

Metallo Beta Lactamase Producing *Pseudomonas Aeruginosa* and its Association with Diabetic Foot

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Abstract *Pseudomonas aeruginosa* strains that produce metallo beta lactamases (MBLs) are becoming increasingly prevalent in wound infections. The aim of the present study is to determine the clinical features, incidence, and to find out the antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* in diabetic foot infections. Pus samples for bacterial culture were collected from 310 patients admitted with diabetic foot infections. Antimicrobial sensitivity testing was performed by the Kirby-Bauer disc diffusion method. Carbapenem resistance screening and confirmation of MBL was done by the modified imipenem-ethylenediaminetetraacetic acid (EDTA) double disc synergy test. A total of 54 *Pseudomonas aeruginosa* was isolated from 310 diabetic foot cases. Males were affected more than females with an M:F ratio of 1.6:1. Most patients belonged to the fifth decade of life with a mean age of 49 ± 16.8 years. All the patients were previously diagnosed with diabetes mellitus with duration of the disease at 16 ± 10.2 years and 63% were prescribed oral hypoglycaemic agents. Wound characteristics were classified according to Wagner's classification majority of *Pseudomonas aeruginosa* were isolated from Wagner's II and III grade wound. A number of 26 (89.7%) patients underwent debridement, while 9 (31%) patients underwent toe disarticulation, and 7 (24.1%) patients underwent below-the-knee (BKA) ampu-

tation. Antibiotic sensitivity testing revealed 20.3% of *Pseudomonas aeruginosa* were resistance to carbapenem and 81.8% of these were MBL mediated resistance. Infection with multi drug resistance organisms (MDROs) is common in diabetic foot ulcers and is associated with inadequate glycemic control and increased requirement for surgical treatment. There is a need for continuous surveillance of resistant bacteria to provide the basis for empirical therapy and reduce the risk of complications.

Keywords Diabetic foot · *Pseudomonas aeruginosa* · Metallo beta lactamase

Introduction

Foot ulcers are among the leading causes of morbidity in diabetics and are the most common indication for admission in this population [1, 2]. Devitalized tissue is the site where the bacteria responsible for the non-healing ulcers inflict damage [1]. Infectious agents are associated with amputation of the infected foot if not treated promptly [3]. *Pseudomonas aeruginosa* is one of the important nosocomial pathogens and multiple factors contribute to it; for example, injudicious administration of broad spectrum antibiotics [4], instrumentation and intrinsic resistance of microorganisms towards antimicrobial agents [5]. The introduction of carbapenems into clinical practice was of great help in the treatment of serious bacterial infections caused by β -lactam resistant bacteria and is the drug of choice for the infection caused by cephalosporin-resistant gram negative infections [6]. There is not enough information from the Indian subcontinent regarding the prevalence of metallo beta lactamase (MBL) mediated

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resistance among *Pseudomonas* in diabetic foot infection. The aim of the present study is to determine the incidence and to find out the antimicrobial susceptibility pattern of *Pseudomonas aeruginosa*.

Materials and Methods

Over a one and half year period from July 2008 to December 2009, 54 isolates of *Pseudomonas aeruginosa* were cultured from diabetic foot infection admitted to the hospital. The specimen were collected, transported, and processed in the laboratory without any delay. The isolates were identified by standard laboratory techniques [7]. Antimicrobial sensitivity testing was performed on Mueller-Hinton agar plates with commercially available discs (Hi-Media, Mumbai) by the Kirby-Bauer disc diffusion method [8]. The results were recorded and interpreted as per Clinical and Laboratory Standard Institute (CLSI) recommendations [8]. *Pseudomonas aeruginosa* ATCC 27853 was used as control. Carabapenem resistance was done by the Kirby Bauer disc diffusion method. Further screening for detection and confirmation of metallo-beta lactamase was done by the modified imipenem-EDTA double disc synergy test [9].

Results

Out of 310 specimens screened for bacteria, 54 samples yielded growth for *Pseudomonas aeruginosa*. Out of 54 patients infected with *Pseudomonas aeruginosa*, 32 (59.3%) were male and 22 (40.7%) were females. The mean age of all the patients was 49 years (SD 16.8) and the mean duration of diabetes was 16 years (SD 10.2). Out of 54 known diabetic patients prior to admission, 34 (63.0%) were maintained on OHA and 20 (27%) were maintained on insulin. 15 (27.8%) of the subjects had leukocytosis during admission. Majority of *Pseudomonas aeruginosa* were isolated from Wagner's II and III grade wound.

In this study, 31 (57.4%) patients presented with diabetic complications. 12 (38.7%) patients had peripheral neuropathy, 09 (29.0%) nephropathy, 6 (19.4%) retinopathy, and 4 (12.9%) with coronary artery disease and these were the most frequent complications. A number of 25 (46.3%) patients were managed with antibiotics alone while 29 (53.7%) underwent surgical intervention with concomitant antibiotics. Debridement was the most frequent surgical intervention in 26 (89.7%) patients followed by toe disarticulation in 9 (31.0%) and below-the knee (BKA) amputation in 7 (24.1%). Repeated procedures were done in 14 (48.3%) patients.

Overall microbial sensitivity to the different antibiotics is as follows: amikacin 35%, gatifloxacin 22.2%, piperacillin/tazobactam 42.6%, piperacillin 22.2%, aztreonam 7.4%, cephotoxime 18.5%, ceftriaxone 18.5%, ciprofloxacin 22.2%, imipenem 79.7%, sparfloxacin 25.9%, and cefixime 13.0% (Table 1). Again, 9 of 11 isolates which were resistant to imipenem showed significant zone size enhancement with the EDTA impregnated discs when compared with the plain antibiotic discs (MBL resistant) (Table 2).

Discussion

Diabetic foot ulcer is the most common complication requiring hospitalization among diabetic patients [2]. It is also the most common cause of non-traumatic lower extremity amputations [3]. Physicians have an important

Table 1 Demographic, clinical and microbiologic characteristics of patients with diabetic foot infections

Patients characteristic	Number	Percentage
Demographic characteristics		
Sex: Male	32	59.3
Sex: Female	22	40.7
Age (years) Mean \pm SD	49 \pm 16.8	
Clinical characteristics		
Duration of DM (years) Mean \pm SD	16 \pm 10.2	
DM foot duration (days) Mean \pm SD	16 \pm 10.2	
Duration of hospital stay	22 \pm 5.58	
Mortality	07	12.9
Medications:		
OHA	34	63.0
Insulin	20	27.0
Wagner's classification:		
W1	08	14.8
W2	25	46.3
W3	16	29.6
W4	05	9.3
W5	Nil	
Leukocytosis on admission	15	27.8
Diabetic complications		
Peripheal neuropathy	12	38.7
Nephropathy	09	29.0
Retinopathy	06	19.4
Coronary artery disease	04	12.9
Treatment		
Antibiotic alone	25	46.3
Surgical intervention	29	53.7
Toe disarticulation	09	31.0
BKA amputation	07	24.1

Table 2 Antibiotic resistant pattern of *Pseudomonas aeruginosa* isolated from diabetic foot

Antibiotics	Sensitive (%)	Resistant (%)
Amoxicillin	01 (1.9)	53 (98.1)
Amikacin	19 (35.2)	35 (64.8)
Ciprofloxacin	12 (22.2)	42 (77.8)
Cefixime	07 (13.0)	47 (87.0)
Gentamicin	04 (7.4)	50 (92.6)
Cephotaxime	10 (18.5)	44 (81.5)
Piperacillin	12 (22.2)	42 (77.8)
Piperacillin + tazobactam	23 (42.6)	31 (57.4)
Imipenem	11 (20.3)	43 (79.7)
Sparfloxacin	14 (25.9)	40 (74.1)
Aztreonam	04 (7.4)	50 (92.6)

role in the prevention, early diagnosis, and management of diabetic foot complications. Management, however, entails an extensive knowledge of the major risk factors for amputation and preventive maintenance with special reference to drug resistance in bacteria.

In the present study, there were significant differences in the following variables. More males were affected with an M:F (1.5:1) and the wounds were classified as Wagner Grade II-III on admission. Length of stay was 22 ± 16.1 days. Mortality rate was at 12.9% as compared to other study which revealed an M:F (0.7:1), wound grade of Grade II on admission, length of stay of 7–30 days and mortality rate of 0.3% [10].

In the present study, 63% of patients were previously diagnosed and maintained on oral hypoglycaemic agents but a large portion of them were non-compliant. Poor blood glucose control may contribute to increased mortality and morbidity since hyperglycemia is contributory to the development of infection and increased susceptibility to tissue injury. During acute episodes of hyperglycemia, there is decreased tissue oxygen utilization, causing preferential shunting of glucose through the sorbitol pathway instead of the glycolytic pathways, thereby decreasing mitochondrial pyruvate utilization and decreased energy production. This is termed as hyperglycemia-induced pseudohypoxia. This explains the development of decreased peripheral pulses and eventually gangrenes in the distal extremities of diabetics [11]. Neuropathy, on the other hand, predisposes a diabetic to unrecognized injury due to loss of sensation. In our study, nine patients (16.7%) had peripheral neuropathy. Patients with diabetic foot ulcer more often than not present with advanced stages of ulcers because of this. It is therefore, important to educate patients on preventive foot care. *Pseudomonas aeruginosa* was isolated in 17.4% cases which is less compared to other Indian studies.

Antibiotic sensitivity testing revealed that more than 96% of *Pseudomonas aeruginosa* were resistant to more than two drugs (MDR). In various studies across the world, varying rates of resistance (4–60%) have been reported for imipenem and meropenem. In the present study, 20.3% of *Pseudomonas aeruginosa* were resistant to imipenem. Among the Indian workers, Gladstone et al. reported 42.8% [12], whereas Taneja et al. reported 36.4% [13].

Resistance to carbapenem is due to decreased outer membrane permeability, increased efflux systems, alteration of penicillin binding proteins, and the production of carbapenem hydrolyzing enzymes, that is, carbapenemases. The resistance may also be due to the production of MBLs [14].

There are frequent reports of MBL production in *Pseudomonas aeruginosa* from the Asian and the Pacific countries, namely Japan, Taiwan [14], and Hong Kong [14]. In this study, we observed 20.3% resistance to carbapenem among the *Pseudomonas aeruginosa* screened and 81.8% of this was MBL mediated as per the screening results. Other study from south India reported 12% MBL-mediated imipenem resistance in *P. aeruginosa*.

MBL production is a significant problem in hospital isolates of *P. aeruginosa*. With increasing isolation of extended spectrum beta lactamase (ESBL) producing isolates in the diabetic foot necessitating the use of carbapenem, the problem of MBL production is also increasing and as the result it is mandatory to identify MBL-producing *Pseudomonas aeruginosa* isolates in diabetic foot ulcers so that appropriate treatment can be provided.

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