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Risk Factors Associated with Multi-drug Resistant Organism (MDRO) Isolates in Infected Diabetic Foot Ulcers (DFU): An Analytical Cross-sectional Study

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Abstract

Background. Increasing use and abuse of antibiotics has led to a rise in multi-drug resistant organisms (MDRO) isolates among infected diabetic foot ulcers (DFUs) and is seen in more than half of all diabetic foot infection cases. This study aimed to identify risk factors for MDRO infection in DFUs since no Filipino studies are available to establish such association.

Methodology. A cross-sectional, analytical study was conducted to evaluate risk factors for MDRO in DFUs. Adult patients admitted in a tertiary hospital from January 2019 - December 2023 for DFU were included in the study. Based on retrospective chart review, patients with DFU described to be infected with accompanying superficial or deep wound cultures were studied. Patients were classified under the MDRO-positive group if their cultures reported any of the following organisms: methicillin-resistant Staphylococcus aureus (MRSA), extended-spectrum beta-lactamases (ESBL), amp C and carbapenamases-producing Enterobacteriaceae, Metallo-beta-lactamases (MBL)-producing Pseudomonas, Acinetobacter species, or any isolate with at least one drug resistance in three different antibiotic classes. Those who did not fall into this definition, including cultures without growth, were classified under the MDRO-negative group. Clinical risk factors and pertinent laboratory findings were obtained from their data.

Results. One hundred fifty-one (151) patients were included in the study and 52 (34.43%) patient had MDRO isolates. The most common isolates seen were Klebsiella pneumoniae for gram-negative organisms and Staphylococcus aureus for gram-positive organisms. For gram-negative isolates, erythromycin had the most resistance at 17.53% followed by oxacillin and clindamycin both at 13.4%. For gram-positive isolates, highest resistance were seen with ampicillin at 18.99% followed by cefuroxime at 13.57% and amoxicillin-clavulanate at 11.63%.

Higher ulcer grade (Crude OR = 1.11 [95% CI 1.00 - 1.23], *p*-value 0.04; Crude OR = 1.29, [95% CI 1.04 - 1.60], *p*-value 0.02), as well as previous admission for diabetic foot ulcer (Crude OR = 2.28, [95% CI 1.10 to 4.73], *p*-value 0.04) were associated with multi-drug resistance. Logistic regression showed that only a previous admission had significant association for MDRO positivity (Adjusted OR = 3.00 [95% CI: 1.04 – 8.62], *p*-value 0.04).

Conclusion. There is a high burden of multi-drug resistant organism infection in diabetic foot ulcers with significant association in higher ulcer grades. A previous admission for diabetic foot ulcer significantly increases the odds for MDRO infection in succeeding diabetic foot infections.

Key words: diabetic foot infection, diabetic foot ulcer, diabetic complications

INTRODUCTION

Diabetic foot ulcers (DFUs) are a frequent complication of diabetes mellitus (DM) with a prevalence rate of 15%. Similar rates are seen in the Philippine setting, usually in men. Associated co-morbidities are peripheral neuropathy and peripheral artery disease. Recurrence rates are also common and are usually seen in patients with DFU who have multiple lesions, plantar location, and presence of peripheral neuropathy.[1]

The most common isolates seen in DFUs are *Staphylococcus aureus (S. aureus)* and *Pseudomonas aeruginosa (P. aeruginosa)* with note of increasing risk for multi-drug resistance (MDR).[2,3] More than half of DFU cases are associated with MDR organisms (MDRO) which can also be a risk factor for recurrence.[4]

Several studies have determined the following variables as independent predictors for the presence of MDRO in patients: duration and control of diabetes, ulcer grade

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and size, smoking, uncontrolled metabolic factors, and concurrent peripheral neuropathy and peripheral artery disease.[5-11] A systematic review by Dai et al., in 2020 looked at 11 retrospective and prospective studies investigating associated risk factors for MDRO infection in DFU and this review showed that poor wound characteristics namely: ischemic ulcers, increased ulcer size and grade, and presence of osteomyelitis as well as previous antibiotic use and hospitalization were risk factors for MDRO-positive cultures.[12] Another systematic literature review by Matta-Gutiérrez et al., in 2021 had shown that MDRO positivity, although not influencing healing time, increased rates of amputation and mortality.[13]

No study has been done to identify independent predictors for DFUs infected with MDRO in the Philippines. It is important to verify and determine additional predictive risk factors to guide appropriate antibiotic selection while awaiting culture sensitivity reports.

METHODOLOGY

A cross-sectional, analytical study was conducted using retrospective data from patients admitted for DFUs in De La Salle University Medical Center, Cavite, Philippines from January 2019 to December 2023 after approval from the technical review board and independent ethics committee of the institution.

The minimum sample size required was 151 and was based on a method by Hsieh et al. with the following equation and parameters:

$$n = \frac{(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta})^2}{P(1-P)\beta^2}$$

where P = OR/(1+OR), $z_{(1-\alpha/2)}$ set at 1.96, $z_{(1-\beta)}$ set at 0.842, odds ratio (OR) set at 2.5, and confidence interval set at 95%.[14] All admissions and referrals for adults ≥19 years old with diabetic foot ulcers were reviewed for microbiologic profile, and the following risk factors: duration and control of diabetes, use of insulin, ulcer grade using University of Texas Wound Classification Score (UTWCS) and Perfusion, Extent, Depth, Infection, Sensation (PEDIS) Score, presence of osteomyelitis and sepsis, any previous admissions for diabetic foot ulcers, any previous antibiotic and surgical treatment of ulcers, presence of peripheral artery disease, presence of diabetic neuropathy, presence of coronary artery disease, presence of cerebrovascular disease, presence of diabetic nephropathy, presence of diabetic retinopathy, and socioeconomic status. The following laboratory markers were also obtained: hemoglobin, white blood cell count (WBC), serum albumin, quantitative C-reactive protein (CRP), glycated hemoglobin (HbA1c), and serum creatinine. Patients admitted for other causes of foot ulcers and unavailability of wound or tissue cultures were excluded from the study.

Ulcer grade and infection was determined by wound description and clinical status showing local or systemic infection based on IWGDF guidelines as described in medical records.[15,16] Fluid or tissue specimens were subjected to automated minimum inhibitory concentration and disc diffusion distribution testing. Classification of MDRO positivity was based on pre-defined criteria as follows: methicillin-resistant *Staphylococcus aureus* (MRSA), extended spectrum beta-lactamases (ESBL), amp C and carbapenamases-producing Enterobacteriaceae, Metallo-beta-lactamases (MBL)-producing Pseudomonas, Acinetobacter species, or any isolate with at least one drug resistance in three different antibiotic classes. Those who did not fall into this definition, including cultures without growth, were separated into the MDRO-negative group. Previous wound care was defined as any antibiotic or wound debridement done in the outpatient department within the last three months and re-admission was defined as any previous admission for diabetic foot management.

Descriptive statistics was used to summarize the demographic and clinical characteristics of the patients. Frequency and proportion were used for categorical variables, median and interquartile range for non-normally distributed continuous variables and mean and standard deviation for normally distributed continuous variables. Independent Sample T-test, Mann-Whitney U test and Fisher's exact/ Chi-square test were used to determine the difference of mean, rank and frequency, respectively, between patients with and without MDRO. Odds ratio and corresponding 95% confidence intervals from binary logistic regression were computed to determine significant factors of MDRO. All statistical tests were two tailed tests. Shapiro-Wilk test was used to test the normality of the continuous variables. Missing values were neither replaced nor estimated. Null hypotheses were rejected at 0.05α-level of significance. Microsoft Excel and STATA 13.1 was used for data management and analysis, respectively.

RESULTS

A total of 151 diabetic patients admitted for diabetic foot ulcers were included in this study with 52 patients or 34.44% having multi-drug resistant isolates. Ninety-nine or 65.56% of patients had wound or tissue cultures that were not multi-drug resistant and 15% of these patients did not have any growth in their wound or tissue culture. Table 1 shows the demographic and clinical profile of patients admitted for diabetic foot ulcers. The MDRO-positive group was comparable to the MDRO-negative group in terms of age, sex, and diabetic profile. The mean age is 60.82 years of age and more males were admitted for diabetic foot ulcers in both groups. The duration of diabetes is on average 10 years for both groups with more than half of the patients requiring insulin management. Peripheral arterial disease and peripheral neuropathy were present in more than 80% of patients with diabetic foot ulcers and 78% of patients had to undergo surgical intervention either by wound debridement or amputation during their admission.

Table 1. Demographic and clinical profile of patients

	Multi-drug resistant organism			
	Total, (n = 151)	With, (n = 52, 34%)	Without, (n = 99, 66%)	*P*-value
	Frequency (%); Mean + SD; Median (IQR)			
Age, years	60.82 + 11.03	60.46 + 10.07	61.01 + 11.55	0.773
Sex				0.603
Male	91 (60.26)	33 (63.46)	58 (58.59)	
Female	60 (39.74)	19 (36.54)	41 (41.41)	
Socioeconomic status				0.112
A and B	114 (75.5)	35 (67.31)	79 (79.8)	
C to E	37 (24.5)	17 (32.69)	20 (20.2)	
DM duration, years	10 (3 to 18)	10 (2.5 to 17.5)	10 (3 to 18)	0.830
Insulin therapy	80 (53.98)	31 (59.62)	49 (49.49)	0.303
Macrovascular complication				
PAD (n = 140)	117 (83.57)	43 (84.31)	74 (83.15)	1.000
CAD (n = 150)	40 (26.67)	14 (27.45)	26 (26.26)	1.000
CVA (n = 150)	19 (12.67)	9 (17.31)	10 (10.20)	0.302
Microvascular complication				
Neuropathy (n = 99)	81 (81.82)	33 (80.49)	48 (82.76)	0.796
Nephropathy (n = 146)	72 (49.32)	29 (56.86)	43 (45.26)	0.225
Retinopathy	13 (43.33)	5 (38.46)	8 (47.06)	0.721
Underwent surgery (n = 150)	117 (78)	45 (86.54)	72 (73.47)	0.096
Wound history				
Duration of symptoms, days	22 (14 to 60)	23.5 (14 to 60)	21 (9 to 60)	0.480
Size (n = 144)				0.287
0 to 3.9 cm	330 (20.83)	8 (15.38)	22 (23.91)	
≥ 4 cm	114 (79.17)	44 (84.62)	70 (76.09)	
UTWCS	8 (4 to 12)	10 (8 to 12)	8 (4 to 12)	0.035
PEDIS score	9 (7 to 9)	9 (8 to 10)	8 (7 to 9)	0.017
Sepsis	31 (20.53)	7 (13.46)	24 (24.24)	0.141
Received outpatient antibiotics	113 (74.83)	40 (76.92)	73 (73.74)	0.699
Received outpatient wound care	58 (38.41)	26 (50)	32 (32.32)	0.037
Readmission (n = 149)	44 (29.53)	21 (41.18)	23 (23.47)	0.037
Laboratory parameters				
WBC	14.6 (10.7 to 21.5)	14.1 (10.25 to 20)	15.8 (10.9 to 23.4)	0.487
Hemoglobin	112 (95 to 129)	114 (93 to 126)	112 (97 to 132)	0.532
CRP (n = 115)	99.64 (58.7 to 175)	82.18 (49.2 to 150)	121.09 (63.7 to 185)	0.072
HbA1c (n = 118)	9.65 (7.6 to 12.3)	9.8 (7.7 to 12.35)	9.55 (7.3 to 12.3)	0.562
Albumin (n = 112)	31 (26 to 36)	29 (24 to 34)	32 (27 to 36)	0.170
Creatinine (n = 143)	111.9 (81 to 194.3)	121.75 (80.5 to 214.7)	111.3 (81.7 to 191)	0.951
Polymicrobial Isolates	31 (20.53)	15 (28.85)	16 (16.16)	0.089

Both MDRO-positive and MDRO-negative groups were comparable in terms of rates of coronary artery disease, cerebrovascular disease, nephropathy, and retinopathy. Both groups were also comparable in terms of clinical and laboratory markers for infection severity and sepsis. Both groups exhibited anemia and hypoalbuminemia with a mean hemoglobin of 112 g/dL and mean albumin of 31 g/dL.

No significant difference was seen in terms of socioeconomic classification and incidence of MDRO. No difference was seen in presence of MDRO isolates in patients who have received outpatient antibiotic treatment. There was no difference in polymicrobial rates between MDRO-positive and MDRO-negative groups. Distribution of major resistance phenotypes were seen more in MRSA infections at 19.23% followed by ESBL at 13.46%, *Acinetobacter sp.* at 5.77% and lastly, other carbapenemases at 1.92%.

There were a total of 115 gram-negative isolates and 45 gram-positive isolates with a 2.5:1 ratio.

The most common gram-negative isolate was *Klebsiella pneumoniae* (n = 20, 17.4%) with 35% being multi-drug resistant (Figure 1). The most common gram-positive isolate was *Staphylococcus aureus* (n = 15, 33.3%) with 33% having methicillin resistance (Figure 2). *Achromobacter xylosoxidans* ss xylosoxidans for gram-negative isolates and *Staphylococcus epidermidis* for gram-positive isolates particularly had higher multi-drug resistance rates (*p*-value 0.031 and *p*-value 0.013, respectively).

For gram-negative isolates, erythromycin had the most resistance at 17.53% followed by oxacillin and clindamycin both at 13.4%. For gram-positive isolates, highest resistance were seen with ampicillin at 18.99% followed by cefuroxime at 13.57% and amoxicillin-clavulanate at 11.63% as seen in Figure 3A and 3B.

Higher UTWCS 2D through 3D (Crude OR = 1.114 [95% CI 1.008 - 1.230], *p*-value 0.035) and PEDIS Score ≥ 8 (Crude OR = 1.290, [95% CI 1.044 - 1.596], *p*-value 0.017) were

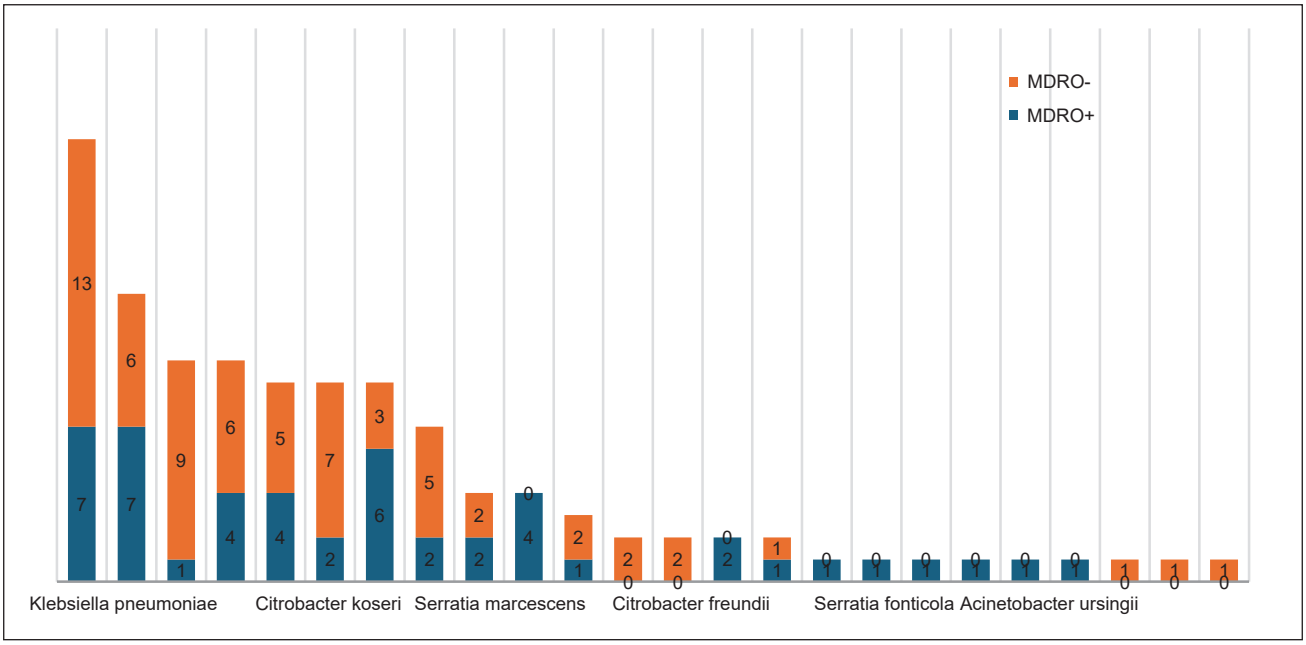

Figure 1. Proportion of MDRO-positive vs MDRO-negative among gram-negative isolates (n = 115).

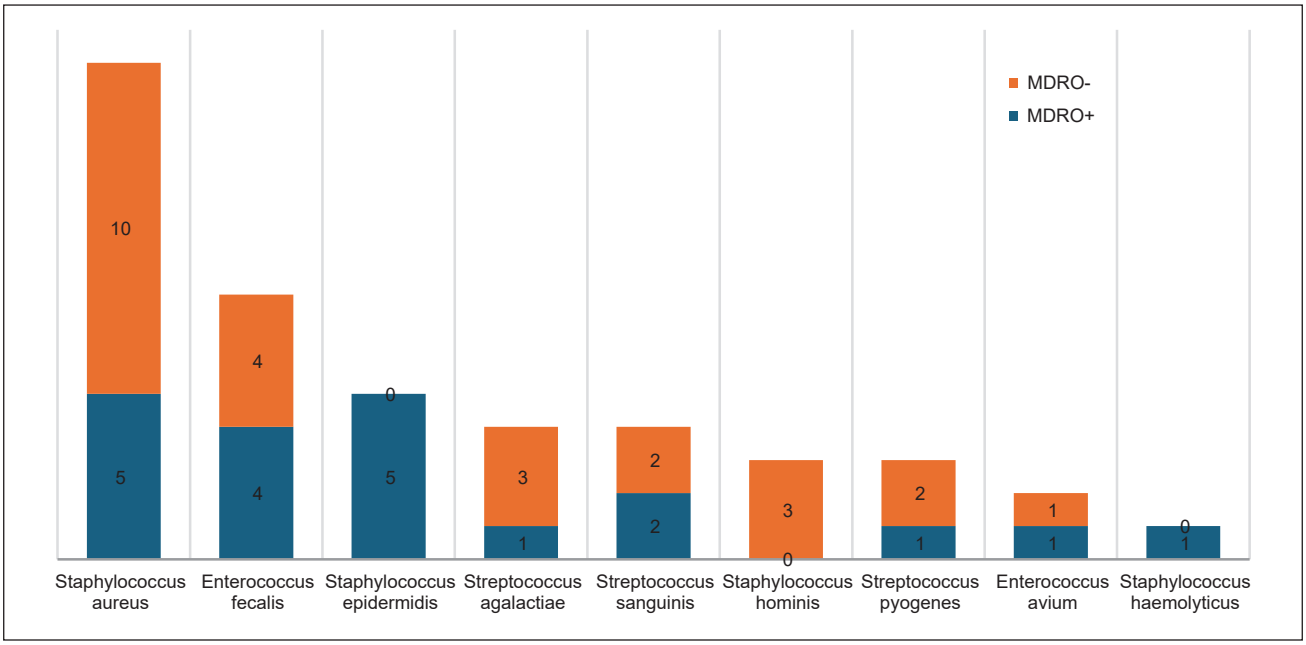

Figure 2. Proportion of MDRO-positive vs MDRO-negative among gram-positive isolates (n = 45).

observed in MDRO-positive cases as well as in patients who were previously admitted for diabetic foot ulcer (Crude OR = 2.283, [95% CI 1.103 to 4.725], *p*-value 0.037) as seen in Table 2. Logistic regression showed that only a previous admission was significantly associated with MDRO positivity (Adjusted OR = 2.997 [95% CI: 1.041 – 8.625], *p*-value 0.042) after correcting for confounders.

Table 2. Univariate analysis of factors associated with multi-drug resistant organism

Parameters	Crude odds ratio	95% CI	*P*-value
UTWCS	1.1138	1.0083 to 1.2303	0.034
PEDIS score	1.2907	1.0437 to 1.5960	0.019
Readmission	2.2826	1.1028 to 4.7246	0.026

DISCUSSION

DFU is a serious complication of DM with a worldwide prevalence rate of 15%.[9] In a clinical profiling of DFU cases in General Santos, Philippines, similar rates are observed. There was also a male prevalence with a ratio of 1.4:1 in their study. Associated co-morbidities were peripheral neuropathy and peripheral artery disease. DFU by itself already causes significant morbidity and mortality. Recurrence rates are common and are usually seen in

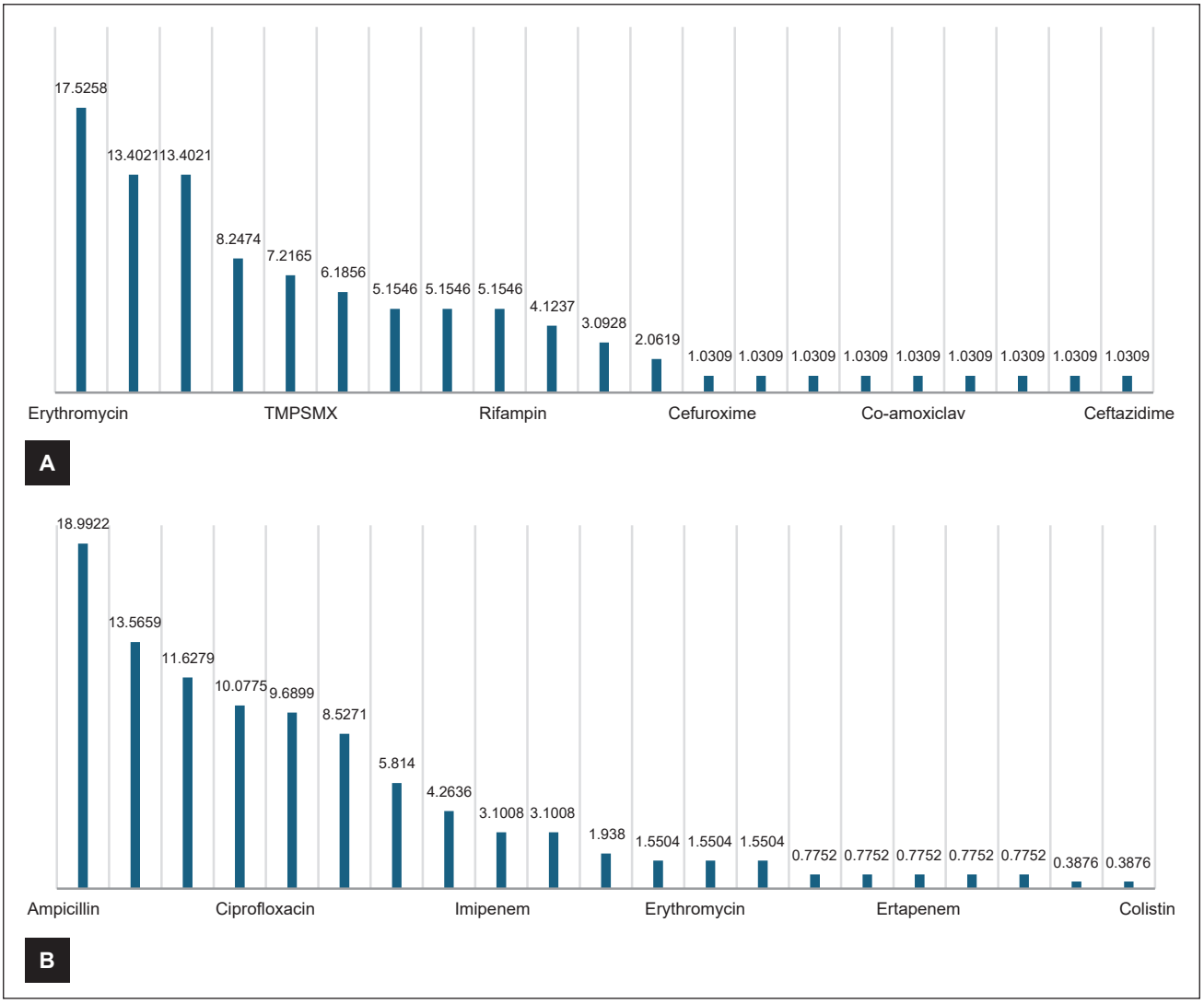

Figure 3. (A) Resistance rates to antibiotics of gram-negative cultures (n = 258) and **(B)** Resistance rates to antibiotics of gram-positive cultures (n = 97)

patients with DFU who have multiple lesions, plantar location, and presence of peripheral neuropathy. 48.00% of patients admitted for diabetic foot ulcers require surgical intervention and 14.00% of those who needed surgical intervention required amputation.[1] Multi-drug resistant infections further complicate outcomes with note of increased amputation rates and mortality rates.[13]

This study observed the associated risk factors for multi-drug resistance in patients with diabetic foot ulcers. MDRO isolates were seen in 34.40% patients admitted for diabetic foot ulcers. This is lower in contrast to known MDRO rates in diabetic foot ulcers at 63.40%.[4]

Microbiologic profile

A study by Jain and Barman in 2017 showed that MDROs are present in both major and minor amputations but gram-negative MDROs were seen more on deep ulcers while gram-positive MDRO were seen more on superficial ulcers.[5]

Bacteriologic profiling in a study done in a tertiary care setting in Malaysia in 2020 showed that *P. aeruginosa* and *S. aureus* were the most common isolates in DFUs, followed by Methicillin-Resistant *S. aureus* (MRSA) and Bacteroides. Among the gram-positive isolates, there were high resistance rates noted for penicillin, tetracycline, and erythromycin. For gram-negative isolates, high resistance rates were noted for fluoroquinolones and ampicillin. Clindamycin resistance was notable for anaerobic isolates.[8] The same resistance patterns can be observed in our study.

In another microbiologic study in India in 2018, *S. aureus* and *P. aeruginosa* were also the most common gram-positive and gram-negative isolates, respectively. Four of ninety-one (4.4%) staphylococcal isolates were MRSA and five of sixty-seven (7.56%) of pseudomonal isolates were Extended-Spectrum Beta-Lactamase (ESBL)-positive on resistance screening. Several isolates with pan-resistant phenotypes underwent genotypic testing, confirming MDR in these isolates.[3]

Similarly, *Staphylococcus aureus* was also the most common gram-positive isolate with 33% of isolates having methicillin resistance. Although *Klebsiella pneumoniae* was the most common gram-negative isolate, this was followed by *Enterobacter cloacae* (11.30%), *Escherichia coli* (8.70%), and *Pseudomonas aeruginosa* (8.70%). In a study looking at outcomes of MDRO infected DFUs, *Klebsiella pneumoniae* infection was found to have a high fatality rate with OR = 7.7 (95% CI 1.24 -47.96).[17]

Risk factors

Several studies are available in assessing patient-related risk factors for MDRO. In a study by Kathirvel et al. in South India in 2018, after logistic regression, they found that high grade ulcers and recurrent ulcers were significant risk factors for MDRO infection. They also saw a higher gram-negative to gram-positive infectivity with a 2.3:1 ratio.[8] Other Indian population studies, confirmed these risk factors along with diabetes duration >20 years.[18,19]

Three publications from China for risk factors for MDRO DFU determined that hospitalizations within the last 12 months for diabetic foot is a risk factor for MDRO. Liu et al. in 2022 showed that previous hospitalization, ulcer size >4 cm, surgical therapy, and CRP were independent risk factors for having MDR isolates in diabetic foot ulcers.[1,10]

Ji et al., in 2014 showed that previous hospitalization, higher ulcer size and grade, previous antibiotic use, and osteomyelitis were significant risk factors for MDRO after logistic regression.[20]

Several diabetic foot classification systems are available and are recommended by the International Working Group for on the Diabetic Foot (IWGDF) 2023 to aid healthcare workers in communicating wound characteristics as well as in stratifying healing likelihood and amputation risk. UTWCS is one of the more common scoring systems used.[21] PEDIS is another validated scoring system.[22] A higher UTWCS score has more correlation with the presence of neuro-ischemic pathology and osteomyelitis whereas a higher PEDIS score has more correlation with peripheral artery disease, neuropathy, severe infection, tissue involvement, and large ulcer size. Increased scores correlated with difficulty healing and higher amputation rates.

Although this study was unable to establish diabetes duration and severity as well as ulcer size ≥ 4 cm as MDRO risk factors, univariate analysis showed that higher grade ulcers, as shown by UTWCS score 2D-3C and PEDIS Score ≥8, have significant risk for MDRO infection.

On logistic regression it showed that patients who had any previous hospitalization for diabetic foot ulcer management were 3 times more likely to present with MDRO infection as supported by Liu and Ji's publications.[10,20] This may be due to exposure to antibiotics which may lead to mutations for drug resistant genes or may be due to exposure to opportunistic organisms during previous hospitalizations.

Several limitations in this study include its retrospective nature especially since wound description and wound scoring may have inter-observer variability.[16] A prospective study may better classify wound characteristics and avoid variability. Anaerobic sampling is not routinely done in this institution and was not available for all patients hence no report can be made regarding drug resistance in anaerobic organisms.

CONCLUSION

There is high burden of multi-drug resistant infections in diabetic foot ulcers. High wound classification by UTWCS and PEDIS scoring are significant risk factors for presence of MDRO isolates in diabetic foot ulcers. Any previous hospitalization for diabetic foot ulcer is associated with MDRO infection in succeeding admissions.

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Statement of Authorship
Both authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
AVP: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Resources, Data Curation, Original Draft Preparation, Reviewing, Editing, Visualization, Project Administration; **DAP:** Conceptualization, Methodology, Validation, Resources, Reviewing, Editing, Supervision, Project Administration.

Data Availability Statement
Datasets are not publicly available because participants in the study did not give written consent for their data to be shared.

Author Disclosure
Both authors declared no conflict of interest.

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