Original Article

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Changing Antibiogram Profile of *Acinetobacter baumannii* in Diabetic and Non-Diabetic Foot Ulcer Infections

Microbiology Section

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ABSTRACT

Introduction: Foot ulcer infection relies on the hosts immune status and pathophysiological condition. The differences between Diabetic Foot Ulcer Infections (DFU) and non-DFU patients may alter the biofilm-forming capabilities of microorganism and thereby, play a key role in regulation of ulcer healing. Extended Spectrum of β-Lactamase (ESBL) and Metallo-β-Lactamase (MBL) producing *Acinetobacter baumannii* isolates are reported as important causative agents of infection.

Aim: To determine the antimicrobial susceptibility, ESBL, MBL production, and biofilm formation in *A.baumannii* among diabetic and non-diabetic foot ulcer patients.

Materials and Methods: This is a hospital based study, done for a period of 10 months. Samples were collected from general surgery outpatients and inpatients suffering from foot ulcer infections and also include wagner grade II to V. Pus/tissue was collected and processed for standard methods of culture, antimicrobial susceptibility, biofilm formation assay, ESBL, MBL, Modified Hodge Test (MHT) and Minimum Inhibitory Concentration (MIC).

Results: A total of 70 bacterial isolates were obtained from 400 patients with DFU and non-DFU from pus and tissue specimens. Antibiogram profiles of DFU isolates were sensitive to colistin and resistant to all the major groups of antibiotics classes. Non-DFU isolates were sensitive to amikacin, ceftriaxone, gentamicin piperacillin/tazobactam, imipenem, meropenem, colistin and resistant to ceftazidime, cefotaxime, ciprofloxacin, co-trimoxazole, piperacillin, tetracycline. The result showed that out of 35 DFU isolates 13 (37.14%) produced ESBL, 10 (28.57%) produced MBL and 9 (25.71%) formed strong biofilm. Further in 35 non DFU isolates 8 (22.85%) produced ESBL, 5 (14.28%) produced MBL and 3 (8.57%) formed strong biofilm. Almost all the isolates of Multi-Drug Resistance (MDR) are ESBL and MBL producer as well as biofilm formers.

Conclusion: Colistin is the drug of choice for the efficient treatment of multi-drug resistant isolates of foot ulcer patients. The rapid spreading of ESBL, MBL producers, and MDR require the implementation of not just surveillance study but also proper and rational selection of antibiotics, especially for MDR for better clinical outcomes.

Keywords: Antimicrobial susceptibility, Biofilm, Extended Spectrum of β-Lactamase, Metallo- β-Lactamase

INTRODUCTION

Diabetes mellitus is one of the major threats in the public health [1]. Almost 25% of diabetic patients develop foot ulcer infections during their lifespan due to poor glycolic control and low immune status. According to the International Diabetes Federation (IDF), approximately 69.2 million people have been affected nationwide and 415 million worldwide people are having diabetes [2]. DFU patients have 10 times more risk factors of infection than non-DFU patients and risk of infections is more common in male patients compared to female patients [3,4]. In non-DFU patients, once infection has developed, due to peripheral arterial disease and peripheral neuropathy of lower limb, it limits other secondary infections. However, it may lead to foot condition such as callosities, fissure, and trauma by delay in treatment [5]. Foot ulcer infections include: abscess, necrotizing fasciitis, gangrene, septic arthritis, tendonitis, cellulitis, and osteomyelitis [6].

Many studies have been reported along the DFU patients who had been commonly encountered with predominant pathogens such as *P.aeruginosa, E.coli*, *Staphylococcus* spp., *Klebsiella* spp., *Acinetobacter* spp., *Proteus* spp. Only a very few of them reported DFU and non-DFU patients with *A.baumannii* infections with threatening drug-resistant and limb amputation [7].

A.baumannii is the second most common isolated micro-organism from non-fermented bacteria in foot ulcer infections. Although, *Acinetobacter* wound infection showed an isolation rate of 11.1%, it poses a problem to clinician whether to treat it aggressively as a pathogen or as a colonizer [8]. *A.baumannii* is regarded as a rare colonizer whose prevalence is 0.5% on human skin in temperate climates where it is commonly found in humid climates [9,10]. It is an opportunistic pathogen affecting severe illnesses like immunodeficiency, diabetes prolong hospital stay due to risk of developing hospital acquired infection and prolonged exposure to antimicrobial agents [11,12].

Many factors lead to the emergence and survival of MDR *A.baumannii* in diabetic and non-diabetic foot ulcer patients. Most common among them is biofilm production, that may lead to ineffective penetration of antibiotics due to recruitment of leukocytes into the foot ulcers. Bacteria in the biofilm matrices possess antiphagocytic properties which can facilitate and alter the gene expression of intercellular communications [13]. Hence, peripheral arterial disease and neuropathy are often present in patients with foot ulcer infection and may contribute to poor penetration of antibiotics into the lower limb tissues which promotes resistance to bacteria [14]. More than 90% isolates of *A.baumannii* are resistant to all beta-lactams, fluoroquinolones, and aminoglycosides antibiotics. Several studies have reported the prevalence of ESBL and MBL producers which may promote the multi-drug resistance [7,15]. Colistin was the drug of choice for efficient handling of non-healing ulcer with *A.baumannii* infections [16]. This study was conducted in order to understand the severity of the multi-drug resistance and biofilm formation by

A.baumannii in DFU compared to the non-DFU patients, which may lead to development of secondary infections and non-healing ulcer and lead to amputation of lower limb extremities. This study is aimed to recognise the differences between the isolates among diabetic and non-diabetic foot ulcers. The present investigation is done in order to determine the prevalence, antibiotic susceptibility pattern, ESBL, MBL and biofilm formation of *A.baumannii* isolates in diabetic and non-diabetic foot ulcer patients.

MATERIALS AND METHODS

A hospital based, prospective study was conducted over a period of 10 months from Sepember 2016 to June 2017 at a tertiary care hospital, Mangalore after obtaining Institutional Ethics committee approval (Reg. No- YU2016/172). Informed consent was obtained from all partcipants.

Study Design and Sample Collection: Samples (pus and exudates, tissues) were collected from patients who presented with foot ulcer infections. A total of 70 non-duplicate isolates were obtained from 400 patients screened. Thirty five isolates of *A.baumannii* were taken in each, among DFU and non-DFU patients. Inclusion criteria for DFU included only diabetic foot ulcer infection and age of > 18 years and exclusion criteria was the duplicate sample of the same patient. Inclusion criteria for non-DFU included ulcer with peripheral arterial disease and neuropathy and age > 18 years; and exclusion criteria - prior treatment, if any, and Meggit Wagner classification system grading of foot ulcer: 0–I [17].

Bacteria Isolation and Identification

The collected pus/tissues was processed for Gram staining and cultured on 5% Sheep blood agar and Mac Conkey agar (Hi-Media Laboratories, India) for aerobic culture and incubated at 37°C overnight. Tissues were homogenised before inoculation and all isolates were confirmed using the BD Phoenix 100 system (Becton Dickinson, USA).

Antimicrobial Susceptibility Testing (AST)

AST was carried out by Kirby-Bauer method on Mueller Hinton agar (MHA) and the results were interpreted according to CLSI 2016 guidelines [18]. Antibiotic disks tested were amikacin (30 µg), imipenem (10 µg), meropenem (10 µg), piperacillin (100 µg), piperacillin/tazobactam (110 µg), levofloxacin (5 µg), ciprofloxacin (5 µg), co-trimoxazole (Trimethoprim/sulfamethoxazole) (1.25/23.75 µg), ceftriaxone (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefepime (30 µg), gentamicin (10 µg), tobramycin (10 µg), tetracycline (30 µg) (Hi-Media Laboratories, India). For control, *E.coli* (ATCC® 25922™), *E.coli* (ATCC® 35218™) and *P. aeruginosa* (ATCC® 27853™) were used.

Minimum Inhibitory Concentration (MIC)

The MIC of colistin was determined for all the MDR *A.baumannii* isolates by using the E-test strips and agar methods according to the manufacturer's instructions. The MIC breakpoints of ≤ 2 µg/L were regarded as susceptible and ≥ 4 µg/L as resistant [18,19].

Extended Spectrum of β-Lactamase (ESBL)

Detection of the ESBL phenotype was performed by combined disk diffusion method on Mueller Hinton agar (MHA) plate using ceftazidime (30 µg), ceftazidime/clavulanic acid (30/10 µg) [18]. *E.coli* (ATCC® 25922™) were used as the negative control and an in-house ESBL producing *A.baumannii* (ATCC® 19606™) isolate was used as the positive control. The test was considered positive when an increase in the diameter of the zone of inhibition was ≥5 mm around ceftazidime/clavulanic acid against ceftazidime alone [19-21].

Metallo-β-Lactamase (MBL)

Detection of MBL was done by using Imipenem-EDTA combined disk test. The overnight broth cultures of test isolates along with standard control strains (opacity adjusted to 0.5 McFarland) was lawn cultured on MHA plate. After drying, 10 µg imipenem disks were placed on the lawn culture with 20 mm distance from center to center of the disks. Another 10 µg imipenem impregnated with 750 µg of Disodium EDTA was added to one of the imipenem disks and incubated overnight [18]. Isolates showed ≥7 mm increased with the inhibition zone size of imipenem – EDTA disk than the imipenem disk alone, was considered as MBL producers. Positive control used was *P.aeruginosa* (ATCC® 27853™) [22,23].

Modified Hodge Test (MHT)

Modified Hodge test was performed in all isolates. It is a screening test which helps in detection of carbapenemases. *E.coli* (ATCC® 25922™) an indicator organism sensitive to carbapenems was cultured in peptone water to achieve 0.5 McFarland opacity standard and lawn cultured onto a MHA plate using a sterile cotton swab [18]. After drying, 10 µg imipenem disk was placed at the centre of the plate on the lawn culture, and an overnight growth of test strain was heavily streaked from the edge of the imipenem disk outwards, to the periphery of the plate in one direction. After streaking positive and negative control from the edge of the disk outwards to the periphery of the plates, they were incubated at 37°C overnight and the presence of a distorted zone - clover-leaf shaped zone of inhibition was considered as a positive test [24,25].

Biofilm Formation Assay

Biofilm forming ability of all the isolates was performed as mentioned previously with certain modification [26]. Briefly, bacterial cells were grown overnight at 37°C in 5 mL Trypticase-Soy Broth (TSB) in test tube. A 96-well flat bottomed polystyrene tissue culture plate was added with 200 µL of sterile TSB, inoculated with 10 µL of overnight culture and incubated at 37°C for 24 hours. After incubation, layer from each well was removed and washed carefully three times, with 200 µL of phosphate buffered saline (pH-7.2) in order to remove free-floating bacteria. Adherent bacteria were added with 200 µL of 99% methanol for 15 minutes. The plates were decanted, dried and stained for 7 minutes with 200 µL of 0.1% Hucker crystal violet. Excess stain was rinsed off with tap water. The plates were air dried and dye bound to the adherent cells was resolubilised with 160 µL of 33% (v/v) glacial acetic acid per well. The Optical Density (OD) of each well was measured at 630 nm using ELISA reader (FLUOstar Omega, BMG LABTECH, Germany). The biofilm producing strain *A.baumannii* (ATCC® 19606™) and *P.aeruginosa* (ATCC® 27853™) were taken for Positive Control (PC). Wells inoculated with sterile broth were used as Negative Control (NC).

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS 23.0 version software (SPSS Inc., Chicago, IL). The Chi-square test and Fisher's-exact two-tailed test analysis were done in this study. Statistical significance was regarded as a p-value < 0.05.

RESULTS

Of the 70 isolates of *A.baumannii* obtained from 400-foot ulcer patients (17.50%), 35 isolates of 15.90% (35/220) recovered from DFU and 35 isolates of 19.44% (35/180) non-DFU patients. The mean age group of the patients was 56.57±10.34. Most of the isolates recovered from the foot ulcer patients who were 44 to 60-year-old [Table/Fig-1] and Wagner grading II to IV [Table/Fig-2]. Infections on males were predominant 77.15% (27/35) compared to females 28.85% (8/35) among DFU and non-DFU patients. In our study, DFU patients were suffering from associated diseases such as hypertension, neuropathy, retinopathy, and nephropathy. Few patients also presented with Ischemic heart diseases. Below the age of 30, patients suffer from Type-2 diabetes mellitus and non-DFU patients below the age of 30, having the history of smoking/alcohol drinking and above the age of 30, patients had hypertension.

Age (Years)	DFU (35/220=15.90%)		NDFU (35/180=19.44%)	
	Male	Female	Male	Female
18-30	2	1	5	3
31-43	3	1	8	1
44-56	8	4	7	1
≥ 57	14	2	7	3
Total	27	8	27	8

[Table/Fig-1]: Distribution of age group and sex affected with DFU and NDFU in *Acinetobacter baumannii* infections.
Notes: DFU- Diabetic foot ulcer; NDFU- Non-diabetic foot ulcer

Antibiotic disc	*Acinetobacter baumannii*[*]	Sensitive	Resistant	p-value[*]
AMK	DFU	4(11.43%)	31(88.57%)	0.00062
	NDFU	18(51.43%)	17(48.57%)	
CPM	DFU	3(8.57%)	32(91.43%)	0.000167
	NDFU	18(51.43%)	17(48.57%)	
CAZ	DFU	1(2.86%)	34(97.14%)	0.000678
	NDFU	12(34.29%)	23(65.71%)	
CTX	DFU	2(5.71%)	33(94.29%)	0.001208
	NDFU	14(40.00%)	21(60.00%)	
CTR	DFU	4(11.43%)	31(88.57%)	0.000144
	NDFU	19(54.29%)	16(45.71%)	
CIP	DFU	4(11.43%)	31(88.57%)	0.026617
	NDFU	12(34.29%)	23(65.71%)	
LEV	DFU	6(17.14%)	29(82.56%)	0.001093
	NDFU	20(57.14%)	15(42.86%)	
COT	DFU	3(8.57%)	32(91.43%)	0.000402
	NDFU	17(48.57%)	18(51.43%)	
GEN	DFU	5(14.29%)	30(85.71%)	0.001907
	NDFU	18(51.43%)	17(48.57%)	
IPM	DFU	6(17.14%)	29(82.56%)	0.002409
	NDFU	19(54.29%)	16(45.71%)	
MRP	DFU	8(22.86%)	27(77.14%)	0.000144
	NDFU	24(68.57%)	11(31.43%)	
PIP	DFU	4(11.43%)	31(88.57%)	0.000402
	NDFU	17(48.57%)	18(51.43%)	
PIT	DFU	6(17.14%)	29(82.56%)	0.005056
	NDFU	18(51.43%)	17(48.57%)	
TET	DFU	3(8.57%)	32(91.43%)	0.010239
	NDFU	12(34.29%)	23(65.71%)	
TOB	DFU	7(20.00%)	28(80.00)	0.011862
	NDFU	18(51.43%)	17(48.57%)	

[Table/Fig-2: Antimicrobial susceptibility pattern of *Acinetobacter baumannii* in DFU and NDFU.
Notes: [*]Fisher exact two-tailed test (p-value < 0.05 and significant difference between DFU and non-DFU patients for all antibiotics).
[*]Acinetobacter baumannii isolated from diabetic and non-diabetic foot ulcer patients (Tissue, Pus and exudates)
Abbreviations:AMK – Amikacin ; CPM – Cefepime ; CAZ – Ceftazidime ; CTX – Cefotaxime ; CTR – Ceftriaxone ; CIP – Ciprofloxacin ; LEV – Levofloxacin ; COT – Co-trimoxazole ; GEN – Gentamicin ; IPM – Imipenem ; MRP – Meropenem ; PIP – Piperacillin ; PIT – Piperacillin / tazobactam ; TET – Tetracycline ; TOB – Tobramycin ; DFU- Diabetic foot ulcer; NDFU- Non-diabetic foot ulcer

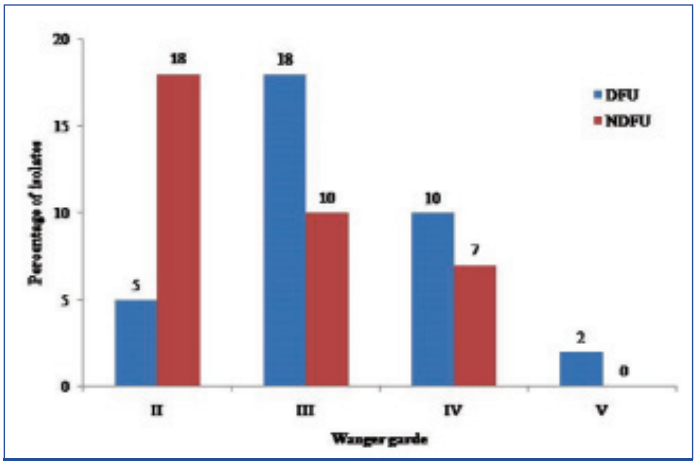
Antimicrobial susceptibility pattern of *A.baumannii* among DFU and non-DFU patients is depicted [Table/Fig-3]. All the DFU patients isolates of *A.baumannii* were resistant to major groups of antibiotics in comparison to non-DFU. DFU were sensitive to 100% colistin and resistant to ceftazidime (97.14%), cefotaxime (94.29%), 91.43% (cefepime, co-trimoxazole, and tetracycline), 88.57% (amikacin, ceftriaxone, ciprofloxacin, piperacillin), gentamicin (85.71%), 82.56% (levofloxacin, imipenem, piperacillin/tazobactam), tobramycin (80%) and meropenem (77.14%). NDFU were sensitive to 51.43% (amikacin, cefepime, gentamicin, tobramycin, piperacilin/tazobactam), 54.29% (ceftriaxone, imipenem), levofloxacin (57.14%), meropenem (68.57%), 100% colistin and resistant to 65.71% (tetracycline, ceftazidime, ciprofloxacin), cefotaxime (60%), and 51.43% (piperacillin, co-trimoxazole).

ESBL and MBL producing isolates among *A.baumannii* were 37.14% (13/35) and 28.57% (10/35). Non-DFU isolates were ESBL 22.85% (8/35), and MBL 14.28% (5/35) respectively. All isolates of DFU and non-DFU isolates which are MBL positive were confirmed by MHT. MDR isolates of *A.baumannii* were 62.85% (22/35) in DFU and 28.57% (10/35) in non-DFU patients [Table/Fig-4]. Biofilm formation was seen in *A.baumannii* from DFU and more compared to non-DFU isolates [Table/Fig-5a,b]. Based on the tissue culture plate assay, the isolates were classified as strong [Optical Density (OD) >0.350], moderate (OD between 0.200–0.350) or weak (OD 0.041-0.200) biofilm-forming *A.baumannii.* A chi-square (χ^2) test showed that there was a significant difference between isolates from DFU and non-DFU patients with *A.baumannii* infections {p-value < 0.05(0.034); χ^2 value= 6.71 and degree of freedom (df) =2} which correlated with ESBL, MBL production and MDR with biofilm formation [Table/Fig-6a,b]. The hospital stays of patients in whom ESBL, MBL producer, biofilm formation, and MDR were isolated ranged between 15-40 days.

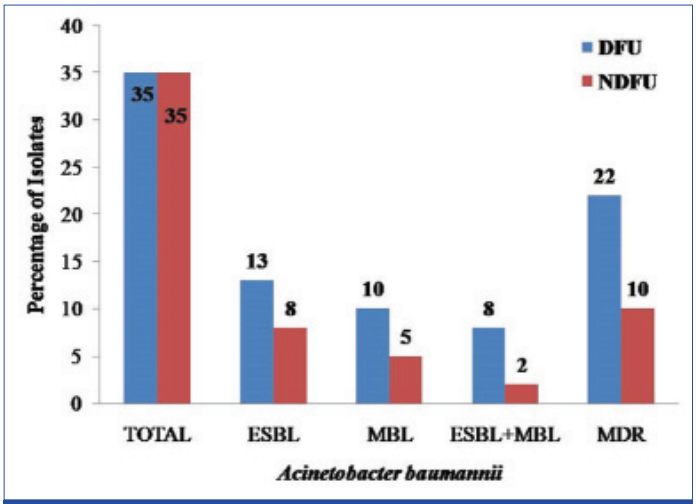
DISCUSSION

Foot ulcer infection complications are frequent clinical problems among non-communicable diseases leading to hospitalization. Most commonly foot ulcer infection seen in patients who tend to have immune deficiency, mono-microbial, and poly-microbial infections. Foot ulcer infections are predominantly poly-microbial with the ability to form changing trends of susceptibility, ESBL, MBL, MDR and biofilm, are an important causative agent resulting in treatment failure and increased risk of amputation.

This study shows *A.baumannii* infections in 15.90% DFU and 19.44% non-DFU patient, which is slightly higher than Murali TS et



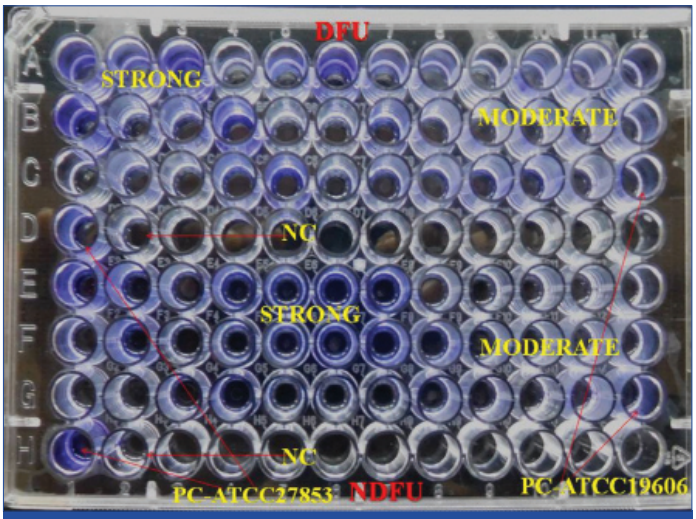
[Table/Fig-3]: Isolate distribution in different grades of ulcer in DFU and non-DFU patients.



[Table/Fig-4]: Percentage of ESBL, MBL producer and MDR *Acinetobacter baumannii.*

Biofilm Formation	DFU (n=35)	NDFU (n=35)	
Weak (0.041 – 0.200)	03(8.57%)	10(28.57%)	$\chi^2 = 6.71$, df =2, p=0.034
Moderate (≥0.200 – 0.350)	23(65.71%)	22(62.86%)	
Strong (≥0.350)	09(25.72%)	03(8.57%)	

[Table/Fig-5a]: Comparison of biofilm formation in *Acinetobacter baumannii* among DFU and non-DFU isolates.
Notes: Chi-square (χ^2) test; Significant p-value = <0.05, df= Degree of Freedom; DFU- Diabetic Foot Ulcer; NDFU- Non-Diabetic Foot Ulcer.



[Table/Fig-5b]: Biofilm formation in *Acinetobacter baumannii* in DFU and NDFU isolates.
Note: PC- Positive Control, NC- Negative Control, DFU- Diabetic Foot Ulcer, NDFU- Non-Diabetic Foot Ulcer

Acinetobacter baumannii	Biofilm Formation-DFU		
	Strong (09/35)	Medium (23/35)	Weak(03/35)
ESBL	08	05	0
MBL	08	02	0
MDR	09	11	02

[Table/Fig-6a]: Correlation of ESBL, MBL and MDR *Acinetobacter baumannii* with Biofilm formation in DFU
ESBL-Extended Spectrum of β-Lactamase, MBL- Metallo- β-Lactamase, MDR- Multi-Drug Resistant, DFU- Diabetic Foot Ulcer, NDFU- Non-Diabetic Foot Ulcer

Acinetobacter baumannii	Biofilm Formation-NDFU		
	Strong (03/35)	Medium (22/35)	Weak (10/35)
ESBL	02	05	01
MBL	02	03	0
MDR	03	07	0

[Table/Fig-6b]: Correlation of ESBL, MBL and MDR *Acinetobacter baumannii* with Biofilm formation in NDFU.
Notes: ESBL-Extended Spectrum of β-Lactamase, MBL- Metallo-β-Lactamase, MDR- Multi-Drug Resistant, DFU- Diabetic Foot Ulcer, NDFU- Non-Diabetic Foot Ulcer

al., they reported 14% in both isolates [13]. Another similar study by Karmaker M et al., reported the prevalence of 10% *Acinetobacter* spp. in DFU and non-DFU infections [27]. El-Din RA et al., reported 33% of *A.baumannii* isolated from DFU patients [15]. Jyothylekshmy V et al., reported the prevalence of 5.3% *Acinetobacter* spp. in DFU patients [28]. In our study, DFU isolates were 100% sensitive to colistin and resistant to ceftazidime (97%), cefotaxime (94%), 91% (cefepime, co-trimoxazole, and tetracycline), 89% (amikacin, ceftriaxone, ciprofloxacin, piperacillin), gentamicin (86%), 83% (levofloxacin, imipenem, piperacillin/tazobactam), tobramycin (80%) and meropenem (77.14%). Mendes JJ et al., reported sensitivity to 100% colistin and resistant to all routine antibiotics in DFU isolates [16]. As per Shanmugam P et al., reported antibiotic resistant were 83% ceftazidime, 67% ciprofloxacin, 50% (cefepime, gentamicin, tetracycline, tobramycin), 33% (amikacin, cotrimoxazole), 17% (imipenem, piperacillin/ tazobactam) in DFU isolates [7]. Another similar study, Murali TS et al., [13] reported in DFU isolates resistant to amikacin (76%), cefotaxime (94%), ciprofloxacin (84%), gentamicin (90%). *Acinetobacter* spp. was resistant to cefotaxime (88%), gentamicin (59%), amikacin(53%), ciprofloxacin (36%), and 29% (ceftazidime, piperacillin, piperacillin/tazobactam) in DFU isolates [29]. Similarly, Turhan V et al., in their study reported *Acinetobacter* spp. were resistant to piperacillin/tazobactam (88%), ciprofloxacin (84%), co-trimoxazole (75%), ceftazidime (63%), amikacin (53%), imipenem (29%) [30]. Akhi MT et al., reported antibiotic resistance to 100% (tetracycline, cefepime, ceftriaxone), and 50% (gentamicin, ciprofloxacin, imipenem, piperacillin /tazobactam) [31]. Results of our study indicated significant difference between in DFU and non-DFU patients for all isolates, p-value < 0.05 [Table/Fig-3].

Non-DFU patient's isolates were susceptible to 100% colistin, meropenem (68.57%), levofloxacin (57.14%), 54.29% (ceftriaxone, imipenem), 51.43% (amikacin, cefepime, gentamicin, tobramycin, piperacillin/tazobactam) and resistant to 65.71% (tetracycline, ceftazidime, ciprofloxacin), cefotaxime (60%), and 51.43% (piperacillin, co-trimoxazole). Murali TS et al., reported antibiotic sensitivity to non-DFU isolates were 25% (amikacin, ciprofloxacin, gentamicin) and resistance to 100% cefotaxime, and 75% (amikacin, ciprofloxacin) [13].

Extended spectrum of β-lactamase and Metallo-β-lactamase production in *A.baumannii* was reported on many studies but unfortunately CLSI guidelines does not support or advocate. *A.baumannii* producing ESBL was first reported from Turkey in 2001 and India in 2007. Similarly, MBL production in *A.baumannii* was reported from South Korea in 2001 and India in 2011. A very few studies are reported on diabetic foot ulcer infection in *A.baumannii.* This study shows that among DFU isolates, 37.14% (13/35) *A.baumannii* were ESBL producer and MBL 28.57% (10/35). Shanmugam P et al., reported 33.33% were ESBL and 16.6% MBL producers [7]. Another study by El-Din RA et al., reported 34.61% MBL producers [15]. Our study shows that, non-DFU isolates were producing ESBL 22.85% (8/35) and 14.28% (5/35) MBL. Surprisingly, none of the studies has been reported ESBL and MBL production in non-DFU isolates of *A.baumannii*. Some studies reported but it is not clear whether specimens/sample taken for the patient of peripheral arterial and peripheral neuropathy or not. Hence, this study is first which reports ESBL and MBL production in *A.baumannii* among non-DFU.

Almost all MDR isolates of *A.baumannii* produce biofilm in DFU and non-DFU patients. Biofilm formation was categorized into strong, moderate, and weak. In this study 25.71% (9/35), 65.71% (23/35), 8.57% (03/35) prevalence was reported as strong, moderate and weak respectively. Significant difference was observed between diabetic and non-diabetic groups [Table/Fig-5a,b]. Similar to our study, Swarna SR et al., reported 23.07% strong biofilm formation and 15.38% moderate which was lower too much than observed in our study and 38.46% weak which was higher compared to our study [32]. Murali TS et al., reported biofilm formation in DFU isolates was 39.13% [13]. Another study by Zubair M et al., reported 60% strong and 40% weak biofilm formation in DFU isolates [33]. Similarly, Vatan A et al., reported 59.25% strong and 40.75% weak biofilm formation [34]. Di Domenico et al., reported 8.9% strong biofilm formation in DFU isolates [35].

Biofilm formation in non-DFU patients of *A.baumannii* in our study shows that 8.57% (03/35) strong, 62.86% (22/35) moderate, and 28.57% (10/35) weak. Amazingly, we found that biofilm formation in DFU isolates correlating with ESBL, MBL and MDR *A.baumannii* were more predominant than non-DFU isolates [Table/Fig-6a,b]. Murali TS et al., reported the correlation of biofilm and MDR isolates in DFU and non-DFU patients that were more virulent compared

to non-DFU isolates leading to limb amputation [13]. In our study, we found wide variation and changing trend of antimicrobial susceptibility pattern compared to other studies. DFU patients compared to non-DFU patients had significant difference of healing ulcer due to their compromised immune status. There is a need to understand the characteristics of *A.baumannii* in non-healing ulcer infections among DFU and non-DFU isolates.

LIMITATION

In the present study, the MIC of netilmicin and polymixin B was not determined as duration of sample collection was limited. The study did not include any molecular approach to find out ESBL, MBL, and drug resistance genes.

CONCLUSION

This study highlights the need to establish antimicrobial susceptibility surveillance for *A.baumannii* to determine the appropriate empirical treatment regimen. Periodical checked up in a tertiary care hospital with the help of infection control and wound/ulcer management committee to inhibit the spreading of *A.baumannii* infections. The biofilm formation in DFU compare to non-DFU patients is the most severe threat to the non-healing ulcers. The rapid spreading of ESBL, MBL producers, and MDR require the implementation of not just surveillance study but also the proper and rational selection of antibiotics, especially for MDR which help clinician in the treatment of diabetic and non-diabetic foot ulcer infection patients should be done.

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