

**PREVALENCE OF MRSA AND MSSA INFECTION IN DIABETIC FOOT ULCER****J. UMASANKARI*¹, M.C. VANITHA² AND C. SEKAR¹**

1. Departments of Microbiology, Faculty of Agriculture, Annamalai University, Chidambaram, India.
2. Centre for Marine Bioprospecting, Amet University, Chennai, India.

ABSTRACT

This study investigated the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin sensitive *Staphylococcus aureus* in infected diabetic foot ulcers of 100 patients. Pus samples were collected and they were cultured by using appropriated methods. The *S. aureus* was the most common pathogen among the Gram-positive bacteria isolated from ulcers. Of the 65 *Staphylococcus aureus* isolated, 50 (76.92%) were found to be Methicillin resistant (MRSA) and 15 (23.07%) were Methicillin sensitive (MSSA). Methicillin resistance *Staphylococcus aureus* were tested for vancomycin resistance. It was concluded that patients with diabetes for long duration (more than 10-15 yrs) with high blood sugar level (350-450mg/dl) were predominantly affected and the foot ulcers were significantly higher in males (80%). The Methicillin resistance *Staphylococcus aureus* (MRSA) was sensitive to vancomycin and imipenem antibiotics and the minimum inhibitory concentration (MIC) of vancomycin for MRSA is 0.01µg concentration and MIC of methicillin for MSSA is 60 µg concentration.

KEYWORDS: Diabetes, Diabetic foot ulcer, Microbial infection, MRSA, MSSA.

*corresponding author

J. UMASANKARIDepartments of Microbiology, Faculty of Agriculture,
Annamalai University, Chidambaram, India.

INTRODUCTION

Foot lesions in diabetic patients are a major medical, social and economic problem. Infectious agents are associated with amputation of the infected foot if not treated promptly. Approximately one-in-four people with diabetes will develop an ulcer during their lifetime and as many as half of these ulcerations will develop an infection^{12 14}. Infection with multidrug-resistant organisms may increase the duration of hospital stay and cost of management, as well as morbidity and mortality^{10 1 21}. *Staphylococcus aureus* is the most common pathogen among Gram-positive bacteria isolated from ulcers. Almost 50% of *S. aureus* isolates are methicillin-resistant *S. aureus* (MRSA). MRSA has been increasingly isolated from diabetic foot ulcers, and several studies have found its emergence in as many as 15–30% of diabetic wounds^{8 18 21}. Furthermore, there is evidence that MRSA colonization of chronic ulcers is associated with delayed healing times⁴. Strategies to eliminate MRSA from colonized wounds are therefore essential and should include the use of simple, low-cost, effective treatments. MRSA emergence may be associated with adverse prognosis. Identifying risk factors for MRSA infections could improve prevention and treatment in diabetic foot infections, reduce resistance patterns, and reduce healthcare costs. The objective of this study was to determine the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin sensitive *Staphylococcus aureus* (MSSA) infection in diabetic foot ulcers.

MATERIALS AND METHODS

The study was carried out in the Division of Microbiology, Rajah Muthiah Medical College, Annamalai University, Chidambaram, India. The study material consisted of samples from 100 diabetic foot ulcer patients treated at the Department of Diabetology, Rajah Muthiah Medical College and Hospital, Chidambaram, India. Comprehensive particulars of the patient are meticulously noted before

collecting the sample. The particulars included name, age, sex, patient number, monthly income of the family, date of admission, presenting complaints, site affected, duration of the complaint, history of injury and the duration of diabetes and blood sugar level. The condition of the patient on the day of discharge is also observed, and the details of the medical and surgical treatment for each patient are recorded.

COLLECTION OF SPECIMENS

Pus samples are collected using sterile swabs from the foot ulcers, before applying antiseptic dressing to the wound and before antibiotic treatment.

One swab is used for making smears and another one for culture.

PROCESSING OF THE SPECIMENS

Macroscopic examination

The diabetic foot ulcer appeared reddish in colour, with foul smelling discharge. In some cases, gangrene of the foot fingers is noted. The collected specimens are evaluated macroscopically for colour, consistency and odour. The colour of the pus specimens ranged from green-yellow to brown-red. The red colour is due to admixture with blood or haemoglobin. In some cases, *Pseudomonas aeruginosa* gave rise to blue-green stained pus due to the pyocyanin pigment.

Culture method

All the bacterial isolates are identified by their characteristic colony morphology, gramstaining, motility, catalase test, oxidase test, coagulase test and biochemical reactions based on the guidelines described in the Koneman's Colour Atlas and Textbook of Diagnostic Microbiology⁶.

Antimicrobial Susceptibility Testing

All the clinical isolates are subjected to antimicrobial susceptibility testing on Mueller Hinton agar using the Kirby-Bauer method. The procedures outlined in the fourteenth edition of

Bailey and Scott's diagnostic Microbiology is followed³.

MRSA and MSSA detection method

Studying the antibiogram pattern of the *Staphylococcus aureus* isolates, the strains which are sensitive to oxacillin (5µg) are considered as Methicillin sensitive *Staphylococcus aureus* (MSSA) and the strains which are resistant to oxacillin are considered as Methicillin resistant *Staphylococcus aureus* (MRSA).

Detection of MIC of Vancomycin in MRSA strains and Methicillin in MSSA using E test (Epsilometric test)

The E test is an automated system for measuring the Minimum inhibitory concentration (MIC) of the bacterial isolate. It is a very simple test as compared to broth and agar dilution methods which are technically cumbersome. Hi comb MIC test pack from Himedia Private Ltd, Mumbai is used. It contained two types of strips, A and B. It contained the drug in various concentrations. The E test package is stored according to the manufacturer's (HiMedia, India) instructions. Mueller-Hinton agar (MHA) plates are used to perform the test. The agar surface is allowed to dry. The test organism is inoculated. The E strips of vancomycin (the drug concentration in strip A ranges from 240µg to 0.01µg, the drug concentration in B strip ranges from 4µg to 0.001 µg) are applied to the agar surface using a sterile forceps, on a 90 mm MHA plate. After incubating for 18-24 hrs at 37° C, an elliptical zone of growth inhibition is seen around the strip. The reading is taken where the eclipse intersects the scale. The same procedure is carried out for the detection of Minimum inhibitory concentration (MIC) of Methicillin in Methicillin sensitive *Staphylococcus aureus* (MSSA) starting using Epsilometric test (E test). The concentration of drug in E strip of oxacillin strip A 240µg to 0.01 µg, strip B 4µg to 0.001µg.

RESULTS AND DISCUSSION

Worldwide, diabetic foot lesions are a major

medical, social and economic problem and are the leading cause of hospitalization for patients with diabetes. This study presents a comprehensive clinical and microbiological survey of infected diabetic foot ulcers in hospitalized patients. Majority of the diabetic patients suffering from diabetic foot ulcers belong to the age group between 60-80 yrs (41%). This correlates with the study of Ikem et al¹¹.

Prevalence of diabetic foot ulcers

The diabetic foot ulcers are significantly higher in males (80%) and also in low socio economic status (77%). Bentkover and Champion², reported in his study that the males are predominantly (85.0%) affected with the age group of 50-80 and they belong to low socio-economic status. This study showed that patients with diabetes for long duration (more than 10-15 yrs) with high blood sugar level (350-450mg/dl) are predominantly affected. This correlates with the study of Viswanathan et al²².

Profile of micro organisms

A total number of 205 isolates are detected from 100 diabetic foot ulcer specimens. 85% of the patients had polymicrobial infection and 15% of the patients had single microbial infection in our study. Sapico et al¹⁷ have reported polymicrobial infection in their study (154 bacterial strains were isolated from 32 patients with diabetic foot ulcer). In our study, *Staphylococcus aureus* is the single largest isolate (31.70%), *Pseudomonas aeruginosa* is the second most responsible organism, for diabetic foot infection (20.97%), compared to *Enterobacteriaceae* like *klebsiella sp* (20.48), *E.coli* (12.19%), *proteus* (7.31%) and last one is *serratia sp* (0.97%). Fungi, are isolated from two patients (Table – 1). Louie et al¹⁵, reported Group D *Streptococci* as most frequent isolate in their study. However, other studies have shown *Proteus sp.*, to be the common isolate. Gibbons and Ellopoulos⁷ have reported *Staphylococcus aureus* and *Staphylococcus epidermis* (60%) as the commonest isolates. Shanker et al¹⁸ from south India, reported the prevalence of the causative organism as follows *Staphylococcus aureus* (25%), followed by

proteus sp (23%), *E. coli* (22%) *Pseudomonas aeruginosa* (18%) and *Klebsiella sp* (12%). Narayan Krishna Bhowmik¹⁶ from Bangladesh reported the pathogens as *Staphylococcus aureus*, *Streptococci* (group D and B) followed by Gram negative bacilli. In all these studies, *Staphylococcus aureus*, *proteus*, *E.coli*, *Pseudomonas aeruginosa* and *Streptococci* are the predominant organisms isolated from diabetic foot ulcer patients and this is accordance with our study. Gram positive organisms accounted for 37.07% of the total organisms isolated and Gram negative organisms accounted for 61.95% and fungi for 0.48%. This correlates with the study of Axler¹ reported 33.3% of Gram positive and 51.4% of Gram negative organisms as etiological agents of diabetic ulcer.

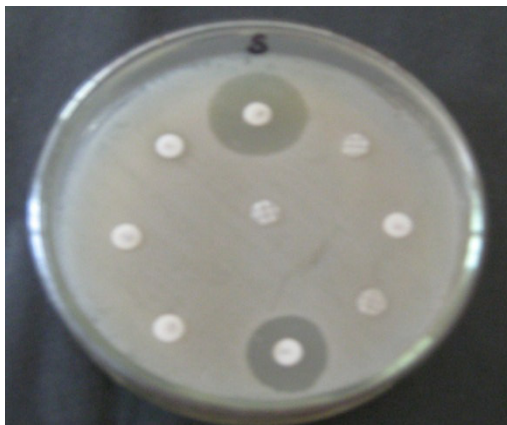
Prevalence of MRSA

Of the 65 *Staphylococcus aureus* isolated in our study, 50 (76.92%) are found to be Methicillin resistant (MRSA) and 15 (23.07%) are Methicillin sensitive (MSSA) (Fig-1). A study by Dhawan et al⁵ from India tertiary care hospital reported 44.0% MSSA and 56.0% MRSA from diabetic foot ulcer and Tentolouris et al²¹ reported the prevalence of MRSA as 46.2%. In the above mentioned studies the prevalence of MRSA is considerably lower than our study. The minimum inhibitory concentration (MIC) of vancomycin for MRSA is 0.01µg concentration and MIC of methicillin for MSSA is 60 µg concentration (Fig – II).

Table 1
Microbiological profile of diabetic foot ulcers

Organism isolated	No. of isolates	Percentage
<i>Staphylococcus aureus</i>	65	31.70%
<i>Pseudomonas aeruginosa</i>	43	20.97%
<i>Klebsiella</i>	42	20.48%
<i>E. coli</i>	25	12.19%
<i>Proteus</i>	15	7.31%
<i>Stneptrococcus pyogens</i>	11	5.36%
<i>Serratia</i>	2	0.97%
<i>Candida</i>	1	0.48%
<i>Dermatophytes</i>	1	0.48%
Total	205	100%

Figure -1
Methicillin resistant Staph aureus



Staph aureus resistant to Penicillin, oxacillin, ofloxacin, pefloxacin, ampicillin, gentamicin, ciprofloxacin, Sensitive: vancomycin, imipenem

Figure - II
MIC of Vancomycin in MRSA



MIC of vancomycin for MRSA is 0.01 μ g concentration

MIC of Methicillin in MSSA



MIC of methicillin for MSSA is 60 μ g concentration

CONCLUSION

The age and patients with diabetes for long duration (more than 10-15 yrs) with high blood sugar level (350-450mg/dl) and size of the ulcer were the risk factor for MRSA infection. The Methicillin resistance *Staphylococcus aureus* (MRSA) and MSSA were sensitive to imipenem and vancomycin antibiotics and the minimum inhibitory concentration (MIC) of vancomycin for MRSA is 0.01 μ g concentration. Thus the above study indicates that imipenem and vancomycin can be used as a drug of choice for treating the infection with MRSA in diabetic foot ulcers. The diabetic foot ulcers are preventable or treatable with patient education, properly designed and fitted foot wear, and careful periodic monitoring. In future study, pomegranate peel extract will be used for the management and treatment of

diabetic foot ulcers. Recently, the diabetic foot ulcers are treated by using a broad spectrum of antibiotics. This antibiotics treatment leads to emergence and subsequent rapid growth of drug resistant bacteria and it also causes side effects. This pomegranate peel extract is cheap, cost effective and easily available which may be act as an alternative therapy and also overcome emergence of drug resistant bacteria.

ACKNOWLEDGEMENT

My sincere thanks to Dr.M. Jeya MBBS., MD., PhD., Professor, Department of medical microbiology, RMMC & Hospital, Annamalai University, Chidambaram, Tamil Nadu, India

REFERENCE

1. Arumugam Suresh., Gopal Muthu., Seetharaman Srivani. Detection of ESBL and MBL among gram negative enterobacteriaceae isolates from diabetic foot ulcer in Chennai, International Journal of Pharma and Bio Sciences, 5 (3): 965 – 973, (2014)
2. Axler D.A., Microbiology of diabetic foot infections, J. Foot sur, 26: 53-6, (1987)
3. Bentkover J.D., Champion A.H. Economic evaluation of alternative methods of treatment for diabetic foot ulcers patients, Wound care clinics, 5: 207-215, (1999)
4. Betty AF., Daniel FS., Alice SW, Bailey & Scott's Diagnostic Microbiology, 13th Edn, Mosby publisher: 1056, (2013)
5. Dang CN., Prasad YD., Boulton AJ, Jude EB. Methicillin-resistant *Staphylococcus aureus* in the diabetic foot clinic: a worsening problem, Diabet Med, 20:159 – 161, (2003)
6. Dhawan B., Mohanty S., Das BK., Kapil A. Antimicrobial susceptibility patterns of *Staphylococci* in tertiary care hospital, Natl. Med. J. India, 17: 52-53, (2004)
7. Elmer W., Koneman, Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th Edn, Lippincott Williams and Wilkins Publisher: 1736, (2005)
8. Gibbons G.W., Ellopoulos G.H. Infection of the diabetic foot, Am. J.med, 8: 97-102, (1984)
9. Goldstein EJ., Citron DM., Nesbit C.A. Diabetic foot infections. Bacteriology and activity of 10 oral antimicrobial agents against bacteria isolated from consecutive cases, Diabetes Care, 19: 638–641, (1996)
10. Hamza K., Zahidullah K., Inamullah Khan., Jamal D., Sadiq R., Bughdad K. Factors contributing to the development of diabetic foot ulcers and role of health literacy, Rawal Med J, 36: 34-37, (2011)
11. Hartemann-Heurtier A., Robert J., Jacqueminet S., Ha Van G., Golmard JL., Jarlier V., Grimaldi A. Diabetic foot ulcer and multidrug-resistant organisms: risk factors and impact, Diabet Med, 21:710 – 715, (2004)
12. Ikem RT., Kolawole BA., Ikem IC. The prevalence, presentation and outcome of diabetic foot lesions in a Nigerian teaching hospital, Trop Doct, 32: 226-227, (2002)
13. Lavery LA., Armstrong DG., Murdoch DP., Peters EJ., Lipsky BA. Validation of the

- Infectious Diseases Society of America's diabetic foot infection classification system, Clin Infect Dis, 44: 562-5, (2007)
14. Lawrence A., Lavery DPM., Javier La Fontaine MPH., Kavita Bhavan DPM., Paul MD., Kim J., Jayme R., Williams DPM., Nathan A. Risk factors for methicillin-resistant *Staphylococcus aureus* in diabetic foot infections, Diabetic Foot & Ankle, 5: 235-75, (2014)
 15. Lipsky BA., Berendt AR., Deery HG., Embil JM., Joseph WS., Karchmer AW. Diagnosis and treatment of diabetic foot infections, Clin Infect Dis, 39: 885-910, (2004)
 16. Louie TJ., Bartlett JG., Tally FP., Gorbach. Aerobic and anaerobic in diabetic foot ulcers, Ann. Intern. Med, 85: 461-463, (1976)
 17. Narayan KB., Systematic review of antimicrobial treatments for diabetic foot ulcers, Ind.J.Med.Microbiology, 9: 492 – 97, (1996)
 18. Sapico FL., Witte JL., Canawati HM., Montgomerie JZ., Bessman A.N. The infected foot of the diabetic patient. Quantitative microbiology and analysis of clinical features, Rev infect dis, 6 (1): 171-176, (1984)
 19. Shankar E., Mohan M., Premalatha V., Srinivasan., AR. Usha AR. Bacterial etiology of diabetic foot infections in South India, Eur J Intern Med, 16: 567–570, (2005)
 20. Shao-Hua W., Lin Sun Z., Yi-Jing Guo., Quan Y., Yang B., Qiong Wei., Ping Ye K. Risk factors for methicillin-resistant *Staphylococcus aureus* in diabetic foot infections, Journal of Medical Microbiology, 59: 1219–1224, (2010)
 21. Suresh A., Muthu G., Srivani R., Moses A. Aerobic bacterial resistance in diabetic foot ulcer from Chennai, International Journal of Pharma and Bio Sciences, 2(2): 517-528, (2011)
 22. Taha AB., Relationship and susceptibility profile of *Staphylococcus aureus* infection diabetic foot ulcers with *Staphylococcus aureus* nasal carriage, Foot Edinb, 23: 11-16, (2013)
 23. Tentolouris N., Jude EB., Smirnof I., Knowles EA., Boulton AJ. Methicillin-resistant *Staphylococcus aureus*: an increasing problem in a diabetic foot clinic, Diabet Med, 16, 767–771, (1999)
 24. Viswanathan, V., Jasmine JJ., Snehalatha C., Ramachandran A. Prevalence of pathogens in diabetic foot infection in south Indian type 2 diabetic patients, J. Assoc. physicians India, 50: 1013-1016, (2002)