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BACTERIOLOGICAL PROFILE OF DIABETIC FOOT ULCER IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Diabetic foot ulcer (DFU) is one the most serious complication of Diabetes mellitus and leading cause of hospitalization and increase expenditure in health care system. Bacteriological profile and antibiotic susceptibility pattern to microbes in DFU has been undergoing changes over period of time. Pus, exudate from base of diabetic foot ulcer were taken after cleaning the area with saline gauge. Bacterial identification and sensitivity patterns were done by VITEK 2 Compat system. Maximum cases were seen in the age group between 46–65 years and males were affected more. Pseudomonas aeruginosa was the most common organism isolated (24.54%) followed by Methicillin resistant staphylococcus aureus (MRSA) (16.36%) , Escherichia coli(12.72%), klebsiella pneumoniae(10.90%) Enterococcus species (7.27%), Acinetobacter species(5.45%), Proteus mirabilis (4.54%) , Enterobacter cloacae (4.54%) ,Methicillin sensitive staphylococcus aureus(4.54%), Enterobacter aerogenes(3.63%) Methicillin resistant coagulase negative staphylococcus(03.63%), and Morganella species (1.81%).All staphylococcus aureus were sensitive to vancomycin and linezolid. All the gram-negative bacilli showed highest sensitivity to piperacillin tazobactam, meropenem and imipenem overall. Least sensitivity by all gram-negative organisms were shown to ciprofloxacin.

Keywords: Diabetic mellitus, Diabetic foot ulcer, Antibiotic sensitivity, Pseudomonas aeruginosa, staphylococcus aureus

INTRODUCTION

Diabetes Mellitus (DM) is one of the most common metabolic disorders which results in serious complications. As per WHO reports DM was direct cause of 1.5 million deaths and 48 % of deaths occur before 70 years of age.[1] Diabetic foot ulcer (DFU) is one the most serious complication of DM and leading cause of hospitalization and increase expenditure in health care system. The etiology of DFU is multifactorial with triad of diabetic peripheral neuropathy, peripheral vascular disease (PVD) and foot deformity. [2]

DFU are infections resulting from high sugar level providing ideal media for organisms to grow. The individuals with diabetes have 10-fold increase risk of hospitalization for soft tissue and bone infections.[3]DFU usually require use of multiple prolonged broad spectrum antibiotic usage which finally results in resistance and causing morbidity and mortality.[4] Poor glycemic control, lifestyle factors, and smoking showed increased risks for foot ulcer complications[5]

Bacteriological profile and antibiotic susceptibility pattern to microbes in DFU has been undergoing changes over period of time. Continuous use of broad-spectrum antibiotics may lead to resistance and limit further treatment options. This study was undertaken to identify the bacteriological profile of DFU and to evaluate antibiotic susceptibility pattern to formulate the policy of empirical antibiotic therapy to minimize resistance to micro-organisms and complications of DFU.

MATERIALS AND METHODS

This was a prospective study undertaken for a period of 01 year and 06 months and was conducted in all samples received in Microbiology laboratory of MVJMC & RH in Bangalore from patients suffering from DFU . The only exclusion criteria were patients with foot ulcer without DM. Ethical committee approval from the institution was obtained for conducting this study.

Samples were collected from respective departments on first day of admission. Pus, exudate from base of ulcer were taken after cleaning the area with saline gauge. Samples were transferred to sterile bottles and labelled and sent to Microbiology laboratory immediately. Swabs were also collected by swabbing base of ulcer with 02 sterile swabs.

All the procedures were be done according to standard operation procedures for tissue samples. Samples received in Microbiology laboratory was processed as soon as possible from the time of receival. Age, gender, treatment taken by the patient were documented. Direct smears from samples were done immediately by Gram stain. Specimens were inoculated on blood agar, Mac-conkey agar and thio-glycolate broth and incubated at 37ºCelsius overnight. It was examined for growth following day. Further processing was done depending on colony morphology and Gram's stain from the colony smear. Bacteriological identification and antibiotic susceptibility were done by VITEK 2 Compat system.

Results

In the present study, total 94 samples (pus, exudates) were obtained from different departments in MVJMC &RH and were processed according to standard operative procedures for detection of organism and antibiotic susceptibility. Bacterial etiology was identified in 90 samples out of 94 samples obtained (95.74%). No growth was detected in 4.25% of total samples.

Among 90 cases maximum cases (38) were seen in the age group between 46 – 55 years. (Table 1)

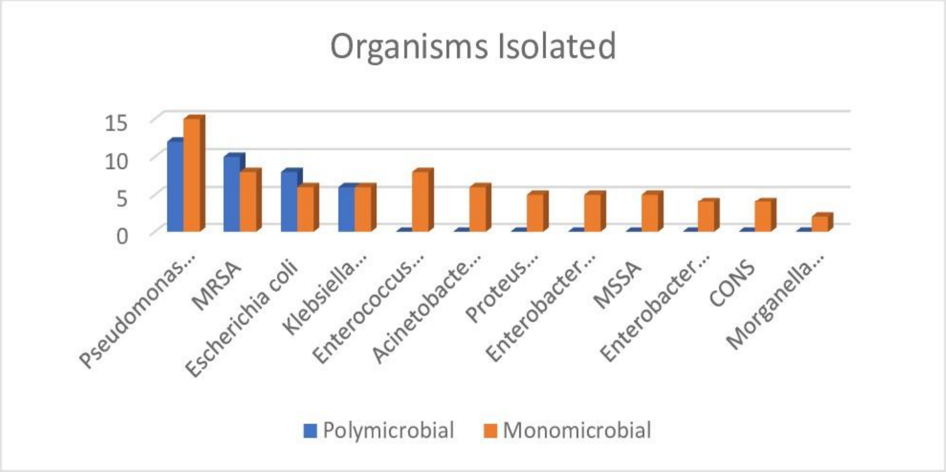
Table 1- Age wise distribution

Age group	No of cases
< 25 years	Nil
26-35 years	02
36-45 years	10
46-55 years	38
56-65 years	20
66-75 years	15
>75 years	5

Males were more affected with DFU (64%) compared to females (36%) making males to female ratio 1.78 in the present study. Majority of patients gave history of trauma (66.66%) compared to spontaneous DFU (33.33%). Out of 90 cases, 80 patients (75.55%) were on insulin treatment and others (24.44%) were on oral hypoglycemic drugs.

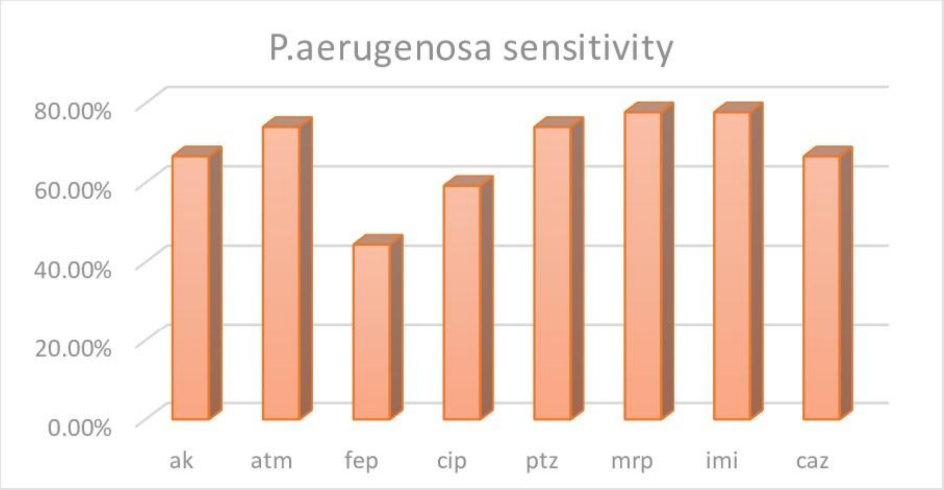
Out of 90 samples 110 organisms were isolated. Among it 20% (18) samples showed polymicrobial growth. Predominant organisms were gram negative bacilli (68.18%) compared to gram positive cocci (31.81%). Pseudomonas aeruginosa was the most common organism isolated (24.54%) followed by Methicillin resistant staphylococcus aureus (MRSA) (16.36%) , Escherichia coli(12.72%), klebsiella pneumoniae(10.90%) Enterococcus species (7.27%), Acinetobacter species(5.45%), Proteus mirabilis (4.54%) , Enterobacter cloacae (4.54%) ,Methicillin sensitive staphylococcus aureus(4.54%) Enterobacter aerogenes(3.63%), Methicillin resistant coagulase negative staphylococcus(03.63%), and Morganella species (1.81) %). (Figure 1)

Figure 1 -Bacteria isolated



Pseudomonas aeruginosa susceptibility pattern – The Vitek showed highest sensitivity of 77.77% for imipenem(imp) and meropenem(mrp) and 74.07% sensitivity for piperacillin tazobactam (ptz) and aztreonam(atm). This was followed by amikacin(ak) and ceftazidime (caz) which showed 66.66% each. Lowest sensitivity was seen for ciprofloxacin (cip) and cefepime (fep) (59.25% and 44.44% respectively). (Figure 2)

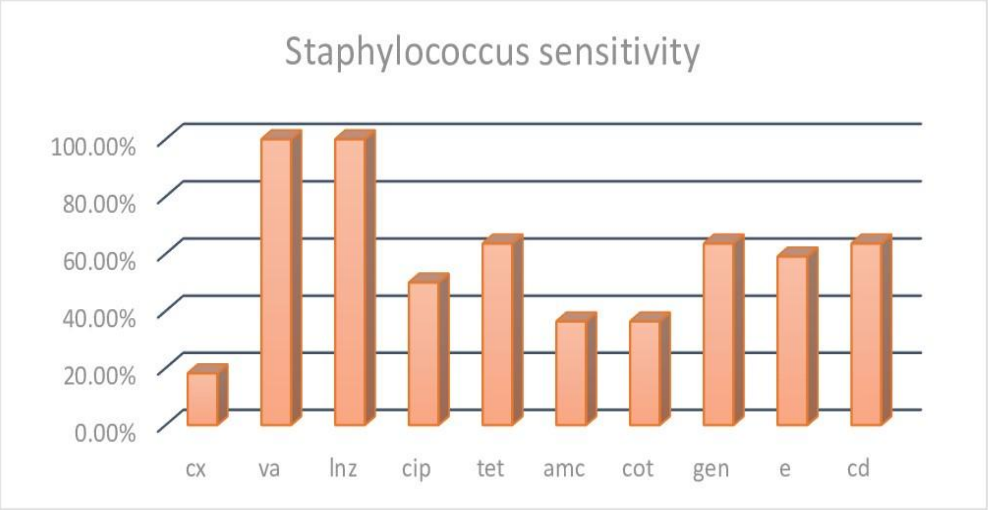
Figure 1 – Pseudomonas aeruginosa sensitivity



(ak -amikacin, atm- aztreonam, fep- cefepime, ptz – piperacillin and tazobactam, mrp – meropenem, imi- imipenem, caz- ceftazidime)

All staphylococcus aureus were sensitive to vancomycin(va) and linezolid(lnz) Out of total staphylococcus aureus 81.81% (18) were resistant to cefoxitin (Methicillin resistant staphylococcus aureus). Among all, 63.33% were sensitive to gentamycin(gen) and tetracycline(tet) each. Ciprofloxacin(cip), clindamycin(cd) and erythromycin(e) showed 50% ,63.63% and 59.09% susceptibility respectively. Out of total, 36.36 % were sensitive to both cotrimoxazole (cot) and amoxicillin with clavulanic acid. (amc) (Figure 3)

Figure 3 – Sensitivity of Staphylococcus aureus



(cx-cefoxitin,va-vancomycin,lnz-linezolid,tet- tetracycline, amc-amoxicillin clavulanate-trimoxazole-,gen-gentamycin, e-erythromycin, cd -clindamycin)

Antibiotic susceptibility for gram negative organism (Table -2)

Antibiotic susceptibility							
0rganism Isoloated	**ptz**	**mrp**	**imp**	**atm**	**ak**	**fep**	**cip**
Pseudomonas aerugenosa (27)	74%	78%	78%	74%	67%	44%	59%
Escherichia.coli (14)	86%	79%	79%	64%	57%	57%	43%
Klebsiella pnemoniae (12)	83%	83%	83%	67%	58%	50%	42%
Enterobacter spp(09)	89%	78%	78%	56%	44%	78%	33%
Acinetobacter spp(06)	83%	83%	83%	50%	67%	50%	50%
Proteus mirabilis(05)	80%	80%	80%	80%	40%	40%	40%
Morganella spp(2)	100%	100%	100%	50%	100%	100%	100%
n-75 , Average %	**85%**	**83%**	**83%**	**63%**	**62%**	**60%**	**52%**

(ptz – piperacillin tazobactam, mrp – meropenem, imi- imipenem, atm- aztreonam ak -amikacin, fep-cefepime, cip-ciprofloxacin)

All the gram-negative bacilli showed highest sensitivity to piperacillin tazobactam, meropenem and imipenem overall. Least sensitivity by all organism was shown to ciprofloxacin. (Table 2)

Discussion

In the present study a total of 94 samples (pus, exudates) were obtained from different departments and were processed according to standard operative procedures for detection of organism and antibiotic susceptibility.

Males were more affected with DFU (64%) compared to females (36%) making males to female ratio 1.78. In the studies conducted in Karnataka by Banu A et al,[6] Gujarat by Maharaul H et al,[7] Kerala by Obulesu G et al, [8] Kolkata by Jain SK et al,[9] Brazil by Palomo AT et al[4] and Malasia by Goh TC et al[10], male patients were predominantly reported to be affected with DFU compared to females. This reason may be due to more exposure to outside work and hence more exposure to trauma by males. Among the total cases, maximum cases (58) were seen in the age group between 46 – 65 years in the present study. This is similar to most of the studies as mentioned in the table number 3

Table 3

Place of study	**Authors**	**Year of study**	**Highest frequency of age group affected/ mean age**
Kerala[8]	Obulesu G et. Al	2018	46- 55 years
Gujarat[7]	Maharaul.H et al	2021	51-60 years
Iraq[11]	Kadhim FH et al	2021	40-60 years

China[12]	Chai W et al	2021	71 – 80 years
Brazil [4]	Palomo AT et al	2022	71 years
Maharashtra[13]	Kale DS et al.	2023	55.4 years

Elderly age group are mostly affected with DFU as they may be having other comorbidities which act as a risk factor for DFU[14] and skin softens with age and can easily break even with trivial trauma.[7] In the present study 75.55% of patients were on insulin and remaining 24.44 % were on oral hypoglycaemic drugs. This was similar to a study conducted in Kerala where 71% of patients were on insulin and 29% were on oral hypoglycaemic drugs.[8]

Among 110 organisms isolated in our study, 68.18%(75) were Gram negative bacilli and 31.81 % (35) were Gram positive cocci. It is similar to studies conducted by Banu A et al, Chai W et al and Sannathimmappa MB et al in Karnataka, China and Oman respectively where gram negative bacilli were isolated more in number than gram positive organisms.[6,12,15] This was in contrast to studies conducted in Kuwait and Brazil by Alhubail A et al and Palomo AT et al where they isolated 68.1% and 50.5% of Gram-positive organism respectively. [2, 4] In the present study Pseudomonas aeruginosa was the most common organism (24.54%) isolated from the patients with DFU followed by Staphylococcus aureus (20%). There were 18 MRSA among it. Table 4 shows isolation rates of Pseudomonas aeruginosa and Staphylococcus aureus isolated in different parts of world. Previous antimicrobial use, prolonged hospitalizations, chronic wounds, and surgical interventions may be the likely contributors to the comparatively large prevalence of Pseudomonas aeruginosa in this study. This coincides with the studies conducted by Goh TC et al in Malasia, Chai W et al in China, Gopi A et al in Karnataka where pseudomonas was the most common organism isolated in patients with DFU. [10,12,16] Whereas some studies conducted by Alhubail A et al in Kuwait, Sannathimmappa MB et in Oman and Ahmad S et al in Pakistan showed Staphylococcus aureus as most common organism isolated. [2, 15, 17.] In contrast to the present study, Escherichia coli and Staphylococcus aureus were the most common organisms isolated (24.4 % each) in a study conducted in Karnataka by Banu A et al [6]

Table 4- Isolation rates of Pseudomonas aeruginosa and Staphylococcus aureus

Place & Year of Study	**Authors**	**Pseudomonas aeruginosa**	**Staphylococcus aureus**
Karnataka 2015[6]	Banu A et al	17.1%	24.4%
Karnataka,2017[16]	Gopi A et al	27.4%	19.4%
Kerala, 2018[7]	Obulesu G et. Al	32.43%	22.97%
Kuwait, 2020[2]	Alhubail A et al	12.8%	19.9%s
Malasia 2020[10]	Goh TC et al	19%1	11%
Gujarat, 2021[7]	Maharaul.H et al	9.23%	36.92%
Oman, 2021[15]	Sannathimmappa MB et al	17%	19%
China, 2021[12]	Chai W et al	19.6%	18.6%
Pakistan, 2022[17]	Ahmad S et al	15.9%	31%

MRSA isolation rates were 16.36% in the present study. This was comparatively less compared to the studies conducted by Gopi A et al in Karnataka and Y Kavitha et al in Puducherry where they got 36% and 46.81% MRSA respectively.[16, 18] The present study was in contrast to studies conducted in Kuwait and Malasia where MRSA isolation rates were lesser (5% and 8% respectively) probably because of good infection control practices followed in these hospitals.[2, 10]

In the present study, Pseudomonas aeruginosa showed highest sensitivity of 77.77% for imipenem(imp) and meropenem(mrp) and 74.07% sensitivity for piperacillin tazobactam (ptz). Ceftazidime (Caz) and Ciprofloxacin (cip) showed 66.66% and 59.25% sensitivity respectively. Among all Staphylococcus aureus, 63.63%were sensitive to gentamycin and tetracycline and clindamycin each. Ciprofloxacin and erythromycin showed 50% and 59.09% respectively. Out of total, 36.66% were sensitive to both cotrimoxazole and amoxicillin with clavulanic acid in the present study. The sensitivity pattern is almost similar to study conducted in Karnataka where highest sensitivity of Pseudomonas aeruginosa was towards imipenem, meropenem and piperacillin plus tazobactam. Staphylococcus aureus showed 100% sensitivity to vancomycin and linezolid similar to our study.[16] In the studies conducted in Kuwait , Malasia and China Staphylococcus aureus showed 100% sensitivity to vancomycin.[2,10,12] In contrast to our study, it showed only 33.3% and 20.8% sensitivity to tetracycline and ciprofloxacin respectively in China.[12] Resistance pattern for Staphylococcus were also different for gentamycin, tetracycline, cotrimoxazole and amoxicillin with clavulanic acid (44.8%, 62% and 61 % and 61% respectively) in the study conducted in Malasia by Goh TC et al.[10] Studies conducted by Alhubail A et al and Goh TC et al showed lesser resistance of Pseudomonas aeruginosa to ciprofloxacin (22.8% and 18% respectively) in contrast to the present study [2, 10]

All the gram-negative bacilli showed highest sensitivity to piperacillin tazobactam, meropenem and imipenem overall. Least sensitivity by all organism were shown to ciprofloxacin in the present study. Study conducted by Gopi A et al showed that gram negative isolates were susceptible to Piperacillin plus tazobactam, and increased resistance to Imipenem and Cephalosporins due to their irrational use.[16] A similar study in Bangalore also showed highest sensitivity of gram-negative organisms to Piperacillin plus tazobactam and imipenem.[6] Study conducted in Brazil by Palomo AT et al showed that Enterobacterales had great susceptibility to piperacillin-tazobactam and meropenem and least susceptibility to ciprofloxacin similar to our study. [4] Study conducted in Kerala also showed less sensitivity of gram-negative bacilli to Ciprofloxacin. Amikacin sensitivity was 70.79% in their study in contrast to 62% in the present study.[7] Ciprofloxacin is the agent with the lowest susceptibility to Gram-negatives, most likely because it is an oral antibiotic, which patients must have used before hospitalization and whose previous use induced resistance.[4]

Conclusion

From the present study we conclude that gram positive cocci are most sensitive to Vancomycin and linezolid and gram-negative bacilli are most sensitive to imipenem, meropenem and piperacillin plus tazobactam. The bacteriological profile and sensitivity patterns of DFU are different in different parts of the world. So, the knowledge of microbiological profile and sensitivity of DFU is essential to guide empirical therapy and for adequate management of DFU. This further helps in controlling the emergence of drug-resistant organisms, reduction in health-care costs and better patient outcomes.

Ethical Clearance

Approval was obtained from the ethical committee of the institute for this study.

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None.

Conflict of interest

The authors declare that there is no conflict of interest**.**

Authors' contribution

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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