

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Prevalence and Antibiotic Susceptibility Pattern of Methicillin Resistant *Staphylococcus aureus* in Diabetic foot Ulcer.

MS Nandini*, and Kiran Madhusadhan.

Sree Balaji Medical College and Hospital, Bharath University, Chrompet, Chennai -600044, Tamil Nadu, India.

ABSTRACT

Diabetic foot ulcer (DFU) is the most common complication in diabetic patients, infection has led to increased mortality and sometimes may lead to amputation of limb. *Staphylococcus aureus* is the most common organism causing infection in DFU, and there is high prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) among them. MRSA is a virulent organism causing delayed wound healing and mortality. To determine the prevalence of Methicillin resistant *Staphylococcus aureus* in diabetic foot ulcer and to determine antimicrobial susceptibility pattern among MRSA strains. A prospective study pus samples / wound swab were cultured from 100 patients who attended out patients and in patients at diabetic foot clinic with diabetic foot ulcer. Specimens were subjected to routine microbiological study, and antibiotic susceptibility was done by Kirby- Baurer disk diffusion test. Out of 100 wound samples 143 organisms were isolated, of which 31 (21.67%) were *Staphylococcus aureus*. These isolates were tested for Methicillin susceptibility and was found that 13 (41.93%) were resistant and 18 (57.8%) were susceptible. These MRSA strains showed high resistance to Ampicillin (100%), Penicillin (100%), tetracycline (70.3%), Amoxicillin (69.23%), and MRSA isolates were sensitive to Vancomycin (100%), Linezolid (100%) Erythromycin (66%) and Amoxicillin/clavulanic acid (69.3%). There is high prevalence of MRSA among diabetic foot ulcers.

Keywords: Diabetic foot ulcer, *Staphylococcus aureus*, Methicillin resistant *Staphylococcus aureus* (MRSA) antibiotic susceptibility pattern.

*Corresponding author

INTRODUCTION

Diabetic foot ulcer, associated with infection is a major financial burden for the patients. It increases mortality, morbidity and duration of stay in the hospital [1]. With proper treatment of DFU, 43.8% of limb amputation can be avoided [2]. Among the organisms isolated from DFU, *Staphylococcus aureus* is the most common [3]. Methicillin resistant strains among *Staphylococcus aureus* is most virulent strain, is emerging as a serious problem in diabetic foot ulcer patients [4]. Hence proper understanding about prevalence of MRSA and their antibiotic susceptibility pattern will help the clinicians in choosing appropriate antimicrobials for empirical treatment of diabetic foot ulcer. Therefore this study was conducted to find out the prevalence rate of MRSA in diabetic foot ulcer and their in vitro antibiotic susceptibility pattern.

MATERIALS AND METHODS

A prospective study was undertaken from December 2014 to June 2015 in a tertiary care hospital in Chennai. Total of 100 patients who attended diabetic clinic with diabetic foot ulcer Wagner's stage 2-5 [5] were included in the study. Wound was thoroughly washed with saline and pus / swab was sent to laboratory immediately. Direct Gram's stained smear were done to find out the probable organism. The samples were inoculated on to nutrient agar, MacConkey agar and blood agar and incubated at 37°C for 18-24 hrs. Robertson cooked meat media was used for culturing anaerobes. The organisms were identified by Gram's staining and biochemical reactions. The Cefoxitin disc (10 µg) in Muller Hinton agar was used to identify MRSA strains, zone of less than 22 mm was considered as methicillin resistance [6]. MRSA strains were confirmed by PCR by detecting *mecA* gene [7]. The isolated MRSA strains were subjected to various antibiotics to detect antibiotic susceptibility pattern in Muller Hinton agar by Kirby Bauer disc diffusion method and CLSI guidelines [8] - Penicillin (10U), erythromycin (15 µg), Amoxicillin (20 µg), ciprofloxacin (5 µg), Amoxicillin/ cloxacillin (20 µg/10 µg), tetracycline (30 µg), vancomycin (30 µg), Ampicillin (10 µg), trimethoprim/sulfamethoxazole (1.24/23.75 µg), Ofloxacin (5 µg), Cephalexin (30 µg), Cefazolin (30 µg), Linezolid (30 µg).

RESULTS

Male diabetic patients with diabetic ulcer were predominant 83% and mean age was 53.5±11.2 years. Diabetic foot ulcer infection is a polymicrobial, total of 143 pathogenic organisms were isolated from 100 ulcer samples, 19 pus samples did not yield any growth. More than one organisms were isolated from single sample, average of 1.4 organisms per sample were isolated. 3 organisms were isolated from single sample in 31 patients, 42 samples had both gram negative and gram positive organisms, both aerobic and anaerobic organisms were isolated from 28 samples. Gram positive isolates were more in number than gram negative isolates, among them *Staphylococcus aureus* was predominant 31 (21.67%). Among *Staphylococcus aureus* MRSA strains were 13 (41.93%) and methicillin sensitive *Staphylococcus aureus* (MSSA) strains 18 (57.8%). Other organisms isolated are given in table (1). Antibiotic sensitivity pattern among MRSA strains are given in table (3).

DISCUSSION

Staphylococcus aureus is the leading cause for infection in diabetic foot ulcer and among them MRSA a virulent strain is increasing being isolated. Various studies [9, 10, 11, 12, 13, 14] [table 2] have showed that there is high prevalence of MRSA in diabetic foot ulcer. Susceptibility pattern of MRSA strains to various drugs in our study showed that, it is 100% resistant to Penicillin and Ampicillin which correlates to the study done by Wang et al [15]. Commonly used drugs like ciprofloxacin and tetracycline showed high resistance (44.5% and 70.3%) which was similar to study done by Nesrene et al [16] (ciprofloxacin 40% and tetracycline 75%). All MRSA strains were 100% sensitive to Vancomycin and Linezolid and many were multi drug resistant.

CONCLUSION

MRSA a virulent strain is found to be more prevalent among diabetic foot ulcer. These strains are emerging multi drug resistant and are sensitive mainly to vancomycin and Linezolid and narrowed our choice of antimicrobials to few drugs. Measures have to be taken for surveillance to monitor and control measures for judicious use of antimicrobials.

Type of organism	Total (n)	Percentage (%)
Gram positive cocci		
Staphylococcus aureus	31	21.77
MRSA	13	41.93
Enterococcus faecalis	8	5.64
Streptococcus pyogenes	10	7.25
Gram negative bacilli		
Pseudomonas aeruginosa	18	12.9
Escherichia coli	10	8.87
Klebsiella pneumonia	10	8.87
Citrobacter koser	9	6.45
Acinetobacter baumannii	7	4.83
Proteus mirabilis	13	8.87
Klebsiella oxytoca	12	8.06
Anaerobes		
Bacteriodes fragilis	3	1.61
Peptococcus species	4	2.41
Peptostreptococcus	2	1.61
Fungi		
Candida albicans	5	3.22
Candida non albicans	1	0.80

Table 1: Organism isolated from diabetic foot ulcer.

Sl. no	Antimicrobials	Resistance (%)
1.	Ampicillin	100
2.	Penicillin	100
3.	Trimethoprim/ sulfamethoxazole	50
4.	Amoxycillin	69.23
5.	Ofloxacin	53.8
6.	Erythromycin	34
7.	Cefazolin	11.5
8.	Cephalexin	38.4
9.	Ciprofloxacin	44.5
10.	Tetracycline	70.3
11.	Vancomycin	0
12.	Linezolid	0
13.	Amoxycillin/ clavulanic acid	30.7

Table 2: Antimicrobial resistant patterns among MRSA strains.

Sl no.	Various studies	Prevalence of MRSA(%)
1.	Goldstein et al[9]	20
2.	Yates et al [10]	23
3.	Ranjini et al[11]	23.8
4.	Gadepalli et al [12]	30
5.	Estrella Cervantes et al[13]	34
6.	Tentolouris et al[14]	46.2
7.	Our study	41.93

Table 3: Prevalence of MRSA in diabetic foot infection among various studies.

REFERENCES

- [1] Driver VR1, Fabbi M, Lavery LA, Gibbons G, J Vasc Surg. 2010 Dec;52(6):1751
- [2] Ghanassia, E., L. Villon, J. F. Thuan Dit Dieudonne, C. Boegner, A. Avignon, et al. Diabetes Care (2008), 31(7): 1288-1292.

- [3] Lipsky BA1, Berendt AR, Deery HG, Embil JM, Joseph WS, Karchmer AW, LeFrock JL, Lew DP, Mader JT, Norden C, Tan JS; Infectious Diseases Society of America, 2006 Jul-Sep;4(3):295-301. 2006 Jun;117(7 Suppl):212S-238S.
- [4] Tentolouris N, Jude E, Smirnof I, Knowles E, Boulton AJM, Diabet Med 1999; 16: 767– 771.
- [5] Wagner FW, Foot ankle. 1981 sep;2(2): 64-122.
- [6] Clarence J. Fernandes, Lorna A. Fernandes, Peter Collignon , and on behalf of the Australian Group on Antimicrobial Resistance (AGAR), Oxford journal of Antimicrobial chemotherapy volume 55, issue 4> Pp 506-510.
- [7] D. Jonas, M. Speck, F. D. Daschner, and H. Grundmann, Journal of clinical microbiology. 2002 may; 40 (5): 1821- 1823.J.
- [8] CLSI 2011. Performance Standards for antimicrobial susceptibility testing, 21st Informational Supplement. Approved Standard M100-S21. Wayna, pa.
- [9] Goldstein EJ, Citron DM, Nesbit CA, Diabetes Care,1996;19(6):638-41,
- [10] Christopher Yates, Kerry May, Thomas Hale, Bernard Allard, Naomi Rowlings, Amy Freeman, Jessica Harrison, Jane McCann and Paul Wraight, Diabetes Care. 2009 Oct; 32(10): 1907–1909.
- [11] Ranjini Chittur Yerat, Vidhya Rani Rangasamy, Int J Med Public Health 2015;5:236-41
- [12] Gadepalli R, Dhawan B, Sreenivas V, Kapil A, Ammini AC, Chaudhry R, Diabetes care. 2006 Aug; (8): 1727-32.
- [13] Estrella Cervantes-García, Estrella Cervantes-García, Rafael García-González, Rafael García-González, Aldo Reséndiz-Albor, Aldo Reséndiz-Albor, Paz Maria Salazar-Schettino, Paz Maria Salazar-Schettino. The International Journal of Lower Extremity Wounds (Impact Factor: 1.19). 01/2015; 14(1),
- [14] Tentolouris N1, Jude EB, Smirnof I, Knowles EA, Boulton AJ. Diabet Med. 1999 Sep;16(9):767-71.
- [15] Shao-Hua Wang, Zi-Lin Sun, Yi-Jing Guo,Bing-QuanYang,Yang Yuan,Qiong Wei, and Kuan-Ping Ye, Correspondence, Zi-Lin Sun, Journal of Medical Microbiology (2010), 59,1219–1224.
- [16] Nesrene .Omar,M. Elnahas,H. Elsothy, M.Eltarshoby and O.Stet, Egyptian Journal of Medical Microbiology, January 2007, Vol. 16, No. 1, 29.