Surveillance on Multi Drug Resistant Organism (MDRO) associated with Diabetic Foot Ulcers in Pondicherry
R Sasikala, R Latha, N Muruganandam, K Senthilkumar

Citation

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 admissions , . 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 wounds , (Photo 1, 2,3)
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.

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

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>329</td>
<td>101</td>
<td>430</td>
</tr>
<tr>
<td>No. of patients infected</td>
<td>301</td>
<td>94.48</td>
<td>395</td>
</tr>
<tr>
<td>Age of patients screened</td>
<td>Less than 50 years</td>
<td>Between 51 – 50</td>
<td>More than</td>
</tr>
<tr>
<td>Male</td>
<td>Female</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td>63/329</td>
<td>109/329</td>
<td>172/329</td>
</tr>
<tr>
<td>Age of infected patients</td>
<td>Less than 50 years</td>
<td>Between 51 – 50</td>
<td>More than</td>
</tr>
<tr>
<td>Male</td>
<td>Female</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>No. of diabetic patients screened</td>
<td>a Type 1</td>
<td>b Type 2</td>
<td>a Type 1</td>
</tr>
<tr>
<td>Male</td>
<td>Female</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>No. of Patients screened with ulcer for Duration of</td>
<td>Less than 3 months</td>
<td>More than 3 months</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>Female</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>No. of Patients infected with ulcer for Duration of</td>
<td>Less than 3 months</td>
<td>More than 3 months</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>Female</td>
<td>Total</td>
<td></td>
</tr>
</tbody>
</table>

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 method.

**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

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 + Tazobactum 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 + Tazobactum. 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
**Table 4: Frequency of antibiotic resistance pattern of gram negative isolates**

**Figure 6**

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Staphylococci aureus (n=126)</th>
<th>Enterococci spp (n=50)</th>
<th>CONS (n=23)</th>
<th>Streptococci spp (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>112 (88.9)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>42 (33.3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>126 (100)</td>
<td>50 (100)</td>
<td>100 (100)</td>
<td>12 (85.7)</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>98 (77.8)</td>
<td>4 (80)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>42 (33.3)</td>
<td>21 (42)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ciprofloxacain</td>
<td>21 (16.9)</td>
<td>7 (14)</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>28 (22.2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Oxacillin was tested only against Staphylococcus aureus*

**Figure 7**

**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 etal. The rate of isolation of the MRSA is comparatively higher than the earlier standard reports. 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 infections. The rate of Staphylococcus aureus is 126 (21.17%), which is higher than as reported by Lipsky et al. 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 al but similar to the reports of study conducted in a South Indian hospital. 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 hospital. Poly microbial involvement was around 10.58 % this is much lesser than the many of the earlier reports. The isolation rate of Enterococci spp was 9.41 % which was much lesser than the isolation rate reported by a study conducted in US.

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 Ciprofloxacain, Proteus resistance to Amikacin, Pseudomonas resistance to Pipercillin, Pipercillin + Tazobactum, 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 study.

As per the results of a study conducted in United States, Pseudomonas exhibited 98 % susceptibility to Pipercillin + Tazobactum combination. The results of our study indicated 100 % sensitivity & hence our result was in concordance to the results of this earlier report. 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 Cefazidime which was lesser than the reports of the same study.
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.

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, 333 % 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 form diabetic foot ulcers was ceftazidime – 73.6 %, ciprofloxacin – 78.4 %, cefuroxime 69.6 %. This was higher when compared to the results of our study.

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 alarming increase in 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).

ACKNOWLEDGEMENT

We are grate full to our Chairman & Prochancellor of Vinayaka Missions University for giving us the moral support to carry out this work. We are also deeply thankful to our Dean Dr. A.Samuel Gnanadass and our Prof & Head Dr.S.Ganeshan for their expert guidance through the study.

CORRESPONDENCE TO

Mrs. R. Sasikala Lecturer Dept of Microbiology Aarupadai Veedu medical college & Hospital Kirumampakkam Cuddalore Pondicherry road Pondicherry. Email Id: sensai0501@yahoo.com Phone No.: 9843133255

References

4. Diane M Citron, Ellie J L Goldstein, C: Vreni Merriam, Benjamin A Lipsky and Murray A Abramson. Bacteriology of Moderate to Severe Diabetic Wound Infections; Jour Clin Mic Sep 2007 - 2819 - 2828
10. Seyed Mohammad Alavi, Azar D. Khoosrav, Abdaulah
Sarami, Ahmad Dashtebazorg, Effat Abasi Montazer
Bacteriologic study of diabetic foot ulcer; Pakistan journal of medical sciences: vol 23, oct-dec 2007 (part 1) No.5
Author Information

R. Sasikala, M.Sc
Department of Microbiology, Aarupadai Veedu Medical College & Hospital

R. Latha, MD
Department of Microbiology, Aarupadai Veedu Medical College & Hospital

N. Muruganandam
M.Sc, Department of Microbiology, Aarupadai Veedu Medical College & Hospital

K. Senthilkumar, M.Sc
Department of Microbiology, Aarupadai Veedu Medical College & Hospital