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Original Research Article

Determination of the incidence of carbapenem-resistant *pseudomonas aeruginosa* in patients suffering with diabetic foot ulcers

Sangeetha C Patil[1,*], Surekha Y A[1]

[1]*Dept. of Microbiology, Vijayanagar Institute of Medical Sciences, Bellary, Karnataka, India*

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ABSTRACT

Introduction: The present study was undertaken to determine the incidence of carbapenem-resistant *pseudomonas aeruginosa* in patients suffering from diabetic foot ulcers.
Materials and Methods: A total of one hundred patients with diabetic ulcer admitted in surgical wards, VIMS, Bellary were studied. Swabs were collected from the depth of the ulcers on the feet of the diabetic patients. From each patient, two swabs were collected. One swab was used for the isolation of aerobic bacteria and the other for preparation of smear for Gram stain. Debrided necrotic material was also collected. After sample collection, the specimens were processed immediately in the laboratory.
Results: Out of 100 cases, 40 (40%) patients presented with ulcer of 2-4 weeks duration. Out of 165 organisms isolated, most common isolate were *staph aureus* 38(23.03%), followed by *Klebsiella spp* 34 (20.6%), *Pseudomonas aeruginosa* 2(16.96%), *E.coli* 26(15.75%), *Proteus spp* 23(13.93%), *Enterococcus fecalis* 8(4.84%), *Citrobacter spp* 4(2.42%) and *Staph epidermidis* 4(2.42%)
Conclusion: The carbapenems seem to be successful against the *Pseudomonas aeruginosa*.

1. Introduction

Diabetes mellitus (DM), a very common endocrine disorder with major public health consequences arising from severe damage to numerous end organs. DM affects all populations worldwide and the prevalence of this disease is increasing at a very alarming rate. At present 31.7 million people are diabetic in India. The International Diabetes Federation (IDF) currently estimates that about 366 million persons in the world have DM, with projections that this will increase to 552 million by 2030.[1] The Indian diabetic population is expected to increase to 57 million by the year 2025.[2]

Diabetes warrants a lot of attention because of its various complications like retinopathy, nephropathy, peripheral neuropathy, cardiovascular disease, peripheral vascular disease (PVD), cerebrovascular accident, hypertension and diabetic foot.[3]

The diabetic foot may be defined as a group of syndromes in which neuropathy, ischemia, and infection lead to tissue break down resulting in morbidity and possible amputation.[4]

The diabetic wounds are mostly infected by pus forming microorganisms like Enterococci spp, Staphylococcus aureus, *Pseudomonas aeruginosa*, *E.coli*, *Klebsiella spp*, *Proteus spp*.[5]

Pseudomonas aeruginosa is an invasive organism that frequently causes severe tissue damage in diabetic foot ulcers. A major problem in *Pseudomonas aeruginosa* infection may be that this pathogen exhibits a high degree of resistance to a variety of antimicrobials including beta lactams. Carbapenems are used as antibiotics for treatment of infections caused by beta- lactam resistant *Pseudomonas aeruginosa*. Carbapenem hydrolysing beta lactamases known as Metallo beta lactamases (MBL) are reported in clinical isolates of *Pseudomonas aeruginosa*. These are resistant to most broad spectrum beta lactams, aminoglycosides, and fluoroquinolones.[6]

* *Corresponding author*.
E-mail address: foryourhelp55@gmail.com (S. C. Patil).

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Proper management of these infections requires microbial isolation and appropriate antibiotic selection. The present study was undertaken to determine the incidence of carbapenem-resistant *pseudomonas aeruginosa* in patients suffering from diabetic foot ulcers.

2. Materials and Methods

The present study "Aerobic bacteriology of diabetic foot with special reference to carbapenem resistance in *Pseudomonas aeruginosa*" was conducted in the Department of Microbiology, Vijayanagar institute of medical sciences, Bellary from January 2012 to December 2012. A total of one hundred patients with diabetic ulcer admitted in surgical wards, VIMS, Bellary were studied. The institutional ethical committee's clearance was obtained before conducting the study.

2.1. History taking and examination

A proforma was filled for each patient documenting, age, sex, address and clinical information including chief complaints, duration of symptoms, predisposing factor and any previous history of treatment.

2.2. Collection of sample

Samples were collected in the surgical wards where the dressing was being done.

The ulcer was cleaned with sterile normal saline and the surrounding area was cleaned with 70% alcohol. Debris, dead and devitalized tissue overlying the ulcer was removed using a sterile forceps and scissors. Swabs were collected from the depth of the ulcers on the feet of the diabetic patients. From each patient, two swabs were collected. One swab was used for the isolation of aerobic bacteria and the other for preparation of smear for Gram stain.[7]

Debrided necrotic material was also collected.[8] After sample collection, the specimens were processed immediately in the laboratory.

2.3. Processing of sample

2.3.1. Direct microscopic examination

(a) Gram smear Smear was prepared on clean glass slide, air dried. Gram stain was done for the smear and examined under oil immersion objective for the presence of pus cells, bacteria and fungi, low power and the high power objectives for fungi.[9]

2.4. Culture

2.4.1. Aerobic culture

The swab was inoculated on nutrient agar, blood agar and MacConkey agar. All plates were incubated aerobically at 37°C and evaluated at 24 hours, 48 hours and 72 hours. The organisms isolated were identified using standard techniques, based on the colony morphology, gram staining of smear from colony and biochemical properties.

Antimicrobial susceptibility of the bacterial isolates to the commonly used antibiotics was done by Kirby-Bauer disc diffusion method.[9,10]

3. Results

One hundred patients with diabetic ulcer admitted in the surgical wards, VIMS bellary.

The clinic-microbiological analysis from the study was as fallows.

Above table shows out of 100 cases, 65 were males and 35 were females.

Among 100 cases, 37(37%) were of age group 51-60 years, out of 37, 27 (41.54%) were males and 10 (28.57%) were females. 31(31%) cases were of age group 61 and above. Out of 31, 21 (32.3%) were males and 10 (28.57%) were females. 27(27%) cases were of age group 41-50 years, 3(3%) cases were of between 31-40 years, 2(2%) cases were of age group group 41-50 years, 3(3%) cases were of between 31-40 years, 2(2%) cases were of age group of 21-30 years.

Above table shows that out of 100 cases, 2(2%) cases were insulin dependent diabetes mellitus and 98 (98%) cases were non-insulin dependent diabetes mellitus.Table 2

Above table shows out of 100 cases, 40 (40%) patients presented with ulcer of 2-4 weeks duration. 30 (30%) patients presented with ulcer of 8 -10 weeks duration. 15 (15%) patients presented with ulcer of 5- 7 weeks duration.12 (12%) patients presented with ulcer of more than 11 weeks and 5(5%) patients had ulcer of less than 1 week.Table 3

Above table shows out of 165 organisms isolated, most common isolate were *staph aureus* 38 (23.03%), followed by *Klebsiella spp* 34(20.6%), *Pseudomonas aeruginosa* 28(16.96%), *E.coli* 26 (15.75%), *Proteus spp* 23(13.93%), *Enterococcus fecalis* 8(4.84%), *Citrobacter spp* 4(2.42%) and *Staph epidermidis* 4(2.42%).Table 4

Above table shows Gram positive and Gram negative organisms were isolated in 44.06% of cases, Gram negative organisms in 40.67%, three organisms isolated in 11.86% of cases and Gram positive organisms in 3.38% of cases.Table 5

The above table shows the antibiotic susceptibility pattern of aerobic organisms isolated in the study. Out of 165 organisms isolated 147(89%) were sensitive to imipenem, 86(52.1%) were sensitive to amikacin, 85(51.5%) were sensitive to ciprofloxacin, 56(33.9%) were sensitive to gentamycin, 55(33.3%) were sensitive to ceftriaxone, 48(29%) were sensitive to cephalexin, 43(26%) were sensitive to amoxylave, 40(24.2%) were sensitive to cefotaxime. From the above antibiogram most sensitive antibiotics were Imipenem, amikacin, ciprofloxacin and

Table 1: Showing age and sex distribution

Age group	Male		Female		Total	
	No	%	no	%	no	%
21-30	2	3.08	0	0	2	2
31-40	1	1.54	2	5.72	3	3
41-50	14	21.53	13	37.14	27	27
51-60	27	41.54	10	28.57	37	37
>60	21	32.31	10	28.57	31	31
Total	65	100	35	100	100	100

Table 2: Showing type of diabetes mellitus

Type of diabetes mellitus	Number of cases	Percentage
IDDM	2	2
NIDDM	98	98

Table 3: Showing the duration of diabetic ulcer

Duration in weeks	Number of cases	Percentage
<1 week	5	5
2-4	40	40
5-7	15	15
8-10	30	30
>11 weeks	12	12
Total	100	100

Table 4: Showing the different aerobic organisms isolated

Type of organism	Number of organisms	Percentage
Gram positive organisms	50	30.3
Staph aureus (38)	38	23.03
Staph epidermidis (4)	4	2.42
Enterococcus fecalis (8)	8	4.84
Gram negative organisms	115	69.6
Pseudomonas aeruginosa (28)	28	16.96
Klebsiella pneumonia (32) Klebsiella oxytoca (2)	34	20.6
E.coli(26)	26	15.75
Proteus mirabilis (21) Proteus vulgaris (2)	23	13.93
Citrobacter freundii (3) Citrobacter koseri (1)	4	2.42

Table 5: Showing distribution of organisms in polymicrobial flora

Type of organism	Number of cases	Percentage
Gram positive organisms	2	3.38
Gram negative organisms	24	40.67
Gram positive and Gram negative organisms	26	44.06
>- 3 organisms	7	11.86
Total	59	100

Table 6: Showing antibiotic susceptibility pattern of isolates

Antibiotics	*Staph aureus* (n=38)	*Staph epidermidis* (n=4)	Enterococcus spp.(n=8)	Pseudomonas spp.(n=28)	*Klebsiella spp* (n=34)	*E. coli* (n=26)	*Proteus spp* (n =23)	*Citrobacter spp* (n=4)	Total n=165
Amikacin	17(44.7)	1(25)	3(37.4)	14(50)	19(55.8)	14(53.8)	15(65)	3(42.9)	86 (52.1)
Amoxyclave	15(39.4)	3 (75)	5(62.5)	0(0)	6(17.6)	7(26.9)	7(30.4)	0(0)	43(26)
Gentamycin	10(26.3)	1(25)	2(25)	9(32.1)	13(38.2)	11(42.3)	8(38)	1(33.3)	56(33.9)
Ciprofloxacin	13(34.2)	3(75)	5(62.5)	11(39.2)	21(61.7)	18(69.2)	11(52.4)	3(42.9)	85(51.5)
Ceftriaxone	12(31.5)	2(50)	4(50)	3(10.7)	12(35.2)	12(46.1)	9(39.1)	1(25.3)	55(33.3)
Cefotaxime	6(15.7)	2(50)	1(12.5)	1(3.5)	9(26.4)	12(46.1)	8(38)	1(25.3)	40(24.2)
Imipenem	34(89.4)	3(75)	6(75)	25(89.2)	30(88.23)	24(92)	21(87)	4(100)	147(89)
Cephalexin	11(28.9)	2(50)	2(25)	1(3.57)	21(61.7)	4(15.3)	7(33.3)	1(25.3)	48(29)

gentamycin.Table 6

4. Discussion

In the present study diabetic ulcer was more common in the age group of 51-60 years (37%) and above 61 years (31%) which is similar to study of Bona et al,[11] in an epidemiological study conducted in Fortaleza, capital of Ceara State, retrospectively analyzed 67 medical records of patients hospitalized for infected diabetic foot, and found a higher frequency of female patients (52%), with an age range similar to that found in the present study.

All E. faecalis isolated showed high levels of sensitivity to the different antibiotic classes. Shettigar et al,[12] in a prospective epidemiological study with 100 patients, found E. faecalis with high rates of resistance to antibiotics such as erythromycin (94%), tetracyclines (91%) and ciprofloxacin (89%). In the present study 165 aerobic organisms isolated. The most predominant organisms isolated were staphylococcu aureus 38(23.03%), followed by *Klebsiella spp* 34(20.6%), *Pseudomonas aeruginosa* 28 (16.96%), *E.coli* 26(15.75%), *Proteus spp* 23 (13.93%) , *Enterococcus fecalis* 8(4.84%), *Citrobacter spp* 4(2.42%) and *Staph epidermidis* 4(2.42%). The reason could be the similar geographical locations where the 2 studies were conducted. Zubair et al,[13] reported Escherichia coli (26.6%) and *Pseudomonas aeruginosa* (10.6 %) as the predominant gram negative isolates. In the study of Benwan et al,[14] which was done in Kuwait, they reported that more gram-negative pathogens (51.2%) were isolated than grampositive pathogens (32.3%) or anaerobes (15.3%).

In the present study 10.72% of *pseudomonas aeruginosa* were resistant to carbapenem (imipenem) antibiotic. Carbapenem resistance in A. baumannii limits therapeutic options and is largely manifested by β–lactamases, and Metallo-β-lactamase that plays a significant role in mechanism of drug-resistance in diabetic patients. Acinetobacter may cause deep tissue or bony infection in a small but significant proportion of diabetic patients with chronic foot ulcers.

Carbapenemases are specific β-lactamases that hydrolyses the Carbapenems. According to various documentations, the production of β-lactamases has been found to be the most widespread cause of resistance. There has been an increase in number of class A carbapenemases (KPC and GES enzymes), class B metallo β-lactamases(VIM,IMP and NDM) and class D carbapenemases (OXA-23,- 24/40,48,51,55,58 and 143).[15–18] The resistant genes that code for carbapenemases can be exchanged between different gram negative bacteria through genetic packets called transposons or plasmids (jumping genes). Enterobacteriace possessing this carbapenemase genes (CP-CRE) are of great public health concern as their resistance has spread around the globe.

5. Conclusion

A pre-existing medical illness, prolonged operating time, the wound class, and wound contamination strongly predispose to wound infection. The study also directs us that proper management of diabetic foot ulcers with appropriate antibiotics such as carbapenems along with good glycemic control must be implemented to cure the disease. This evidence–based study will definitely lead to a well guided approach to the management of foot ulcers in diabetics in our centre. We conclude that the carbapenems seem to be successful against the *Pseudomonas aeruginosa*.

6. Conflicts of Interest

All contributing authors declare no conflicts of interest.

7. Source of Funding

None.

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Author biography

Sangeetha C Patil, Assistant Professor

Surekha Y A, Professor

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