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Evaluation of various risk factors associated with multidrug-resistant organisms isolated from diabetic foot ulcer patients

Priya Datta, Jagdish Chander, Varsha Gupta, Gursimran Kaur Mohi, Ashok K. Attri[1]

Abstract:

AIMS: Diabetic foot ulcer is a dreaded complication of diabetes. Diabetic foot ulcer patients are often infected with multidrug resistant organism (MDRO) due to chronic course of the wound, inappropriate antibiotics treatment, frequent hospital admission, neuropathy, nephropathy, and peripheral vascular disease.

MATERIALS AND METHODS: This prospective study was conducted in our 750 bedded hospital for a period of 6 months. The present study was undertaken to isolate various MDRO methicillin resistant *Staphylococcus aureus*; Gram-negative bacteria producing enzymes such as extended spectrum beta-lactamases (ESBL), Amp C, Carbapenamases; *Pseudomonas* and *Acinetobacter* species producing metallo-beta-lactamases (MBL). In addition we attempted to identify risk factors for association of diabetic foot ulcer and MDRO.

RESULTS: A total of 149 bacterial isolates were identified. Of the total isolates 73.2% were Gram-negative and remaining 26.8% were Gram-positive bacteria. Among *Enterobacteriaceae* 59% were ESBL producers and 48% were Amp C producers. In addition, 41.5% of the isolates produced both ESBL and Amp C and 13.4% were carbapenem resistant *Enterobacteriaceae*. Among 20 *Pseudomonas* and *Acinetobacter* isolates, 5 were MBL producers (25%). Furthermore, in the study, 56% of patients with diabetic foot ulcer harbored MDRO. The risk of multidrug-resistant infection is significantly more in patients having diabetes duration >20 years and size of ulcer more than 4 cm^2.

CONCLUSION: The detection of MDRO in patients of diabetic foot ulcer changes the treatment strategies limits the antimicrobial options and causes higher complications among them.

Key words:

Diabetic foot ulcer, multidrug resistant organisms, risk factors

Introduction

Diabetic foot ulcer is a dreaded complication of diabetes. Various reasons for this include diabetic neuropathy, peripheral vascular disease, foot deformity, and trauma. The life time risk of developing diabetic foot among diabetic population is 25% in India compared to 15% worldwide. This is credited to various practices like lack of proper foot care practices, barefoot walking, and inadequate diabetic control.[1]

To worsen the matter diabetic foot ulcer patients also have functional changes in microcirculation, cellular activity and growth factor activation processes. Therefore, foot ulcer in diabetic cases differs from those in nondiabetic cases. In fact, these ulcers are highly susceptible to infections and if an infection occurs, it can spread quite rapidly leading to overwhelming tissue destruction

Departments of Microbiology and [1]Surgery, Government Medical College and Hospital, Chandigarh, India

Address for correspondence:
Dr. Gursimran Kaur Mohi, Department of Microbiology, Government Medical College and Hospital, Sector 32, Chandigarh - 160 030, India.
E-mail: gkmohi@gmail.com

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and subsequent amputation.[2] Diabetic foot ulcer patients are often infected with multidrug resistant organisms (MDRO) due to inappropriate antibiotic treatment, chronic course of the wound, frequent hospital admission, neuropathy, nephropathy, and peripheral vascular disease.[3] Furthermore, due to peripheral arterial disease, there is poor penetration of antibiotics into the lower limb tissue, thereby promoting selection of resistant bacterial strains. Just as features of foot ulcer differs between diabetic and nondiabetic cases, so do predisposing factors for MDRO between diabetic and nondiabetic cases.[4]

Therefore, the objectives of this prospective study was to isolate various MDRO i.e. MDRO are defined as methicillin resistant *Staphylococcus aureus* (MRSA) and extended spectrum beta-lactamases (ESBL), Amp C and Carbapenamases producing *Enterobacteriaceae* and metallo-beta-lactamases (MBL) producing *Pseudomonas* and *Acinetobacter* species from diabetic foot. Also to identify risk factors for multi resistant infections and to study the antimicrobial susceptibility pattern of these MDRO in patients of diabetic foot infection.

Materials and Methods

Setting and sampling techniques

This prospective study was conducted in our 750 bedded hospitals for 6 months (January 2016–June 2016). The department of surgery runs a special clinic – "Centre for Treatment and Research in Diabetic Foot and vascular disease." On an average 60 patients attend per week and 15–20 new patients are seen every month in this clinic. This study has been approved by the Institutional Ethical Committee.

First hundred new patients (within 6 months) with diabetic foot ulcer, attending Diabetes clinic were enrolled for this project. A detailed history, general physical examination was taken and pro forma regarding risk factors for MDRO was filled up. Written consent was obtained from all subjects in the study. Various risk factors included age, sex, type, and duration of diabetes, duration of ulcer, grading of ulcer, size of ulcer, complications (such as nephropathy, neuropathy, and peripheral vascular disease).

Diabetic nephropathy was defined as the presence of albumin (even trace amount) in urine as detected by dipstick test. Diabetic peripheral neuropathy was defined as absence of perception of the Semmes–Weinstein monofilament (5.07, 10 g) at two of ten standardized plantar sites on either foot.

Inclusion criteria

Patients attending Diabetic foot clinic and willing to participate.

Exclusion criteria

i. Grade 0 patients - pre- or post-ulcerative site that has healed
ii. Patient already on antimicrobial treatment coming from other institute.

Microbiological study

The wound swab was taken after superficial debridement to avoid colonizer using sterile swabs introduced deep into the wound.

According to University of Texas Wound Classification system, grading of wound is:

i. Grade 0 wound – preulcerative or postulcerative site that has healed
ii. Grade 1 wound – wound not involving tendon, capsule, or bone
iii. Grade 2 wound – wound penetrating tendon or capsule
iv. Grade 3 wound – wound penetrating bone or joint.

Wound swabs belonging to grade 1, 2, and 3 were taken and transported immediately.

Standard microbiological procedures were to be performed for all swabs to isolate the pathogenic bacteria.[5] Antimicrobial susceptibility was performed according to CLSI guidelines and MRSA was defined according to it.[6]

Detection of extended spectrum beta-lactamases

Detection of extended spectrum beta-lactamases was done according to CLSI guidelines. The presumptive ESBL production was confirmed by double disc synergy method.[6]

Detection of Amp C

Presumptive test for inducible Amp C β-lactamases was considered positive if zone diameter of bacterial strain for cefoxitin was ≤18 mm.[6] Confirmatory test for Amp C β-lactamases production was done by Black Amp C disk test.[7]

Detection of carbapenamases producing *enterobacteriaceae*

Test for carbapenamases was considered positive if zone diameter of any bacterial strain for meropenem ≤19 mm and for ertapenem ≤18 mm.[6] Confirmatory test was done by Modified Hodge test.[6]

Detection of metallo-beta-lactamases production

In *Pseudomonas* and *Acinetobacter* was confirmed by Imipenem-ethylenediaminetetraacetic acid double disc synergy test.[8]

Results

Out of these 100 samples, 8 were sterile and 92 showed growth of bacteria. A total of 149 bacterial isolates were identified and 73.2% of these isolates were Gram-negative and remaining 26.8% were Gram-positive bacteria. The Gram-negative isolates included *Escherichia coli* (38), *Proteus mirabilis* (18), *Klebsiella pneumoniae* (15), *Citrobacter* species (7), *Klebsiella oxytoca* (6), *Proteus vulgaris* (5), *Pseudomonas* species (15), *Acinetobacter* species (5), *S. aureus* (26), and *Enterococcus* species (14).

Of the 89 isolates belonging to Family *Enterobacteriaceae*, 16 strains (18%) produced only ESBL, 6 strains (6.7%) produced only Amp C and 37 strains (41.5%) produced both ESBL and Amp C. In addition, 13.4% were carbapenem resistant *Enterobacteriaceae*. Amongst 20 *Pseudomonas* and *Acinetobacter* isolates, 5 were MBL producers. Among the *S. aureus*, 18% were MRSA. A total number of 56 patient's harbored MDRO pathogens in their diabetic foot ulcer and 44 patients had infection without MDRO.

Among the *Enterobacteriaceae*, high level of resistance percentage to various ß-lactam and cephalosporin was seen in Table 1. The resistance to amoxyclav varied from 40% to 73%. The third generation cephalosporin (ceftriaxone and ceftazidime) resistance varied from 22% to 100%. The resistance for ceftazidime/clavulanic acid was 17%–60%. Similarly, higher resistance was seen to ciprofloxacin (45%–83%). Among the various species of *Enterobacteriaceae*, *P. mirabilis* showed maximum sensitivity to these antimicrobials. Piperacillin/tazobactam was a good antimicrobial with resistance as low as 17%–57%. Furthermore, resistance to imipenem among *Enterobacteriaceae* varied from 5% to 20%. *Pseudomonas* species and *Acinetobacter* species showed higher degree of resistance to ceftazidime, tobramycin, aztreonam (33%–60%).

Among the patients, 68% were in the age group of 50–60 years and 74% were males. Most of the patients (82%) had type 2 diabetes. The mean duration of diabetes was <10 years in 14% of patients, between 10 and 19 years in 29% of patients and 56% of the patients had diabetes of >20 years duration. In nearly, 36% of patients, the duration of diabetic foot ulcer was ≤3 months and majority (64%) had ulcer for more than 3 months. Size of ulcer was >4 cm^2 in 70% of the patients. Neuropathy was the most common complication associated with diabetic foot ulcer patients (80%) while 12% had nephropathy and only 8% had peripheral vascular disease.

The risk of multidrug-resistant organism is significantly more in patients having duration of diabetes >20 years and also if the size of ulcer is more than 4 cm^2. Furthermore, the risk of Multi drug resistant organism is more in people <50 years of age (odds ratio [OR] = 1.5), Grade 2 ulcer and Grade 3 ulcer (OR = 3.6 and 2) and patients on medical treatment (OR = 1.8), but these are not statistically significant [Table 2].

Discussion

This study highlights the correlation between the clinical profile of diabetic foot ulcer patients and MDRO in these patients. Diabetic foot ulcer patients have greater chance of having MDRO isolated from their wound which leads to increased mortality and morbidity.

In our study, Gram-negative bacteria (73.2%) were more commonly isolated than Gram-positive as in a similar study by Gadepalli *et al*.[3] in a similar study by Jog *et al*. done on patients with Type II diabetes having foot ulcer, 58.3% of isolates were Gram-negative.[9] The

Table 1: Antimicrobial susceptibility pattern of various bacterial isolates from diabetic foot ulcer

Antibiotics	*Escherichia coli* (*n*=38), *n* (%)	*Klebsiella pneumoniae* (*n*=15), *n* (%)	*Klebsiella oxytoca* (*n*=6), *n* (%)	*Proteus mirabilis* (*n*=18), *n* (%)	*Proteus vulgaris* (*n*=5), *n* (%)	*Citrobacter* spp. (*n*=7), *n* (%)	*Pseudomonas* spp. (*n*=15), *n* (%)	*Acinetobacter* spp. (*n*=5), *n* (%)
Cefepime	18 (47)	10 (67)	1 (17)	4 (22)	3 (60)	3 (43)	-	-
Ceftazidime	21 (55)	13 (87)	3 (50)	4 (22)	3 (60)	3 (43)	5 (33)	3 (60)
Ceftriaxone	23 (60)	15 (100)	4 (67)	4 (22)	3 (60)	4 (57)	5 (33)	3 (60)
Cefoxitin	20 (53)	8 (53)	3 (50)	3 (17)	4 (80)	5 (71)	-	-
Amoxyclav	24 (63)	11 (73)	4 (67)	3 (17)	2 (40)	5 (71)	-	-
Ceftazidime/clavulanic acid	16 (42)	9 (60)	2 (33)	3 (17)	1 (20)	3 (43)	-	-
Tobramycin	-	-	-	-	-	-	5 (33)	3 (60)
Ciprofloxacin	17 (45)	9 (60)	5 (83)	6 (33)	3 (60)	5 (71)	8 (53)	5 (100)
Piperacillin/tazobactam	16 (42)	7 (47)	2 (33)	3 (17)	2 (40)	4 (57)	-	-
Amikacin	15 (39)	12 (80)	2 (33)	3	1 (20)	3 (43)	7 (46)	2 (40)
Imipenem	5 (13)	3 (20)	1 (17)	1 (5)	1 (20)	1 (14)	3 (20)	2 (40)
Aztreonam	-	-	-	-	-	-	5 (33)	3 (60)
Colistin	-	-	-	-	-	-	2 (13)	0
Polymyxin	-	-	-	-	-	-	1 (7)	0

Table 2: Comparison of various risk factors in diabetic foot patients harboring multidrug resistant organisms and nonmultidrug-resistant organisms pathogens

Risk factors	MDRO (*n*=56)	Non-MDRO (*n*=44)	OR (95% CI)	*P*
Age (years)				
<50	20	12	1.5	0.36
50-60*	36	32	1	
Sex				
Male	40	34	0.7	0.50
Female*	16	10	1	
Type of diabetes				
Type 1	6	12	0.4	0.12
Type 2*	50	32	1	
Duration of diabetes (years)				
<10	7	8	1	0.02
10-19	18	11	4.2	
>20*	31	25	3.1	
Duration of ulcer (months)				
≤3	18	18	1.4	0.30
>3	38	26	1	
Size of ulcer (cm^2)				
<4	12	18	1	0.03
>4	44	26	2.5	
Grading of ulcer				
Grade 1	13	20	1	0.19
Grade 2	26	11	3.6	
Grade 3	17	13	2	
Complication				
Nephropathy	8	4	0.7	0.20
Neuropathy	42	38	0.4	
PVD	6	2	1	

MROD = Multidrug-resistant organisms, CI = Confidence interval, PVD = Peripheral vascular disease, OR = Odds ratio

present study also highlights that more than half the diabetic foot ulcer patients had infection with MDRO. This has also been reported by Gadepalli *et al*. and Hartemann-Heurtier *et al*. in their studies.[10]

In our study, 59% of *Enterobacteriaceae* isolates were ESBL producers, 48% were Amp C producers and 41.5% isolates produced both ESBL and Amp C. Moreover, among the *Pseudomonas* species and *Acinetobacter* species isolated from diabetic foot ulcer patients, 25% were MBL producers. Similarly, Rawat *et al*. showed 40% of these species to be ESBL producers, 32% Amp C and 37% MBL producers.[11] Jog *et al*. also reported 33.3% of isolates as ESBL producers and 20.63% of isolates as Amp C producers (9). In patients with diabetic foot ulcer having ESBL and/or Amp C, treatment of choice remains carbapenem or combination of beta lactam/beta lactam inhibitor.[12] Furthermore, in our study, 13.4% of *Enterobacteriaceae* isolates from diabetic foot ulcer patients are carbapenem resistant, therefore decreasing the treatment option further. Moreover, piperacillin/tazobactam was an effective antimicrobial as already reported by us in our previous study.[13]

There was a very high rate of antibiotic resistance observed in the present study. This may be because most of these patients had diabetes of more than 10 years and 64% of them had ulcer of more than 3 months. Therefore, widespread use of antimicrobial agent would exert selective pressure on resistance.

Very few studies have documented the risk factors in diabetic foot ulcer patients with MDRO infection or colonization. In the present study, deep bacteriological swabbing was done, so as to rule out colonization. There is importance of knowing the risk factors for the presence of MDRO in these patients when these patients report initially to the health-care setup. The time taken to detect various resistance mechanisms in routine microbiological laboratory would be around 48 h. In addition, the risk factor varies according to geographic area and patient's profile. However, knowing the risk factors beforehand would help in better management of these patients and improve the prognosis.[14]

In this study, the risk factors significantly more associated with MDR infection were duration of diabetes >20 years and ulcer size more than 4 cm^2. Gadepalli *et al*. also found ulcer size >4 cm^2 and additional risk factors such as osteomyelitis, neuropathy, and surgical treatment to be more significantly associated with MDRO. However, there is variation in risk factors associated with MDRO in various studies. Richard *et al*. found that previous hospitalization and proliferative retinopathy significantly increased the risk of MDRO.[14] Likewise Hartemann-Heurtier *et al*. found previous hospitalization for the same wound and presence of osteomyelitis to be significantly associated with MDRO. This variation could be because of different patient profile and varying levels of antimicrobial resistance in different geographical area.

Conclusion

Patients of diabetic foot ulcer having MDRO are a major health-care burden in a country like ours. The presence of MDRO in-patient of diabetic foot ulcer changes the treatment strategies and limits the antimicrobial options. In addition, the detection of MDRO in diabetic foot ulcer patient will have an impact on infection control practices. Stringent methods to prevent cross transmission, good hand hygiene practices, and isolation precautions need to be followed.[10] Improper and delay in treatment, ineffective infection control practices, and ignoring risk factors would lead to higher rate of complications. The treating surgeons in order to provide best results should

identify the presence of MDRO and their associated risk factors.

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Conflicts of interest

There are no conflicts of interest.

References

1. Viswanathan V, Jasmine JJ, Snehalatha C, Ramachandran A. Prevalence of pathogens in diabetic foot infection in South Indian type 2 diabetic patients. J Assoc Physicians India 2002;50:1013-6.
2. Kandemir O, Akbay E, Sahin E, Milcan A, Gen R. Risk factors for infection of the diabetic foot with multi-antibiotic resistant microorganisms. J Infect 2007;54:439-45.
3. Gadepalli R, Dhawan B, Sreenivas V, Kapil A, Ammini AC, Chaudhry R, *et al.* A clinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. Diabetes Care 2006;29:1727-32.
4. Lavery LA, Armstrong DG, Wunderlich RP, Mohler MJ, Wendel CS, Lipsky BA, *et al.* Risk factors for foot infections in individuals with diabetes. Diabetes Care 2006;29:1288-93.
5. Collee JG, Miles RS, Watt B. Tests for the identification of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie & McCartney Practical Medical Microbiology. 14th ed. London: Churchill Livingstone; 1996. p. 151-79.
6. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty First Informational Supplement M100-S21. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2016.
7. Black JA, Moland ES, Thomson KS. AmpC disk test for detection of plasmid-mediated AmpC beta-lactamases in *Enterobacteriaceae* lacking chromosomal AmpC beta-lactamases. J Clin Microbiol 2005;43:3110-3.
8. Galani I, Rekatsina PD, Hatzaki D, Plachouras D, Souli M, Giamarellou H, *et al.* Evaluation of different laboratory tests for the detection of metallo-beta-lactamase production in *Enterobacteriaceae*. J Antimicrob Chemother 2008;61:548-53.
9. Jog AS, Shadija PG, Ghosh SJ. Detection of multidrug resistant Gram-negative bacilli in type II diabetic foot infection. Int J Med Sci 2013;2:186-99.
10. Hartemann-Heurtier A, Robert J, Jacqueminet S, Ha Van G, Golmard JL, Jarlier V, *et al.* Diabetic foot ulcer and multidrug-resistant organisms: Risk factors and impact. Diabet Med 2004;21:710-5.
11. Rawat V, Singhai M, Kumar A, Jha PK, Goyal R. Bacteriological and resistance profile in isolates from diabetic patients. N Am J Med Sci 2012;4:563-8.
12. Datta P, Gupta V, Arora S, Garg S, Chander J. Epidemiology of ESL, Amp C and Carbapenamases producing *Proteus mirabilis*. Jpn J Infect Dis 2014;67:44-6.
13. Gupta V, Datta P, Agnihotri N, Chander J. Comparative *in vitro* activities of seven new beta-lactams, alone and in combination with beta-lactamase inhibitors, against clinical isolates resistant to third generation cephalosporins. Braz J Infect Dis 2006;10:22-5.
14. Richard JL, Sotto A, Jourdan N, Combescure C, Vannereau D, Rodier M, *et al.* Risk factors and healing impact of multidrug-resistant bacteria in diabetic foot ulcers. Diabetes Metab 2008;34:363-9.