World Journal of Pharmaceutical research

Volume 3, Issue 1, 797-808. Research Article ISSN 2277 – 7105

# ANTIBIOTIC SUSCEPTIBILITY PATTERN OF AEROBIC GRAM NEGATIVE BACILLI FROM WOUND INFECTIONS IN DIABETIC PATIENTS

**Dr. Ravishankar Reddy A*, M. Prathyusha[2], Dr. K. Saileela[3], Dr.V. Ramadevi[4], Dr. Srikanth[5], Dr. K. Kamal Chand[6].**

*[1]Associate Professor. Department of microbiology, Kamineni Academy of Medical Sciences & Research Center, Kamineni Hospitals, L. B. Nagar, Hyderabad 500068. A.P

[2] lecturer, Department of microbiology, Kamineni Institute of Medical Science, Sreepuram, Narketpally.

[3]Professor & HOD, KIMS, Kamineni Institute of Medical Science, Sreepuram, Narketpally.

[4]Associate professor, Govt.M.C. Nizamabad.

[5]Associate professor, KIMS.

[6]Associate professor, KAMS&RC.

Article Received on
15 October2013
Revised on 19 November 2013,
Accepted on 17 December 2013

***Correspondence for Author:**

**Dr. Ravishankar reddy A**

Associate Professor.

Department of microbiology,

Kamineni Academy of Medical Sciences & Research Center,

Kamineni Hospitals, L. B. Nagar, Hyderabad 500068. A.P

drravihyd@gmail.com

chandkamal77@gmail.com

## ABSTRACT

All foot ulcers are colonized with potentially pathogenic organisms and Diabetic foot infections and ulcerations are more common in type 2 diabetes mellitus. Especially aerobic pathogens contributing to progressive and widespread tissue destruction, so we aimed to determine the common aerobic gram negative bacteria of diabetic wound infections and their in - vitro susceptibility to routinely used antibiotics after the Ethics Committee clearance. One hundred specimens from patients with diabetic wound infection were obtained and processed. Gram's staining, Culture, Biochemical reactions and antibiotic sensitivity testing was done. Results were tabulated and found that 96 samples were culture positive. 178 organisms were isolated from theses positive cultures. The most common isolate was *P. aeruginosa* 71(39.88%) followed by *Escherichia coli* 52 (29.2%) and *K. pneumonia* 46 (25.84%), showed good sensitivity to Gentamicin (68%), Cefipime (79%) and Amikacin (87%). The Imepenam was the most effective drug among multidrug resistant isolates. We conclude Diabetic foot infections are polymicrobial with predominance of Gram negative *Pseudomonas aureginosa.*

**KEYWORDS**: Diabetic foot infections (DFIs), Double disc synergy test (DDST), Phenotypic confirmatory disc diffusion test (PCDDT).

## INTRODUCTION

Diabetic foot infections and ulcerations are more common in type 2 diabetes mellitus[1]. The centre for disease control estimate that 50% of these diabetic foot problems leads to subsequent amputations. This can be eliminated by educating both the patients and early treatment[2]. Patho-physiology of foot ulceration [2,3] include 30-50% of the patients suffer with Sensory neuropathy where inability to detect the pain signals that warn impending trauma, the insensitive foot is exposed to increased pressure that hasten tissue damage leading to ulceration. The Autonomic neuropathy can cause arteriovenous shunting, producing vasodilatation resulting distension of foot veins and edema which is not diminished even with foot elevation. Autonomic neuropathy is also responsible for decreased activity of sweat glands of the feet leading to dryness and fissuring of the skin of foot predisposing to the risk of infection. Motor neuropathy in the foot causes weakness and wasting of the small intrinsic muscles, leading to clawing of the toes and plantar flexion of the metatarsal heads. These prominences serve as areas of focal pressure with possible irritation from foot wear. Peripheral vascular changes include thickening of the basement membrane, which act as a barrier to the normal exchange of nutrients and cellular migration, decreases the ability of diabetic foot to fight against infection. Impaired wound healing and infection is due to changes in cellular activity, expression of various growth factors and cytokines that are normally involved in tissue repair. Wound healing and collagen synthesis is altered in diabetic patients. Hyperglycemia can potentially mitigate the cellular activity in inflammatory process; specifically the morphological characteristics of macrophages are transformed so that it impairs the function. There are abnormalities of adherence, chemotaxis, phagocytosis, oxidative burst and intracellular killing .The expression of various growth factors, such as platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF) is also reduced in diabetics[4, 5].

All foot ulcers are colonized with potentially pathogenic organisms. The impaired micro-vascular circulation in patients with diabetic foot limits the access of phagocytes favoring development of infection. *Pseudomonas spp.*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus spp.* and *Enterococcus* spp. are the most frequent aerobic pathogens contributing to progressive and widespread tissue destruction. The common anaerobic isolates are

*Peptostreptococcus* species and *Bacteroides* species. Diabetic foot infections are often polymicrobial[6].Many studies have reported on the bacteriology of diabetic foot infections (DFIs), found that *Staphylococcus aureus* is the main causative pathogen, but recent investigations report predominance of gram-negative aerobes. The role of anaerobes is particularly unclear, because specimens were not collected or cultured properly to recover these organisms. These discrepancies are due to differences in the causative organisms occurring over time, geographical variations or the types and severity of infection included in the studies[7]. The increasing association of multi-drug resistant (MDR) pathogens with diabetic foot ulcers is responsible for the increased length of stay, cost of management, morbidity and mortality of the diabetic patients[8]. Appropriate selection of antibiotics based on the antibiogram of the isolates from the lesions is most critical for the proper management of these infections. Nevertheless, the initial empirical therapy is often decided based on the knowledge of the susceptibility profile of the prevalent microbial flora recovered from the previous cases[9]. So, this study was performed to determine the common aerobic gram negative bacilli of diabetic wound infections and their in- vitro susceptibility to routinely used antibiotics.

## MATERIALS AND METHODS

The study was conducted after Institutional Ethics Committee clearance has been taken in the department of microbiology Kamineni institute of medical sciences, Narketpally. One hundred specimens from patients with diabetic wound infection were obtained from department of surgery, KIMS hospital.

**Inclusion criteria:** Diabetic wound infection with open lesions.

**Exclusion criteria:** Patients on antibiotic therapy were excluded from study.

**Specimen collection**[10,11,12].

Specimens were collected, after thorough cleaning of the lesion with sterile normal saline, preferably before administration of antibiotics.

The specimens were 1) Wound curettage by using a sterile scalpel, 2) Aspiration from abscesses by using needle and syringe, 3) Pus by using sterile swab. Two specimens were collected from each patient. One for Gram stain another for aerobic culture. The specimens were immediately transported to the microbiology laboratory.

**SPECIMEN PROCESSING**

Gram's staining, Culture media: Blood agar, Mac Conkey agar, Mueller-Hinton agar: Aerobic culture was done. Identification Scheme for aerobic/facultative Gram- negative bacilli / Biochemical reactions are used as described in Mackie and McCartney[13]. Commercially available dehydrated culture media from Himedia laboratories, Mumbai, were used in the present study.

Preliminary identification was done by colony morphology. All types of colony grown on the agar plates were read and colony description was recorded. This includes size, shape, edge, profile, color, opacity, and haemolysis and pigment production. The tests conducted are Gram stain, Catalase test, Oxidase test, Motility tests. Specific tests for gram negative bacilli, Biochemical reactions done Oxidation / fermentation (OF) reaction, Sugar fermentation tests: the sugars used: Lactose, Sucrose, Glucose, Mannitol, Xylose, Maltose, and Mannose. Indole test, Methyl red and voges –proskauer test, Citrate utilization test, Urease test, Triple sugar iron test, Nitrate reduction test, Phenylalanine deaminase test, Decarboxylase test.

The antibiotic sensitivity testing was done by Kirby Bauer disc diffusion method with commercially available HiMedia discs according to clinical laboratory of standard institute (CLSI) guidelines [14].

The antibiotics to be tested against the isolates were determined according to the standard guidelines and also considering the local susceptibility pattern of the organism. The set of antibiotics tested for susceptibility against different organisms were as follows:

**The antibiotics and disc strength**

| **Antibiotic** | **Disc strength** |
|---|---|
| Ampicillin(A) | 10µg |
| Amoxicillin –clavulanic acid (AC) | 20/10 µg |
| Piperacillin Tazobactem (PT) | 100/10 µg |
| Ceftazidime (Ca) | 30 µg |
| Ciprofloxacin(Cf) | 5 µg |
| Gentamicin (G) | 10 µg/120 µg |
| Amikacin (Ak) | 30 µg |
| Imipenem (I) | 10 µg |

**Extended spectrum beta-lactamase detection done with Double disc synergy test (DDST)[15], Phenotypic confirmatory disc diffusion test (PCDDT)[15] AmpC β lactamase detection[16] and Metallo β-Lactamase detection[17].**

**AmpC β lactamase detection :** Flattening of the Cefoxitin inhibition zone in the vicinity of test disc was considered as Positive test, while an undistorted zone was taken as non- Amp C producer

**Metallo β-Lactamase detection -** An increase in zone size of atleast 7mm around the Imepenem-EDTA disc and Meropenem-EDTA discs recorded as an MBL-positive strain.

## RESULTS

**All the results were tabulated.**

**Table no.1; Sex distribution of patients with diabetic wound infections n= 100**

| Male | Female |
|---|---|
| 61(61%) | 39 (39%) |

In our study males (61%) were predominant than females (39%).

**Table no.2: Age distribution of patients with diabetic wound infections**

| Age(years) | No. Of patients% |
|---|---|
| < 40 | 31 |
| 41-50 | 25 |
| 51-60 | 24 |
| 61-70 | 20 |
| Total | 100 |

Thirty one (31%) of the diabetic patients were in age group of <40 years. Next common group belonged to 41-50 (25%) age group.

**Table no.3: Areas of specimen collection**

| Area of specimen collection | No. of specimens collected |
|---|---|
| Foot | 70 |
| Leg | 10 |
| Palms | 10 |
| Other areas of body | 10 |

Majority of the specimens were from diabetic foot infections (70%). Other site included 10 (10%) of samples, each from legs palms and other areas of body

**Table no.4; Pattern of organisms isolated from diabetic wound infections**

| Isolate | Number % |
|---|---|
| *Pseudomonas. Aeroginosa* | 71 (39.88) |
| *Klebsiella. Pneumonia* | 52(29.21) |
| *E.coli* | 46(25.84) |
| *Proteus. mirabilis* | 09(5.05) |
| Total | 178 (100%) |

Out of 178 isolates, the most common isolate in our study was *P. aeruginosa* 71(39.88%) followed by *K. pneumoniae* 52 (29.21%), *E. coli* 46 (25.84%), and *P. mirabilis* 9 (5%).

**Table no.5; Pattern of organisms isolated from diabetic wound infections**

| Pattern | Number of cases | Number of isolates |
|---|---|---|
| No isolate | 4 | 0 |
| One isolate | 20 | 20 |
| Two isolates | 70 | 140 |
| Three isolates | 6 | 18 |
| Total | 100 | 178 |

Polymicrobial flora was isolated from 76 (76%) of samples, 20(20%) of samples yielded one isolate and no growth was found in 4 (4%) samples.

**Table no.6: Percentage antibiotic resistance pattern of gram negative isolates**

| Antibiotic | *P. aeroginosa* | *P.mirabilis* | *K.pneumoniae* | *E.coli* |
|---|---|---|---|---|
| Ampicillin | 75 | 89 | - | 44 |
| Amoxy-clavulinic acid | 84 | 67 | 58 | 74 |
| Gentamicin | 32 | 22 | 32 | 25 |
| Amikacin | 25 | 11 | 9 | 8 |
| Ceftazidime | 89 | 60 | 75 | 57 |
| Cefatoxime | 85 | 75 | 71 | 59 |

| | | | | |
|---|---|---|---|---|
| Cefipime | 28 | 22 | 7 | 18 |
| Ciproflaxacin | 57 | 52 | 71 | 69 |
| Imipenem | 33 | 0 | 37 | 32 |
| Piperacilin Tazobactum | 12 | 29 | 16 | 18 |

**Table no.7: Multidrug resistance in gram negative isolates from diabetic wound infections**

| Isolate | Resistant to <3 antibiotics | Resistant to >3 antibiotics |
|---|---|---|
| *Pseudomonas. Aeroginosa* | 25(71.4%) | 30(42.25%) |
| *Proteus. Mirabilis* | 7(41.1%) | 10(58.9%) |
| *Klebsiella. Pneumonia* | 9(47.3%) | 10(52.7%) |
| *E.coli* | 19(65.5%) | 10(34.5%) |

Multidrug resistance was observed in all organisms. *P. Mirabilis* (58.9%), *K. pneumoniae* (52.7%), *E. coli* (34.5%), *P. aeruginosa* (42.25%), showed resistance to more than three antibiotics tested.

**Table no.8: Beta lactamase production in gram negative isolates from diabetic wound infections**

| Organism | ESBL producer | MBL producer | Amp C producer |
|---|---|---|---|
| *Pseudomonas. aeroginosa* | 65/71(91.54%) | 24/71(33%) | 2/71 (2.81%) |
| *Proteus. mirabilis* | 06/09(66%) | 0/9(0%) | 0/9 (0%) |
| *Klebsiella. pneumoniae* | 39/52(75%) | 19/52(36.5%) | 15/52 (28.84%) |
| *E.coli* | 31/46(55%) | 15/46(32%) | 8/46 (17.39%) |
| Total | 141/178(79.2%) | 58/178(32.5%) | 25/178 (14.04%) |

91.54% of *P. aeruginosa* (55%) of *E. coli*, 25 (75%) of *K. pneumoniae*, and (66%) of *P. mirabilis* were ESBL producers. Metallo beta-lactamase production was seen in 33% of *P. aeruginosa*, 32% of *E. coli*, 0% of *P. mirabilis*, and 36.5% of *K. Pneumoniae*. Amp C production was seen in 2/71 (2.81%) of *P. aeruginosa*, 15/52 (28.84%) of *K. pneumoniae*, 8/46 (17.39%) of *E. coli* and none of the *P. mirabilis* were Amp C producers

## DISCUSSION

**Table No. 9: Comparison of isolates from diabetic infections from various studies**

| Study | *P. aeruginosa* | *K. pneumoniae* | *E. coli* | *P. mirabilis* |
|---|---|---|---|---|
| Present study* | 39.88 | 29.21 | 25.84 | 5.05 |
| Ekta Bansal, et al[18] | 21.67% | 16.78% | 18.88% | - |
| Nadeem Sajjad Raja et al[19] | 25% | 15% | 9% | 28% |
| R.Sasikala et al[20] | 14.11% | 14.11%), | 10.5% | 21.17% |

In our present study the most common isolate was *P. aeruginosa* (39.88%) followed by next common isolate was *K. pneumoniae* (29.21%). This in comparison with studies done by Ekta Bansal et al[18], but reports from Nadeem Sajjad Raja et al[19] and R.Sasikala et al[20] showed that P. mirabilis was the most common isolate followed by *P. aeruginosa.*

**Table No. 10: Comparison of growth from diabetic infection samples.**

| Study | No growth | One isolate | Polymicrobial |
|---|---|---|---|
| Present Study* | 4% | 20% | 76% |
| Samir Paul et al[21] | - | - | 75.3% |
| Ekta Bansal, et al | 5.9% | - | - |
| Diane M Citron et al | - | - | 83.8% |

Out of 100 samples 4% samples did not yield any growth and majority of samples were polymicrobial (76%) in our study. This finding is in concordance with results of Diane M Citron et al and Samir Paul et al[21] who reported 83.8% and 75.3% samples to be polymicrobial. Ekta Bansal et al reported no growth in 5.9% of samples collected.

**Table 11: Comparision of antibiotic sensitivity pattern in various studies**

| Antibiotic | Gentamicin | Amikacin | Ceftazidime | Imepenam |
|---|---|---|---|---|
| Present study* | 68% | 87% | 60% | 86% |
| Ravishekar Gadepalli et al[22] | - | 51.3% | 46.6% | 100 |
| R Sasikala et al | 30% | 50% | 88% | 100 |
| Ekta Bansal et al | 81% | 85% | 87% | - |

In our study the isolates showed good sensitivity to Gentamicin (68%), Amikacin (87%), and Cefipime (79%). These findings are in comparison with studies done by Ekta Bansal et al. Multidrug resistance was found in all isolates. Resistance to more than three drugs was found in *P. aeruginosa* (42.25%), *K. pneumoniae* (52.7%), *E. coli* (34.5%) and *P. mirabilis* (58.9%). Similar results were reported by different authors like Ravishekar Gadepalli et al[22] (72% MDR), R Sasikala et al (100% MDR).

Even though Imepenam resistance was found among 25% of isolates in our study, it was the most effective drug among multidrug resistant isolates. Ekta Bansal et al and R Sasikala et al also reported that imepenam was the most effective antibiotic for gram negative isolates from diabetic patients.

**Table 12: Comparison of beta-lactamase production in various studies**

| Study | ESBL producers | MBL producers | AMP C producers |
|---|---|---|---|
| Present study* | 79.2% | 32.5% | 14.04% |
| Shukla I, et al[15] | 69% | - | - |
| Singhal S, et al[16] | - | - | 23% |
| Behera B et al[17] | - | 35% | - |

ESBL, MBL and Amp C production in our study was in comparison with studies done by Shukla I, et al, Singhal S, et al and Behera B et al respectively.

**SUMMARY**

Out of 100 patients majority of the patients (70%) had foot ulcer followed by 10% patients had lesions on leg, palms and other body parts. 96 samples were culture positive. 178 organisms were isolated from theses positive cultures. Out of 96 culture positive cases, 20 samples had only 1 isolate, 70 samples had 2 isolates, while 6 samples had 3 isolates. *Pseudomonas spp*. was the most common isolate, accounting for 71(39.88%) of all isolates, followed by *Klebsiella spp*, *Escherichia coli*, and *Proteus mirabilis* comprising of 46 (25.84%), 52 (29.2%) and 9 (5.05%) respectively. Among *Pseudomonas spp*. 87% strains showed sensitivity to Imipenem, 75% were sensitive to Amikacin, 68% were sensitive to Gentamicin. Pipercillin –Tazobactam combination showed sensitivity of 88%. Ceftazidime and Cefotaxime had least activity of 11% and 15% respectively. Imipenem resistance was found in 35% of isolates while all the proteus isolates were sensitive to Imepenem. They were

least sensitivity to Ciprofloxacin, cefotaxime and Ceftazidime. ESBL production was found in all gram negative isolates (79.2%), and 32.5% of isolates were MBL producers. Amp C production was observed in 14.04% of isolates

## CONCLUSION

Diabetic foot infections are polymicrobial in nature. There is a predominance of Gram negative organisms. The common isolates in the present study were *Pseudomonas aureginosa* and other enterobacteriaceae members. There is a high prevalence of multidrug resistant bacteria isolated from diabetic foot infections. Majority of isolates were ESBL producers or Amp C producers. Pipercillin- Tazobactum or Amikacin or Imepenam seems to be the most prudent empirical treatment of gram negative diabetic foot infections. Deesaclation of this empirical therapy can be done later on based on the antibiogram of the isolates from the individual patients.

## REFERENCES

1. P.C. Leung, diabetic foot u1cers- a comprehensive review, the Royal Colleges of Surgeons of Edinburg and Ireland. 2007,219-31.
2. Thanh L Dinh and Aristidis Veves.The diabetic foot.In:R.A Defronzo, E.Ferrannini, H.Keen editors. International text book of Diabetes mellitus. 3rd edition. England: Wiley; 2004.Pg: 1315-32.
3. Frykberg RG. An evidence-based approach to diabetic foot infections. Am J Surg. 2003;186:44S–54S
4. Diane M Citron, Ellie J.CGoldstein, Benjamine A Lipsky. Bacteriology of moderate to severe diabetic foot infection and in vitro activity of antimicrobial agents. Journal of clinical microbiology. 2007;2819-28.
5. Lipsky BA. Infectious problems of the foot in diabetic patients. In: Levin and O'Neal's The diabetic Foot (7th ed), editor. In: Bowker JH, Pfeifer MA, editors. Philadelphia: Mosby Elsevier; 2008. p. 305–318.
6. Jean-Louis Richard, Albert Sotto and Jean-Philippe Lavigne. New insights in diabetic foot infection. World J Diabetes. 2011:2(2): 24-32.
7. Sivaraman Umadevi, Shailesh Kumar, Noyal Mariya Joseph, Joshy M Easow, G Kandhakumari, Sreenivasan Srirangaraj, Sruthi Raj, Selvaraj Stephen. Microbiological Study of Diabetic Foot Infections. Indian Journal of Medical Specialities,2010; 31-5

8. Prompers L, Huijberts M, Schaper N, Apelqvist J, Bakker K, Edmonds M, Holstein P, Jude E, Jirkovska A, Mauricio D, et al. Resource utilisation and costs associated with the treatment of diabetic foot ulcers. Prospective data from the Eurodiale Study. Diabetologia. 2008;51:1826–1834.
9. Dushyant sing Gaur, Amitvarma, Prathima Guptha. Diabetic foot in Uttaranchal. J K Science. 2007:9(1);28-31
10. J.G. Collie, J P Duglid, A. G.Fraser. Laboratory strategy in the diagnosis of infective syndromes. In: Mackie and McCartney eds. Practical Medical Microbiology. 14th edition. Elsevier publishers New Delhi. 2006:66-71.
11. Barbara J.Howard, John F.Keiser. Anaerobic bacteria. In: Barbara J. Howard, D.A., M.T. (ASCP) eds. Clinical and Pathogenic Microbiology. 2nd edition. Mosby publishers St. Louis. 1994: 383-9
12. Lynne S.Garcia, Henry D. Isenberg, editors. Clinical microbiology procedures hand book.Washington:Wiley;2007
13. J. G. Collie, R. S. Miles, B. Watt. Tests for the identification of bacteria. In: Mackie and McCartney eds. Practical Medical Microbiology. 14th edition. Elsevier publishers New Delhi. 2006:131-49
14. National Committee for Clinical Laboratory Standards (NCCLS):2011, Performance standard for antimicrobial susceptibility testing 21st international supplement .NCCLS document M100-S21, Vol-31, No: 1.NCLS, Wayne, PA, USA-Jan2011.
15. Shukla I, Tiwari R, Agrawal M. Prevalence of extended spectrum betalactamase producing *Klebsiella pneumoniae* in a tertiary care hospital. Ind J Med Microbiol 2004;22(2):87-91.
16. Singhal S, Mathur T, Khan S, Upadhyay DJ, Chugh S, Gaind R. et al. Evaluation of methods for Amp C betalactamase in gram negative clinical isolates from tertiary care hospitals. Ind J Med Microbiol. 2005;23(2):120-4.
17. Behera B, Mathur P, Das A, Kapil A, Sharma V. An evaluation of four different phenotypic techniques for detection of metallo-beta-lactamase producing *Pseudomonas aeruginosa*. Ind J Med Microbiol 2008;26(3):233-7.
18. Ekta Bansal, Ashish Garg, Sanjeev Bhatia, AK Attri, Jagdish Chander. Spectrum of microbial flora in diabetic foot ulcers. Indian J Pathol Microbiol. 2008;51:204-8.
19. Nadeem Sajjad Raja, microbiology of diabetic foot infections in a teaching hospital in Malasia:a retrospective studyof 194 cases. Journal of Microbiol Immunol Infect. 2007;40:39-44.

20. R.Sasikala, R.Latha, N.Muruganandam and Senthil kumar. Surveillance of multi drug resistant organism associated with diabetic foot ulcers in Pondicherry. The internet journal of microbiology. 2008;( 5): 1937-8289.
21. Samir Paul, LovelyBarai, Ashraf jahan and J.Ashraful Haq, A bacteriological study of diabetic foot infections in an urban tertiary care hospital of Dhaka city. Ibrahim med coll J. 2009;3(2):50-4
22. Ravishekar Gadepalli, Benu Dhawan, Vishnubhatla Sreenivas. A Clinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. Journal of Diabetes Care. 2006;29:1727-32