

Evaluation of Amputation Risk in Diabetic Foot Ulcer Patients Infected with *Acinetobacter baumannii* from a Tertiary Care Centre in South India

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ABSTRACT

Background: Foot complications are found to be a significant frequent, complication of Diabetes mellitus. Carbapenem-resistant *Acinetobacter baumannii* is gradually becoming dominant among Diabetes patients in urban population. The purpose of this study was to evaluate the clinical relationship of these gram negative bacteria and their resistance mechanisms in patients with diabetic foot and infected ulcer in India.

Methods: An observational study of 532 patients with infected ulcers in diabetic feet was conducted in Hycare for Wounds, a tertiary care centre exclusively dedicated for Wound care and Management between May 2017 and April 2018. Processing and identification was done as per standard guidelines. The bacteriological assessment was performed in deep tissue cultures and amputation was considered major when performed above the foot's middle tarsus.

Results: Out of 532 patients studied, 350 were positive for bacterial growth. Commonest isolate was *Pseudomonas aeruginosa* followed by *Staphylococcus aureus* (MSSA) species, *Klebsiella* and *E. coli*. We observed that 14 patients were infected with *Acinetobacter baumannii* and 50 % were carbapenamase producers.

Clinical Outcomes: Out of 14 patients 6 were major amputees and 5 were minor amputees and the rest 3 patients are under medical management.

Conclusion: Carbapenem-resistant *Acinetobacter baumannii* is a serious threat to Diabetic patients and can be considered as a risk factor for major amputation.

Keywords: *Acinetobacter baumannii*, Diabetes mellitus, deep tissue cultures, amputation.

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INTRODUCTION

International Diabetes Federation has reported that India ranks second in diabetes in the world for number of people with diabetes (72.9 million) in the age group of 20-79 years next to china in the year 2017 and it will conquer the first position by the year 2045 with an increase of 134.3 million diabetic population.^[1] Diabetic foot is one of the most significant complications of diabetes and about 15% of diabetic patients will develop foot ulcers in their lifetime and this is known to precede amputation in 85% of the cases.^[2] Foot complications in diabetic patients account for more hospital admissions than any other long-term complications of diabetes and also result in increasing morbidity and mortality.

Foot ulcers are most commonly due to more than one organism and it is therefore, imperative that appropriate antibiotic therapy is started. Also, infection with multidrug-resistant organisms will increase the morbidity and lead to great financial burden to the patient. *E.coli*, *Proteus* spp, *Pseudomonas* spp, *S. aureus*, and *Enterococcus* spp are the most frequent pathogens which are cultured from diabetic foot ulcers.

Acinetobacter baumannii is an important Gram-negative pathogen that has become an increasingly prevalent cause of bloodstream infections.^[3] It is an aerobic Gram-negative, non-fermenting bacteria associated with multiple resistances to many antibiotics. The organism is considered as an

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important nosocomial infection causing bacteria, during the past decades.^[4]

The present investigation is done in order to determine the prevalence, antibiotic susceptibility pattern and clinical outcomes of *A. baumannii* isolates in diabetic foot ulcer patients.

METHODS

All patients with Diabetic Foot Infections that presented clinically infected wounds or ulcers and received inpatient or outpatient treatment at Hycare centre for Wounds, a tertiary care centre in South India between May 2017 and April 2018 were included in this study. We were allowed to conduct this research by the hospital and the study was approved by the Institutional Ethics Committee and the Institutional Research Board.

2.1 Tissue processing isolation & Identification:

The tissue sample were weighed in a sterile tube, homogenized thoroughly and 0.1ml of original homogenate tissue sample placed in Blood agar plate, Chocolate agar plate, Mac Conkey agar plate and labelled as 10¹.^[5]

Then serially dilute the homogenate using 0.5µl aliquot and 4.5ml of sterile 0.85% NaCl and place 0.1ml of 10⁴ dilutions into Blood agar plate, Chocolate agar plate, McConkey agar plate. Evenly distribute inoculums with sterile rod and incubate at 37°C for 18-24 hours.

2.2 Antibiotic sensitivity testing:

Antibiotic sensitivity test for the isolated bacteria were performed by Kirby Bauer disc diffusion method.^[6] The isolate was interpreted and MIC for multidrug-resistant strains were confirmed with automated vitek 2 systems. ESBL producers, carbapenemase producers, Inducible clindamycin resistance and Methicillin resistance were tested and reported as per CLSI guidelines.^[6]

2.3 Carbapenemase producers:

Modified Hodge test were performed for Enterobacteriaceae, with suspected carbapenemase production. 0.5 McFarland suspension of ATCC E.coli 25922 was diluted 1:10 in sterile saline. This was inoculated on a Muller Hinton agar plate as for the routine disc diffusion testing. The plate was dried and a disc of Meropenem 10µl was placed in the centre of the agar plate, colonies of the test organism were picked and inoculated in a straight line, from the edge of the disc up to a distance of at test 20-25mm in length. The plates were incubated at 37°C overnight and they were examined the next day.

They were checked for enhanced growth around the test organism at the intersection of the streak and zone of inhibition. The presence of an enhanced growth indicated carbapenemase production and the absence of an enhanced growth meant that the test isolate did not produce carbapenemase.^[6]

RESULTS

In this study, we included a total of 350 patients with diabetic foot infections, of which 269 were males (77%) and 81 (23%) were females with the male to female ratio is almost 3:1 as shown in Fig 1

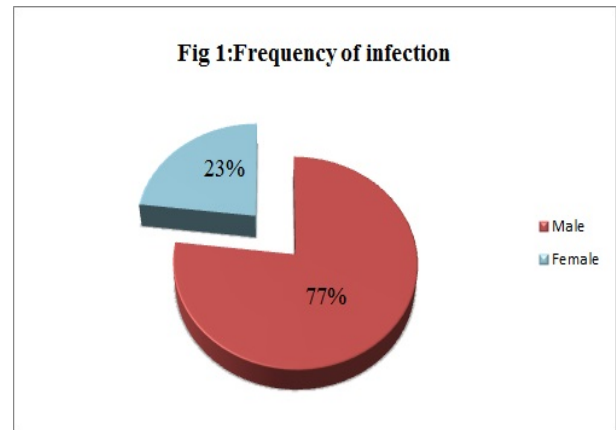


Fig :- 1 Frequency of infection

Types of microbial infection

From 350 diabetic patients with foot infections, 17 organisms (both gram positive and negative) were recovered indicating that there were two types of bacterial infections; monomicrobial infection which is the major 84% and polymicrobial infections is 16% as depicted in Fig.2

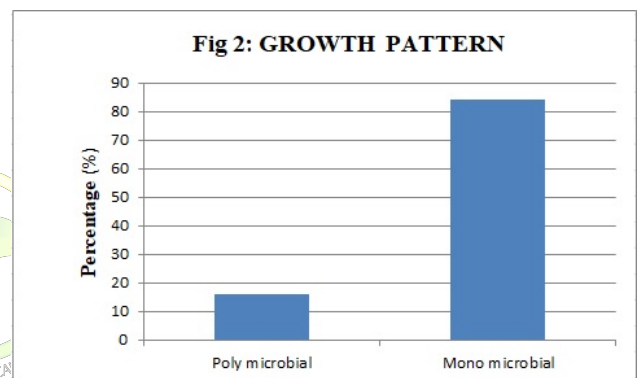


Fig :- 2 Growth Pattern

Type of bacterial isolates

Pseudomonas aeruginosa-134 (45.3%), *Staphylococcus aureus*-63 (MSSA) (57.3 %), *Klebsiella pneumoniae*-57(19.3%), *Escherichia coli*-43(14.5%), *Enterococcus faecalis*-24 (21.8%), *Proteus mirabilis*-20(16.7%), *Acinetobacter baumannii*-14(4.7%), *Staphylococcus aureus*-13(MRSA)(11.8%), *Citrobacter koseri*-8(2.7%), *Morganella morganii*-7(2.4%), *Enterococcus faecium*-6(5.5%), *Proteus vulgaris*-5(1.5%), *Enterobacter cloacae*-4(1.4%), *Streptococcus spp*-4(3.6%), *Providentia rettgeri*-2(0.7%), *Citrobacter freundii* and *Klebsiella oxytoca*-1(0.3%) Fig 3

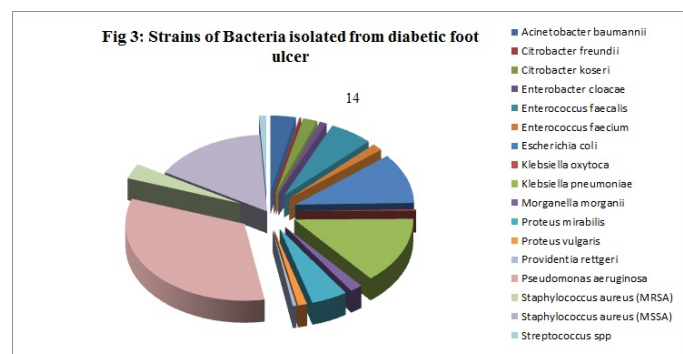
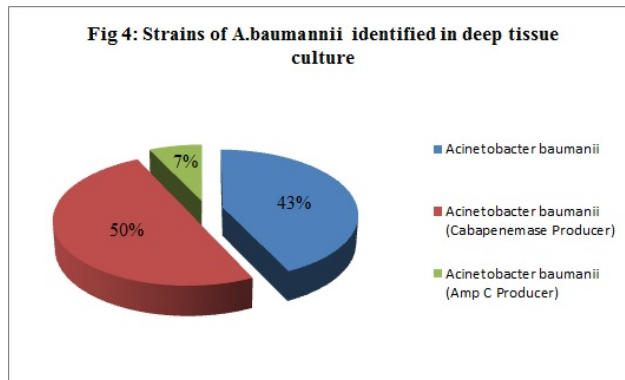


Fig :- 3 Strains of Bacteria isolated from diabetic foot ulcer

Acinetobacter baumannii carbapenemase producer is found to be 50% followed by Amp C producer 7% Fig 4

Fig :- 4 Strains of *A. baumannii* identified in deep tissue culture



A. baumannii isolates identified from Diabetic foot infected patients were sensitive to antibiotics like colistin (93%), tigecycline (100%), Amikacin (50%), Tobramycin and imipenem (43%) and Minocycline and Tetracycline (36%) - Table1

Acinetobacter baumannii strains were found to be resistant to Cefepime (100%), Ceftazidime (93%), Levofloxacin, Trimethoprim, Ciprofloxacin and Gentamycin (79%), Sulbactam (71%), Taxobactam (64%), Imipenem (57%), Azetronam, Meropenem and Cefazolin (50%), Amoxicillin, Ampicillin, Cefotaxime, Cefuroxime and Doripenem (43%) - Table 1

Table- 1: Antimicrobial Susceptibility Pattern of *Acinetobacter baumannii*

Antibiotics	<i>Acinetobacter baumannii</i> (n=14)		
	R	S	I
Amikacin	0	7(50%)	7(50%)
Amoxicillin	6(42.86%)	0	8(57.14%)
Ampicillin	6(42.86%)	0	8(57.14%)
Aztreonam	7(50%)	1(7.14%)	6(42.86%)
Cefazolin	7(50%)	0	7(50%)
Cefepime	14(100%)	0	0
Cefoperazone / Sulbactam	10(71.4%)	2(14.3%)	2(14.3%)
Ceftazidime	13(92.86%)	1(7.14%)	0
Cefotaxime	6(42.86%)	1(7.14%)	7(50%)
Cefuroxime	6(42.86%)	0	8(57.14%)
Ciprofloxacin	11(78.56%)	2(14.3%)	1(7.14%)
Colistin	0	13(92.86%)	1(7.14%)
Doripenem	6(42.86%)	1(7.14%)	7(50%)
Ertapenem	2(14.3%)	4(28.56%)	8(57.14%)
Faropenem	2(14.3%)	1(7.14%)	11(78.56%)
Gentamicin	11(78.56%)	3(21.44%)	0
Imipenem	8(57.14%)	6(42.86%)	0
Levofloxacin	11(78.56%)	2(14.3%)	1(7.14%)
Meropenem	7(50%)	1(7.14%)	6(42.86%)
Minocycline	1(7.14%)	5(35.72%)	8(57.14%)
Piperacillin / Taxobactam	9(64.3%)	4(28.56%)	1(7.14%)
Prulifloxacin	2(14.3%)	1(7.14%)	11(78.56%)
Ticarcillin / Clavulanic Acid	3(21.44%)	0	11(78.56%)
Tetracycline	2(14.3%)	5(35.72%)	7(50%)
Tigecycline	0	14(100%)	0
Tobramycin	1(7.14%)	6(42.86%)	7(50%)
Trimethoprim / Sulfamethoxazole	11(78.56%)	3(21.44%)	0

Fig 5 demonstrates the distribution of patients based on Wagners classification nearly 21% of patients were in Grade 2, 36% in Grade 4 and 43% in Grade 5.

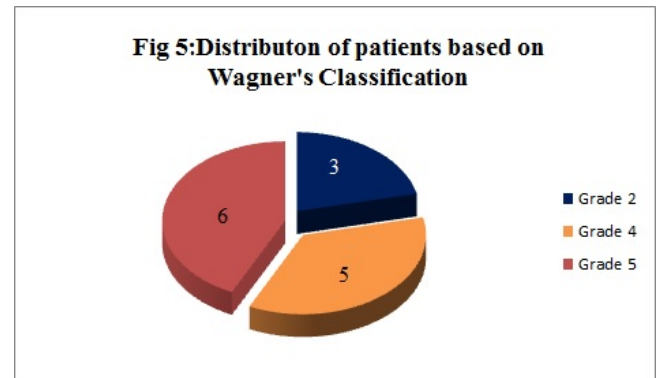


Fig: - 5 Distribution of patients based on Wagner's Classification.

Amputations associated with *A. baumannii* infection:

The percentages of various level amputations are above ankle (major) Amputation 43%, below ankle (minor) Amputation 36% and No Amputation 21%. We observed that most amputated patients were male and only one female patient. Amputations associated with *A. baumannii* infection were depicted in Fig 6.

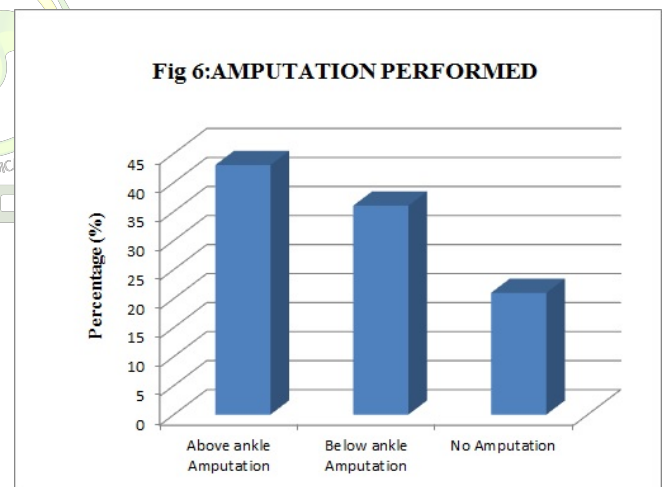


Fig: - 6 Amputation Performed

DISCUSSION

Acinetobacter baumannii has gained importance as a multidrug resistant pathogen and is serious concern among diabetic foot infections. Currently, the emergence of antibiotic-resistant pathogens has made clinicians progressively challenging to select suitable empirical antibiotics for the treatment of diabetic foot infection.^[7] Antimicrobial resistance is a major public health concern which increases morbidity and mortality as well as the cost of health care. *Acinetobacter baumannii* was completely resistant to all the common antibiotics tested not only in our study but also in another study on Antimicrobial susceptibility pattern in diabetic foot ulcer.^[8] The source of the infections in *A. baumannii* is either exogenous or endogenous. The exogenous infection is acquired from the hospital environment and the endogenous is from the skin of the patient himself.^[9]

Although diabetic patients have much serious comorbidity, the infected ulcer is considered to be a risk factor for major amputation.^[10] In the studied sample, the isolation of the bacterial genera *A.baumannii* was a predictive factor for amputation. These bacteria are associated with nosocomial infections and are considered opportunistic pathogens. In addition, these microorganisms can develop antimicrobial resistance, which makes it difficult to treat the infection and therefore, may increase the amputation risk.^[11]

Currently, the increasing number of Carbapenem-resistant *Acinetobacter baumannii* is of major significance in the perspective of resistance to β -Lactams. Indeed, between 1998 and 2004, this rate of resistance in Europe, North America, South America and Asia ranged between 0% and 40%. But in most studies after 2005, the rate of resistance was greater than 50%.^[12] Carbapenem, powerful broad spectrum β -lactam antibiotic are the drugs of choice in the treatment for multidrug-resistant gram negative bacteria; however, a rising number of carbapenemases to this group of drugs is increasingly being reported from different parts of the world.

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.

Clinical outcomes: In our study, we observed that among 14 isolated, 11 patients (78.5%) were amputated. The remainder 21.5% of the patients are under medical management and follow-up protocol.

CONCLUSION

In conclusion, the bacteria *A. baumannii* identified in infected

ulcers of patients with diabetic foot were associated with amputation risk.

REFERENCES

1. IDF Diabetes Atlas, 8th Ed. Brussels, Belgium: International Diabetes Federation, 2017. <http://www.diabetesatlas.org/resources/2017atlas.html>. Accessed 29 October 2018.
2. Shanmugam P, Jeya M. The bacteriology of diabetic foot ulcers, with a special reference to multidrug resistant strains. *J Clin Diagn Res.* 2013;7(3):441.
3. Smolyakov R, Borer A, Reisenberg K, Schlaeffer F, Alkan M, Porath A, et al. Nosocomial multi-drug resistant *Acinetobacter baumannii* blood stream infection: risk factors and outcome with ampicillin-sulbactam treatment. *J Hosp Infect* 2003;54: 32-8.
4. L. Dent, D.R. Marshall, S. Pratap, R.B. Huette. Multidrug resistant *Acinetobacter baumannii*: a descriptive study in a city hospital. *BMC Infect. Dis.*, 10 (2010), p. 196.
5. Lipsky BA, Berendt AR, Deery HG, Embil JM, Joseph WS, Karchmer AW, LeFrock JL, Lew DP, Mader JT, Norden C, Tan JS. Diagnosis and treatment of diabetic foot infections. *Clinical Infectious Diseases.* 2004 Oct 1;39(7):885-910.
6. Clinical and laboratory standards institute. Performance standards for Antimicrobial susceptibility testing. M02 – A12, M07 – A10 and M11 – A8. M100 S. 26th Edition.
7. Lipsky BA, Aragon-Sanchez J, Diggle M, Embil J, Kono S, Lavery L, et al. International Working Group on the Diabetic Foot (IWGDF), Peters EJ. IWGDF guidance on the diagnosis and management of foot infections in persons with diabetes. *Diabetes Metab Res Rev* 2016;24(Suppl 1):45–74.
8. Sekhar S, Vyas N, Unnikrishnan M, Rodrigues G, Mukhopadhyay C. Antimicrobial susceptibility pattern in diabetic foot ulcer: a pilot study. *Ann Med Health Sci Res.* 2014; 4:742-5.
9. Shahi SK, Singh VK, Kumar A, Khan AU. Detection of *Escherichia coli* and Associated β -Lactamases Genes from Diabetic Foot Ulcers by Multiplex PCR and Molecular Modeling and Docking of SHV-1, TEM-1, and OXA-1 β -Lactamases with Clindamycin and Piperacillin Tazobactam. *PLoS One.* 2013;8(7):e68234.
10. Mills JL, Sr., Conte MS, Armstrong DG, Pomposelli FB, Schanzer A, Sidawy AN, et al. The Society for Vascular Surgery Lower Extremity Threatened Limb Classification System: risk stratification based on wound, ischemia, and foot infection (WIfI). *J Vasc Surg.* 2014;59(1):220-34.e1-2.
11. Martins AF, Barth AL. *Acinetobacter* multi resistente - um desafio para a saúde pública. *Sci Med.* 2013;23(1):56-62.
12. Karageorgopoulos DE, Falagas ME. Current control and treatment of multidrug-resistant *Acinetobacter baumannii* infections. *Lancet Infect Dis.* 2008;8(12):751–762.

