

**DETECTION OF MRSA FROM NASAL CARRIER AMONG DIABETIC FOOT ULCER PATIENTS IN TERTIARY CARE HOSPITAL PUDUCHERRY****JAYARANI.K^{*1} AND SUNIL SHYAM SUNDARJI²**¹Department of Microbiology, Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry²Department of Radiology, Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry**ABSTRACT**

The purpose of this study was to evaluate risk factors for *Methicillin-resistant Staphylococcus aureus* (MRSA) from nasal carriers in patients hospitalized for diabetic foot ulcer infections. The study material consisted of nasal swabs collected from diabetic foot ulcer patients admitted at Sri Lakshmi Narayana Institute of medical sciences during the period of 3 months from November 2014 to January 2015. All the samples were subjected to gram staining and bacterial culture and *S. aureus* isolates were screened for MRSA prevalence using microbiological methods. All the clinical isolates are subjected to antimicrobial susceptibility testing on Mueller Hinton agar using the Kirby-Bauer disc diffusion method. *S. aureus* was the most common pathogen among the Gram-positive bacteria isolated from nasal carriers of diabetic foot ulcer patients. Out of 100 samples, 65 *Staphylococcus aureus* was isolated, 40 (40%) were found to be *Methicillin-resistant Staphylococcus aureus* (MRSA) (MRSA) and 25 (25%) were found to be *Methicillin sensitive Staphylococcus aureus* (MSSA). Patients with diabetes with high blood sugar level (350-450mg/dl) were predominantly affected and the foot ulcers were significantly higher in males (70%). Identifying risk factors for MRSA infections could improve prevention and treatment in diabetic foot infections, reduce resistance patterns and reduce health care costs.

KEYWORDS: MRSA, Diabetic foot, Nasal swabs, MSSA.**JAYARANI.K**Department of Microbiology, Sri Lakshmi Narayana
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INTRODUCTION

Foot infection with MRSA has been associated with longer hospital stay, greater costs and greater mortality than infection with MSSA as reported by Lipsky et al¹. Various studies have demonstrated an increase in the incidence of MRSA in patients with diabetic foot infection (DFI)^{2,3} those reports demonstrated 40% of MRSA involvement in DFI. This rate has significantly increased over the past 5 years, more troublesome is the fact that the presence of MRSA appears to significantly increase the risk of lower extremity amputation⁴. Several studies have identified specific modes of transmission via hospital reservoirs such as the anterior nares of the patient, inanimate objects within close proximity of the patient, and direct contamination from health care providers⁵. Screening for carriage of MRSA is fundamental to control Nosocomial infection, both for epidemiologic investigation and day-to-day decisions on barrier isolation⁶, also knowledge of the local predominant pathogens helps in the selection of empirical antibiotic treatment, and the frequent isolation of MRSA from infected foot ulcers must be taken into account when treatment with antibiotics is prescribed⁷. The Aim of the current research is to investigate the prevalence of *Methicillin-resistant S. aureus* (MRSA) from nasal carrier among infected /colonized foot ulcer patients.

MATERIALS AND METHODS

STUDY DESIGN

It was a retrospective study carried out at Department of Microbiology, Sri Lakshmi Narayana Institute of medical sciences during the period of 3 months from November 2014 to January 2015. The study material consisted of 100 samples from diabetic foot ulcer patients treated at the Department of surgery and general medicine, Sri Lakshmi Narayana Institute of medical sciences, Pondicherry, 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, and the duration of diabetes and blood sugar level.

EXCLUSION CRITERIA

Duplicate isolates from a single patient were excluded.

COLLECTION OF SPECIMENS

NASAL SAMPLING

Nasal samples were obtained with sterile cotton-wool swabs from patients both the left and right anterior nares were swabbed by rubbing the swab 4 times around the inside of each nostril while applying an even pressure and rotating the swab without interruption.

PROCESSING OF THE SPECIMENS

CULTURE METHOD

All the samples were subjected to gram staining and bacterial culture, and the *S. aureus* isolates were screened for MRSA prevalence using conventional microbiological methods. Subsequently, the antibiotic sensitivity test was performed for the confirmed MRSA isolates. All the bacterial isolates are identified by their characteristic Colony morphology, Gram staining, 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 Microbiology.

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 Scotts diagnostic Microbiology is followed. The following drugs and concentrations (in brackets) were used to determine the antibiogram of the strains, first line antibiotics: Penicillin G (10U), Ampicillin (10g), Erythromycin (15g), Clindamycin (2g), Cephadrine (30g), Cefuroxime (30g), Gentamicin (10g), Tetracycline (30g) and Trimethoprim-Sulphamethoxazole (1.25/23.75g), The strains resistant to the first line were tested against second line antibiotics: Vancomycin (30g), Amoxicillin/Clavulanate

(20/10g), Fusidic acid(10g), Rifampicin (5g), and Ciprofloxacin (5g).

OXACILLIN DISK DIFFUSION TESTS

The entire surface of the MHA plate was covered with the required inoculum, and the plate was air dried for 15 min before the disks were laid on the surface and incubation was performed for 18 h at the required temperature. Oxacillin resistance was determined with 1g disks according to the NCCLS guidelines.

MRSA and MSSA detection method

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

D-TEST

Inducible resistance to Clindamycin was tested by D Test as per CLSI guidelines. Clindamycin and Erythromycin discs were placed adjacent to each other, the distance from edge to edge being 211mm on a Mueller-Hinton agar. Following overnight incubation at 37°C, Flattening of Zone (D-Shaped) around Clindamycin in the area between two discs, indicated inducible Clindamycin resistance

RESULTS

PATIENT CHARACTERISTICS

Among the 100patients, the diabetic foot ulcers are significantly higher in males (45%) and 12% were found in females. (Table 1) Majority of the diabetic patients suffering from diabetic foot ulcers belong to the age group between 55-77yrs.

**TABLE 1
AGE WISE DISTRIBUTION**

S.NO	GENDER	TOTAL NO.OF PATIENTS	PERCENTAGE OF POSITIVE
1	MALE	70	45%
2	FEMALE	30	12%

This study showed that patients with high blood sugar level (350 - 450 mg/dl) are predominantly affected.

PROFILE OF MICRO ORGANISMS

Out of 100samples, 65 samples showed the growth of *Staphylococcus aureus*, 40(40%)

were found to be *Methicillin resistant Staphylococcus aureus* (MRSA) and 25 (25%) were *Methicillin sensitive Staphylococcus aureus* (MSSA).Among40 MRSA isolates, 12 MRSA isolates are positive For D-test and 28 MRSA isolates are negative for D-test. (See table 2)

TABLE 2

S.NO	ORGANISM	TOTAL NUMBER OF ISOLATES (100)
1	<i>S. aureus</i>	65(65%)
2	MRSA	40(40%)
3	MSSA	25(25%)
4	CONS	35(35%)

FIGURE 1
D-Test showing inducible Clindamycin resistance



DISCUSSION

Samples from nasal carriers of Diabetic foot ulcer patients has recently been reviewed, and the causative infective organisms and their eradication discussed. The commonest single isolate was *Staphylococcus aureus* (65%), with MRSA isolated in 40% of diabetic foot ulcers from nasal carriers. It is felt that the lack of infection control and in appropriate overuse of antibiotics has led to the emergence of MRSA⁸. Growing anti-microbial resistance is now a worldwide issue with MRSA being the most pressing problems. MRSA is the most common cause of hospital acquired infection in the UK. Data from England and Wales show that as a proportion of total *Staphylococcus aureus* bacteraemia MRSA bacteraemia rose from 2% in 1990 to 40% in 2000 one of the highest reported rates in Europe⁹. The overall rate of MRSA bacteraemia in Scotland is very similar to the rate reported in England for the period April 2002 to March 2003¹⁰. For clinicians, the spread of MRSA strains is of great concern as MRSA bacteraemia is associated with a significantly higher mortality compared with MSSA bacteraemia¹¹ and the healing time of MRSA foot ulcers is greater than with MSSA foot ulcers. The prevalence and antibiotic susceptibility patterns of MRSA isolates obtained from diabetic foot ulcer, nasal samples of the patients and health care workers were

determined. 65 *Staph. aureus* were isolated from infected foot ulcer patients, 40 MRSA strains as nasal carriage from the patients and 25 were MSSA. The prevalence of MRSA among diabetic patients was by 40% respectively among infected foot ulcers, which is less than a recent report in Greece (61.1% respectively), their high prevalence of MRSA in patients with foot ulcers may reflect the increased prevalence of MRSA in their community¹². In the current study, we detected that the rate of nasal carriage among infected diabetic foot ulcer was higher in comparison to Saxena et al among the similar studied group of patients with type-II diabetes¹³. Those results confirmed the importance of screening nasal carriage of MRSA among the diabetic patients with foot problem as MRSA colonization of nares increases the risk for MRSA infection as reported by Stanaway et al¹⁴. The diabetic foot ulcers among nasal carriers are significantly higher in males (70%) in our study compared to females. Bentkover and Champion, reported in his study that the males are predominantly (85.0%) affected with the age group of 50-80. 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 al¹⁵. This high

prevalence confirms that patients admitted to our DFU were at particularly high risk for MRSA acquisition, probably because they had a combination of several known risk factors, such as diabetes mellitus, surgical intervention, close contact with hospital personnel, or exposure to a health care setting (particularly if repeated) within six months¹⁶. These studies have identified that a history of MRSA infection, chronic wounds, previous hospitalization, and nasal swabs are associated with the colonization of diabetic foot ulcers with MRSA¹⁷. We identified only one study that evaluated patients admitted to the hospital with diabetic foot infection and their associated risk factors¹⁸. Yates identified previous hospitalization for the same ulceration, chronic wounds, and inpatient treatment as risk factors. However, he did not evaluate nasal swabs, history of MRSA infection, previous antibiotic use, nursing home residence, peripheral arterial disease, or multi-drug-resistant organisms. A

recent study by Taha looked at 222 *S. aureus* isolation. Seventy one (30.87%) of the patients was *S. aureus* infection diabetic foot with nasal carriage. Among diabetic foot infection and nasal carriage patients, 40.85% of *S. aureus* were considered as MRSA¹⁹.

CONCLUSION

Prevalence of MRSA and MRSA antibiotic sensitivity should be considered in order to achieve better control of MRSA colonization or infection in DF and also knowledge of the local predominant pathogens helps in the selection of empirical antibiotic treatment. The frequent isolation of MRSA from infected foot ulcers must be taken into account when treatment with antibiotics is prescribed. The diabetic foot ulcers are preventable or treatable with patient education, properly designed and fitted foot wear, and careful periodic monitoring.

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