

# Spectrum of Antibiotic Susceptibility of *Pseudomonas Aeruginosa* in Case of Diabetic Foot in a Tertiary Care Centre in Kolkata

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## ABSTRACT

**Introduction:** Diabetic foot ulcer is notorious for invasion by different bacteria and *Pseudomonas* is one of them which may be responsible for major cause of mortality in these patients as result of sepsis as this organism exhibits high degree of resistance to different broad spectrum of antibiotics. So, our aim in this study was to determine spectrum of antibiotic susceptibility pattern of *Pseudomonas aeruginosa* isolates from diabetic foot ulcers in our Tertiary care Hospital in Kolkata.

**Material and methods:** In last five years we collected 325 diabetic patients with foot ulcers (male=202, female=123) in our hospital. Pus collected from their wound was sent for culture and sensitivity by sophisticated technique especially in case of polymyxin B and Colistin. We recovered 51 patients as *Pseudomonas aeruginosa* positive along with their culture and sensitivity.

**Results:** We demonstrated male predominance over females in case *Pseudomonas* infection with male to female ratio of 1.64:1. Again, in 51 *Pseudomonas aeruginosa* positive patients male to female ratio was 2.18:1 (male=35, females=16). This organism demonstrated hundred percent resistance to 11 broad spectrum antibiotics but highest positivity to polymyxin B and colistin followed by carbapenem and aminoglycoside group of antibiotics.

**Conclusion:** We demonstrated multidrug resistant *Pseudomonas aeruginosa* with limited sensitivity to few broad spectrum antibiotics. It will help the clinicians to choose proper antibiotics and not to use antibiotics inadvertently and irregularly because this practice may lead to emergence of more and more multi-drug resistance to *Pseudomonas aeruginosa* and it may be responsible for the high mortality in these patients.

**Keywords:** Antibiotic Susceptibility, *Pseudomonas Aeruginosa*, Diabetic Foot

## INTRODUCTION

Diabetes is chronic morbid health problem throughout the world because the incidence of diabetes has been gradually increasing and becoming the burning problem.<sup>1</sup> Approximately one fourth of diabetic patients will develop foot ulcers and half of them will be infected.<sup>2,3</sup> According to the recent report of World Health Organization there are largest in the world (19 million in 1995 which has been increased to 57 million by 2015).<sup>4</sup> A commonly accepted definition of diabetic foot infection is the presence of systemic signs of infection, like, fever and leucocytosis or purulent discharge from the ulcer or two or more local symptoms and signs, like, redness, warmth, and indurations surrounding the ulcer area and local tenderness.<sup>5</sup> The diabetic foot ulcer is very difficult to treat than in non diabetics. Both gram positive and gram negative bacteria are involved in diabetic foot infection but former are three times more common in diabetic individual as compared to non diabetics.<sup>6</sup> Among the gram negative organisms *Pseudomonas aeruginosa* is fairly common. It is very dreadful pathogen because of the following reasons: Firstly, it is able to produce a variety of toxins and

proteases; secondly, it is able to resist phagocytosis; thirdly, the organism is only sensitive to cephalosporin, carbenicillin, polymyxin B, quinolones, gentamicin and streptomycin.<sup>7,8</sup> Though it may be rarely found as normal flora in human being, but it should never be considered as a contaminant specially in diabetic patients because in those patients it may be responsible for sepsis and in very severe case it may lead to amputation.<sup>9</sup> The severity of *Pseudomonas aeruginosa* infection is mainly due to mainly its inherent resistance known as intrinsic resistance. The multiplicity of resistance of this organism is mainly responsible for the failure to control by the antibiotic cycling.<sup>10</sup> This organism is versatile in nature and colonizes in varieties of environment due to its intrinsic resistance to antibiotics.<sup>11</sup> A lot of work s has been done on the antimicrobial sensitivity of *Pseudomonas aeruginosa* throughout the world, but very little works have been progressed in the Eastern Zone of India. So, we have tried to exhibit antibiotic sensitivity pattern in *Pseudomonas aeruginosa* in diabetic foot ulcers in our Tertiary care Hospital in Kolkata.

## MATERIALS AND METHODS:

This study was conducted retrospectively in KPC Medical College and Hospital, Kolkata after getting permission from local ethical committee.

**Sample collection:** Pus samples collectively aseptically from diabetic ulcers of 325 patients and sent to bacteriology department in the form of sterile swab in ice-cold conditions.

**Inoculation into the agar:** The material was inoculated on MacConkey's agar and blood agar by standard methods of inoculation for overnight at 37° C.

**Identification:** *Pseudomonas* was identified by following methods, like, gram staining, motility studies, different biochemical tests, like, catalase test, Oxidase test, blue pigment production, gelatin analysis, arginine dehydrolase test, acid from Hugh-Leifson test, nitrate reduction test and by different methods of growth, like, growth on cetrimide agar and ability to grow at 42° C.<sup>12</sup> This organism is gram negative, glucose oxidizer, positive for oxidase reaction and for arginine dehydrolase test, blue pigment producer.

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**Antibiotic sensitivity:** Antibiotic sensitivity testing was determined Kirby-Bauer disc diffusion method as per guidance of clinical and laboratory standard Institute by using commercially available disc from HI media (Mumbai, India) with standard reference strains of pseudomonas aeruginosa ATCC 27853. Pseudomonas aeruginosa was tested against penicillin, amoxicillin, oxacillin, piperacillin-tazobactam, cefoperazone-sulbactam, cefuroxime, cefotaxime, ceftriaxone, ceftazidime, cefepime, cefixime, erythromycin, azithromycin, ertapenem, imipenem, meropenem, gentamicin, tobramycin, netilmicin, amikacin, fluoroquinolone groups, cotrimoxazole, chloramphenicol, tetracycline, tigicycline, clindamycin, vancomycin, teicoplanin, linezolid, polymyxin B, colistin, ticarcillin and cefoperazone.

**RESULTS**

Total number of diabetic patients with foot ulcers involved in this in this study was 325, amongst them males and females were 202 and 123 respectively with ratio being 1.64:1 (p=0.00). Total number of pseudomonas aeruginosa isolated from the pus was 51 in which male and females were 35 and 16 respectively with ration being 2.18:1 (Table-1,2). Pseudomonas aeruginosa demonstrated 100% resistance to penicillin and semi synthetic penicillin, cefotaxime, erythromycin, tetracycline, clindamycin, vancomycin, teicoplanin and linezolid, nearly 100% resistance to cefuroxime, cefoxitin, ceftriaxone and tigicycline. On the other hand, more than 90% sensitive to polymyxin B, colistin and more than 80% positive to imipenem and meropenem (Table-3).

**DISCUSSION**

In our study amongst total number of patients affected from

foot infection was 325 with male to female ratio being 1.64:1 (male=202, female=123), whereas, in the study done by Sivanmaliappan TS et al. the ratio was exactly 2:1) male=180, female=90).<sup>1</sup> In other similar study it was demonstrated that this male to female ratio was 8:3. So, from the above studies including our present study demonstrated that males were more prone to be affected from diabetic foot with secondary infection because of the following reasons, like, males are more exposed to outside environment for their day to day activities; secondly, they are more exposed to dust, unclean water and soils which contain huge number of bacteria; thirdly, they are exposed to constant tension for his outdoor work activities and family activities which in turn unbalance his blood sugar level and this may make him vulnerable to infection.

In our study 87.69% patients were culture positive (285 patients out of 325 patients) whereas in the study of Sivanmaliappan TS et al. 66.6% diabetic patients were culture positive (180 out of 270 patients).<sup>1</sup> Against 285 culture positive patients only 51 patients were positive for pseudomonas aeruginosa (17.89%) whereas, 70% patients were pseudomonas species positive in the study of Sivanmaliappan TS et al.(126 of 180 culture positive patients).<sup>1</sup> In the same study 18 cases (14.3%) were pseudomonas aeruginosa positive (18 out of 126 pseudomonas species positive). The positivity of pseudomonas aeruginosa in the study done by Dharmasekaran et al. was 18.79%.<sup>6</sup> So values of all the above studies were close to our study. On the contrary in the study done in Private hospital in Chennai, 29.8% patients were positive for pseudomonas aeruginosa.<sup>12</sup>

In our study pseudomonas aeruginosa demonstrated 100% resistance to penicillin, amoxicillin, oxacillin, erythromycin, cefotaxime, tetracycline, clindamycin, vancomycin, teicoplanin, linezolid (ten antibiotics). It was similar to the study done by Sivanmaliappan TS et al. in Tamilnadu where this organism showed multidrug resistance against 11 organisms.<sup>1</sup> This study in Tamilnadu also showed no evidence of 100 percent sensitivity to any antibiotic – this is also similar to our study where highest sensitivity was documented against polymyxin B and colistin (92.15%).<sup>1</sup> This same study demonstrated 68% resistance to gentamicin and imipenem. But our study demonstrated nearly 50% resistance to aminoglycoside group of antibiotics and nearly only 10 to 15% resistance to carbapenem group of

Years	Total Cases (325)	Males (202)	Females (123)
2010	49 (13.79%)	31	18
2011	58 (16.58%)	35	23
2012	54(11.26%)	40	14
2013	69(17.97%)	45	24
2014	58(20%)	40	18
2015	37(20.37%)	25	12

**Table-1:** Year-wise male – female distribution of isolates of bacteria from pus

Bacteria	Males (202)	%	Females (123)	%	P
Pseudomonas aeruginosa (51)	35	58.26	16	41.73	0.01

**Table-2:** Male – female distribution of isolates of pseudomonas species from pus

Organisms	PEN	AMX	OX	AMC	PIPT	CES	CEF	CFT	CXT	
Pseudomonas aeruginosa (51)	0	0	0	0	27 52.94%	25 49.01%	1 1.96%	0	1 1.96%	
	CFZ	CTRX	CFP	AZ	ER	AZT	ERT	IMP	MEP	
Pseudomonas aeruginosa (51)	7 13.91%	1 1.96%	6 11.76%	5 9.80%	0	3 5.82%	6 11.76%	44 86.27%	41 80.39%	
	GET	TOB	NIT	AMK	CIP	OF	LIV	COT	CHLO	
Pseudomonas aeruginosa (51)	26 50.98%	25 49.01%	29 56.86%	31 60.78%	27 52.94%	16 31.37%	29 56.86%	2 3.92%	3 5.88%	
	TET	TIG	CLIN	VAN	TEI	LIZ	POL	COL	TIC	CEFOP
Pseudomonas aeruginosa (51)	0	1 1.96%	0	0	0	0	47 92.15%	47 92.15%	2 3.92%	2 3.92%

**Table-3:** Antibiotic sensitivity in case of pseudomonas aeruginosa

antibiotics. Again, Perim MC et al. in their study demonstrated 100% resistance to ampicillin, 75% resistance to cefotaxime, tetracycline and gentamicin.<sup>13</sup> These different results found in many studies may be due to pattern of microbial infection not consistent in patient with diabetic foot. So, repeated culture and sensitivity is required for proper evaluation of microbial characteristics as well as selection of appropriate antibiotic.<sup>14</sup>

Our study demonstrated 92.15% sensitivity to polymyxin B and colistin. But Perim MC et al. in their study demonstrated very high resistance to polymyxin B as assessed by Kirthy Bauer disc diffusion method.<sup>13</sup> Some other studies also showed poor correlation among the results of different susceptibility tests for polymyxin B, it may be due to poor diffusion of this drug into the agar.<sup>15</sup> The reason is mainly due to presence of high level of cat ion in the agar as it affects the diffusion of this antibiotic.<sup>16</sup> So, the study of Perim MC et al. validated the resistance determined by diffusion test by using broth dilution method and they found only 25% pseudomonas isolates was resistant to polymyxin B.<sup>13</sup> But now-a-days increased and inadvertent use of this antibiotic led to development of pseudomonas highly resistant to polymyxin B.<sup>17</sup>

The multidrug resistance of this organism may be due to selective actions of both disinfections and antibiotics on clinical specimen.<sup>18</sup> So our retrospective study revealed that the pseudomonas aeruginosa was highly sensitive to polymyxin B and colistin, carbapenem group of drugs in case of diabetic foot infection in this region as per our spectrum of antibiotic sensitivity to pseudomonas aeruginosa is concerned. Many studies throughout the world in relation to diabetic foot infections demonstrated contradictory results, in those cases molecular technique application may lead to more accurate characterization of microbial isolates as well as their targeted antibiotic therapy.<sup>19-21</sup>

## CONCLUSION

In our study, amongst 325 patients with diabetic foot infection males were significantly affected as compared to females ( $p=0.00$ ) with male to female ratio of 1.64:1. Number of pseudomonas aeruginosa positive cases was 51. They demonstrated 100% resistance to penicillin and semi-synthetic penicillin, tetracycline, clindamycin, vancomycin, teicoplanin, linezolid, cefotaxime and erythromycin. So it confirmed that the emergence of multidrug resistant pseudomonas aeruginosa in diabetic patients with foot infection. Modern sophisticated accurate technique is necessary to detect sensitivity in different microbial isolates. It is absolutely necessary to evaluate properly pseudomonas aeruginosa in infective wound and antimicrobial susceptibility pattern of this organism. This study showing multidrug resistant pseudomonas aeruginosa in diabetic foot ulcers will definitely help the clinicians to introduce proper antibiotic in proper time. This is our ultimate aim through this extensive study.

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