer for Duration of						
a. Less than 3 months	252/329	76.59	196/231	84.84	448/560	80
b. More than 3 months	77/329	23.40	35/231	15.15	112/560	20
No. Of Patients infected with ulcer for Duration of						
a. Less than 3 months	231/301	76.74	147/175	84	378/476	79.41
b. More than 3 months	70/301	23.25	28/175	16	98/476	20.58

The collected specimen was processed by performing Gram stain from the direct smear, inoculating the specimen onto culture media like Blood Agar, Macconkey Agar, Brain Heart Infusion Agar. The bacterial isolates grown on the media were confirmed by conventional biochemical tests

and antibiotic susceptibility was tested against antibiotics used in treatment of gram positive organism & gram negative organisms. the antimicrobial susceptibility testing was done by Kirby Bauer disc diffusion method₃

RESULT

Among the 560 specimens processed, about 476 (85%) samples yielded pathogenic organisms. No organisms were isolated from 84 samples (15%). All the organisms isolated from the specimens were Multi Drug Resistant Organism (MDRO). The specimens yielded 224 (47.1 %) gram positive isolates and 371 (77.94%) gram negative isolates. The most common isolates were Staphylococcus aureus and Proteus spp, both constituting about 126 (21.17 %) isolates each respectively. Of the 126 strains of Staphylococcus aureus 28 (22.2 %) were found to be MRSA. The other gram positive isolates found to be associated were Enterococci spp. – 56 (9.4 %), Coagulase Negative Staphylococci [CONS] -28 (4.70 %). Streptococcus pyogenes – 14 (2.35 %) & the remaining gram negative isolates were Klebsiella spp – 84 (14.11 %), Pseudomonas spp – 84 (14.11%), E.coli-63 (10.5 %), Citrobacter – 14 (2.35 %). (Table 2)

Table 2: Comparative frequency of the organisms isolated

Figure 5

No. of specimens: 560

No. Positive specimens: 476/560 (85 %)

No. of no growth: 84/560 (15 %)

Total number of organism isolated: 595

Organism	No. of isolates	Percentage %
Gram positive isolates	224/476	47.1
Staphylococcus	126/595	21.17
Enterococci	56/595	9.41
Coagulase Negative Staphylococcus [CONS]	28/595	4.70
Streptococci	14/595	2.35
Gram negative isolates	371/476	77.94
Proteus	126/595	21.17
Klebsiella	84/595	14.11
Pseudomonas	84/595	14.11
E.coli	63/595	10.5
Citrobacter	14/595	2.35
Mixed infections	63/595	10.58
Pseudomonas + Klebsiella	14/595	2.35
E.coli + Staphylococcus	13/595	2.18
E.coli + Klebsiella	12/595	2.01
Enterococci + Pseudomonas	9/595	1.51
Proteus+Staphylococcus	8/595	1.34
E.coli+Proteus	7/595	1.17

A total of 413 (73.75 %) of the specimens yielded single organism infection. Apart from single isolates, few

specimens had polymicrobial involvement. Mixed infection was seen in 63 (10.58 %) specimens. The combinations were E.coli + Staphylococcus aureus – 13 (2.18 %), E.coli+ Klebsiella spp-12 (2.01 %), Pseudomonas spp+ Klebsiella spp-14 (2.35 %), E.coli + Proteus spp-7 (1.17 %), Proteus spp + Staphylococcus aureus -8 (1.34 %), Enterococci + Pseudomonas – 9 (1.51 %).

All the isolates were Multidrug Resistant Organisms (MDRO). The MDROs exhibited different ranges of antibiotic resistance pattern (ie) among the gram positive isolates 100 % of the isolates exhibited resistance to Erythromycin. Staphylococcus aureus showed resistance to penicillin – 112 (88.9%), Cotrimoxazole – 98 (77.8%), Gentamicin – 63 (50 %). Enterococci spp. showed highest resistance to penicillin –35(62.5 %) & CONS showed resistance to Cotrimoxazole – 21 (75 %). When compared to resistance pattern of the other gram positive cocci, Streptococci spp. was relatively sensitive to most of the antibiotics except to Erythromycin. Among the gram negative isolates, Pseudomonas showed the highest resistance level - Ampicillin- 70 (83.3 %), Amikacin – 56 (66.6 %) Cefuroxime –49 (58.3%), Cotrimoxazole – 56 (44.4%). Two strains of Pseudomonas also showed resistance to Piperacillin (16.7 %). All strains of Pseudomonas showed susceptibility to Piperacillin + Tazobactam combination. Proteus showed resistance to Ampicillin – 66.7 %, Ciprofloxacin 55.6 %. Resistance to ampicillin was exhibited by both Klebsiella (100%) and E.coli (66.7 %). Citrobacter was comparatively sensitive to almost all antibiotics. All isolates were sensitive to Vancomycin & Linezolid and were treated with the same. Pseudomonas infections were treated with combinations of Piperacillin + Tazobactam. Detailed antibiotic resistance pattern of all the isolates are described in the Tables 3 & 4.

Table 3: Frequency of antibiotic resistance pattern of gram positive isolates

Figure 6

Antimicrobial agents	Gram positive isolates							
	Staphylococcus aureus (n-126) %	Enterococci spp. (n-56) %	CONS (n-28) %	Streptococci spp. (n-14) %				
Penicillin	112 88.9	35 62.5	0 0	0 0				
Ampicillin	42 33.3	21 37.5	0 0	14 100				
Erythromycin	126 100	56 100	28 100	12 85.7				
Cotrimoxazole	98 77.8	14 25	21 75	0 0				
Gentamicin	63 50	0 0	8 28.5	0 0				
Clindamycin	0 0	0 0	0 0	0 0				
Cefuroxime	42 33.3	21 37.5	0 0	0 0				
Cephalexin	21 16.6	7 12.2	7 25	0 0				
Ciprofloxacin	56 44.4	0 0	8 28.5	0 0				
Oxacillin*	28 22.2	- -	- -	- -				
Vancomycin	0 0	0 0	0 0	0 0				

* Oxacillin was tested only against Staphylococcus aureus

Table 4: Frequency of antibiotic resistance pattern of gram negative isolates

Figure 7

Antimicrobial agents	Gram negative isolates									
	Proteus (n-126) %	Pseudo (n-84) %	Klebsiella (n-84) %	E.coli (n=63) %	Citrobact (n-14) %					
Ampicillin	84 66.7	70 83.3	84 100	42 66.6	7 50					
Cotrimoxazole	35 27.8	56 66.6	21 25	28 44.4	0 0					
Ampicillin + Sulbactam	21 16.7	14 11.1	6 7.1	0 0	0 0					
Gentamicin	42 33.3	42 33.3	7 8	14 22.2	0 0					
Amikacin	42 33.3	56 66.6	0 0	0 0	0 0					
Cefuroxime	28 22.2	49 58.3	8 9.5	7 11.1	0 0					
Ceftazidime	0 0	21 25	0 0	0 0	0 0					
Ciprofloxacin	70 55.6	35 41.7	7 8	21 33.3	0 0					
Imepemem	0 0	0 0	0 0	0 0	0 0					
Piperacillin	- -	14 16.7	- -	- -	- -					
Piperacillin + Tazobactam	- -	0 0	- -	- -	- -					
Linezolid	0 0	0 0	0 0	0 0	0 0					

* Piperacillin & Piperacillin + Tazobactam - tested only against Pseudomonas spp

DISCUSSION

Our study revealed that the no. males (58.75 %) attending the foot infection were more than the no. of females and the main age group involved was patients above the age of 60 years. The majority of the study population had Type 2 diabetes mellitus. All the ulcers in the patients were 3-5 grading in Wagner's grading of foot ulcer. The difference in the grades of the wounds did not have any significant impact on the nature or type of the organism isolated from the wounds.

Our results showed that the 560 specimens yielded about 224 (47.1%) Gram Positive Cocci & 371 (77.94 %) Gram Negative Bacilli. The frequency of gram negative organism was higher to the frequency of gram positive cocci, this result was in accordance to shanker et al₁. The rate of isolation of the MRSA is comparatively higher than the earlier standard reports₄. About 15 % of the specimens did

not yield any growth, the possible explanation is likely to be involvement of anaerobes because anaerobes also have important role in diabetic wound infections₄₅. The rate of Staphylococcus aureus is 126 (21.17%), which is higher than as reported by Lipsky et al₆. Out of 126 Staphylococcus aureus isolated 28 (22.2%) were found to be MRSA (Methicillin Resistant Staphylococcus aureus), which is comparatively lesser than the few earlier reports of gadepalli et al₅ but similar to the reports of study conducted in a South Indian hospital₃. The predominant gram-negative bacilli isolated were Proteus and the predominant gram-positive cocci isolated were Staphylococcus aureus, this was in accordance to study conducted in Indian tertiary care hospital and in an US hospital₄₅. Poly microbial involvement was around 10.58 % this is much lesser than the many of the earlier reports₁₃₄₅. The isolation rate of Enterococci spp. was 9.41 % which was much lesser than the isolation rate reported by a study conducted in US₄

The unique feature about the study was that all the isolates showed resistance to more than 2- 3 antibiotics. When organisms are resistant to more than 2 or 3 antibiotics they can be called as multi drug resistant organisms (MDROs). In our study, the isolation rate of MRSA was 22.2 % which was much lesser when compared to the results of a study conducted in an Indian tertiary care hospital, where the frequency of MRSA was 56 %. Like wise Staphylococci resistance to Ciprofloxacin, Proteus resistance to Amikacin, Pseudomonas resistance to Piperacillin, Piperacillin + Tazobactam, E.coli resistance to Amikacin of the same study was comparatively higher than the results concluded in our study but on the other hand the antibiotic resistance rates of Staphylococcus to Erythromycin, Cotrimoxazole, Pseudomonas spp. resistance to Amikacin, was comparatively lesser than the results of our study₅.

As per the results of a study conducted in United States, Pseudomonas exhibited 98 % susceptibility to Piperacillin + Tazobactam combination. The results of our study indicated 100 % sensitivity & hence our result was in concordance to the results of this earlier report₄. The same study also states the percentage of MRSA isolated was 11.8 %. This was contrary to our results because our result (22.2 %) was much higher. Our results showed 75 % susceptibility to Ceftazidime which was lesser than the reports of the same study₄.

A study from Iran elucidates that Staphylococcus exhibited resistance to Ciprofloxacin (72 %), Clindamycin (54%);

E.coli exhibited 80 % resistance to Ceftazidime & Ciprofloxacin; Pseudomonas exhibited 100 % resistance to Ceftazidime; Klebsiella to Ceftazidime & Ciprofloxacin. It also stated that Citrobacter spp. exhibited high range of drug resistance; this result was completely contrary to our results where our strains isolated exhibited much lesser resistance pattern to all the mentioned antibiotics. ¹⁰

Our study reports that Staphylococcus exhibited 22.2 % resistance to oxacillin., 50 % - Gentamicin, 77.8 % - Cotrimoxazole; Enterococci – 100 % Erythromycin; Pseudomonas – 33.3 % Gentamicin, 66.6 % Amikacin, 25 % Ceftazidime ; Proteus – 33.3 % Gentamicin, 33.3 % Amikacin; E.coli – 25 % Gentamicin, which was much lesser than the reports of a study conducted in Malaysia ,

A study from Nigeria also reports that the resistance percentage of the isolates from diabetic foot ulcers was ceftazidime – 73.6 %, ciprofloxacin – 78 . 4 %, cefuroxime 69.6 %. This was higher when compared to the results of our study ⁸.

The results of our study suggest a definite increase in the prevalence of MDRO in Diabetic foot ulcer than most of the earlier standard results. This alarmingly increasing incidence of Multi Drug Resistant Organism (MDRO) is a potential risk factor in management of diabetic infection. Since improper management MDRO might lead to devastating complications, which includes systemic toxicity, gangrene formation & amputation of lower extremity. Therefore patients with wounds infected with MDRO require an early diagnosis & careful follow up ensure that appropriate and effective medical & surgical regimen is readily available to the patients. In spite of various precautions taken to control & prevent the spread of MDRO, they seem to be an ever-growing problem and are associated with adverse prognosis. These MDRO are frequently resistant to many classes of antibiotic so it is necessary for the clinicians to be completely aware of the prevalence rate of multi drug resistant organism (MDRO) and their management strategies. Hence surveillance about the prevalence of the MDRO should be done at regular intervals to assess the susceptibility patterns of the local strains, in order to adapt proper antibiotic policy to keep a check on the increasing drug resistance.

In addition to proper cleansing, debridement, local wound care, a diabetic foot infection requires a carefully selected broad-spectrum antibiotic therapy. The objective of the study

was to determine the bacteriological profile of DFI in Pondicherry and also to evaluate their level of drug resistance, like wise studies should be frequently conducted in all regions & country to assess the condition prevailing globally. This study also suggests a need for multi center study for evaluation of MDROs to work on strategies of prevention & control of multi drug resistant organism (MDRO).

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