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PHENOTYPIC RELATEDNESS OF CLINICAL & CARRIER METHICILLIN RESISTANT STAPHYLOCOCCUS (MRSA) STAINS ISOLATED AT IIMS&R LUCKNOW.

Microbiology

Manzoor Ahmed Thokar Prof & Head, Dept., of Microbiology IIMS&R,lucknow

Rukaya Rahim* Msc Dept., of Microbiology IIMS&R, lucknow *Corresponding Author

M. N Sidiqqi Director/Chief M.S & Head Dept. of Hospital administration IIMS&R lucknow

ABSTRACT

Introduction - Methicillin resistant *staphylococcus aureus* (MRSA) is a bacterium that causes infections in different parts of body. It is difficult to treat most strains of *Staphylococcus aureus* because of their resistance to commonly used antibiotics.

Materials and methods- This is cross sectional study of clinical and carrier MRSA sample of patients attending Integral Institute of Medical Sciences Research and Hospital Lucknow including IPD, OPD Patients and hospital personnel from January 2019 to June 2019.

Result-In our study, the prevalence of MRSA in clinical samples was 66.7% and in carrier samples prevalence of MRSA was 65.6%. Antibiotic sensitivity once done for both types of clinical and carrier sample of *staphylococcus aureus* showed phenotypic relatedness in around 1.12 % of cases i.e., in four nasal swabs and rest of 98.8% cases of carrier MRSA. Overall antimicrobial sensitivity pattern in *S.aureus* in various samples showed 100 % sensitivity to vancomycin, teicoplanin, linezolid, amikacin, tobramycin, followed by tetracycline and doxycycline which had both 69.77%, clindamycin 65.12%, gentamycin and cotrimoxazole which had both 62.79% and other antibiotics showed lowest sensitivity in which erythromycin 18.60%, followed by levofloxacin 17.44%, nitrofurantion 12.79%, cefoxitin 10.47% and ciprofloxacin 9.30% respectively .

Conclusion- Our study shows that if good infection control practices will be employed in hospitals ,health care and clinical institutions like proper cleaning ,proper waste management ,proper disinfection ,state of art ICU care and on top of all a good hand hygiene being practiced by the staff , then the rate of colonization and transmission of MRSA will decreases by much higher rate.

KEYWORDS

MRSA, Colonization, Transmission, Antibiotic sensitivity pattern

INTRODUCTION

Genus Staphylococcus includes pathogenic organisms in which *Staphylococcus aureus* is the most important. *S.aureus* is the leading cause of gram positive bacterial infections and produces a wide range of spectrum of diseases [1]. Methicillin resistant *S.aureus (*MRSA) is a group of gram positive bacteria that are genetically distinct from other strains of staphylococcus aureus. Methicillin resistant *staphylococcus aureus* (MRSA) is the term used for bacteria of the *Staphylococcus aureus (S. aureus)* that are resistant to the usual antibiotics used in the treatment and are susceptible only to glycopeptides antibiotics such as vancomycin. Traditionally MRSA stood for methicillin resistance but the term increasingly refers to a multi-drug resistant group. Such bacteria often have resistance to many antibiotics traditionally used against *S. aureus.*

Colonization of MSRA

Infections caused by MRSA are the same as other *staphylococcal* infections because the organism itself is not any more virulent (or infectious) than usual type *S. aureus*. Like other S. aureus, MRSA can colonize the skin and body of an individual without causing sickness, and in this way it can be passed on to other individuals unknowingly. Problems arise in the treatment of over infections with MRSA because antibiotic choice becomes very limited [1]. There are three main reservoirs (and hence sources of spread and infection) for MRSA in hospital and institutions: staff, patients and inanimate objects such as beds, linens and utensils. The most important reservoir is patients who may be colonized with MRSA without evidence of infection. The usual sites of colonization with MRSA are the nostrils, skin, groin, axilla, and wounds [1].

Health Hazards caused by MSRA

MRSA can cause urinary tract infection, Outbreaks of hospital-acquired MRSA (HA-MRSA) are typically the result of clonal spread by MRSA being transferred from patient to patient, frequently using healthcare personnel as intermediaries. HA-MRSA strains are generally multidrug resistant. Vancomycin is the standard treatment for serious MRSA infections, but a few cases of vancomycin-resistant. S. Infection by MRSA is more commonly associated with adult patients, particularly in those with the following risk factors prolonged hospital stay, antimicrobial use, invasive procedures, surgeries and patients submitted to the hemodialysis. MSSA infections are more prevalent in neonates, especially those with the following risk factors: premature birth, low weight, breathing syndromes, immunodeficiency, antimicrobial use, prolonged hospital stay, invasive methods and surgical interventions [1]. Moreover, MRSA can rapidly spread from patient to patient and hospital to hospital [2]. Most children and 40% of adults are nasal carriers of S. aureus. Examples of populations with an increased frequency of S. aureus carriage are newborns, hospital workers, hemodialysis patients and those with skin disorders such as eczema. MRSA may be carried for an extremely long period. Therapies to clear nasal carriage of MRSA have been met with some success, but relapse can occur within months of the completion of therapy [3].

S. aureus causes superficial, deep-skin, soft- tissue infections, endocarditis, and bacteremia with metastatic abscess formation and a variety of toxin – mediated diseases including gastroenteritis, staphylococcal scalded skin syndrome and toxic shock syndrome. Prevalence and antibiotic sensitivity pattern of MRSA help the treating clinicians for first line treatment in the referral hospitals and even death [4]. Prior to the advent of antibiotics, staphylococcus aureus emerged as a bacterial pathogen that was capable of causing a variety of significant human diseases leading to a high fatality rate [5]. They grow comparatively under well conditions of high osmotic pressure and low moisture, which partially explains why they can grow and survive in nasal secretions on the skin [6]. *S. aureus* has been recognized as an important cause of disease around the world and it has become a major pathogen associated with both hospital and community-acquired infections[7].

Resistance and susceptibility of MSRA

B-lactam antibiotics, such as penicillin, bind to and inactivate enzymes named PBPs (penicillin binding proteins). These enzymes have their function in the cell wall synthesis. Bacterial resistance to penicillin developed through the production of penicillinases that specifically degrade β-lactam antibiotics [8].

To overcome the resistance problem a new class of antibiotics was developed, namely penicillinase-resistant penicillins e.g., oxacillin and methicillin that were introduced in the late 1950s. Methicillin/oxacillin resistant S. aureus (MRSA) was identified in 1961 and the resistance mechanism developed renders the cells resistant to all known β- lactam antibiotics. Today, most of the MRSA are Multi resistant i.e., resistant to a number of drugs, thus causing a clinical problem as antibiotic treatment becomes useless. Methicillin resistance is almost exclusively caused by production of an additional penicillin binding protein (PBP2a) encoded by the mecA gene, although other mechanisms have been described [8]. This study was

conducted to detect the antimicrobial susceptibility testing of carrier and clinical MRSA At IIMS&R as to formulate the empirical antibiotic protocol for such strains.

MATERIAL AND METHODS

A cross sectional study of clinical and carrier MRSA samples of patients attending Integral Institute of Medical Sciences Research and Hospital Lucknow. Study population composed of IPD, OPD Patients and hospital personnel. Total number of 87 patients and hospital personnel were enrolled. The cases were studied as per the proforma enclosed. A detailed history including age, sex, and distribution of lesion and duration of illness and, prior history of antibiotic intake and any associated risk factors contributing for the illness were elicited from the patients. Samples were collected and processed the Department of Microbiology, Integral Institute of Medical Sciences & Research, Hospital Lucknow. Out of these 50 IPD Patients, 8 OPD Patients and 29 hospital personnel from Integral Institute of Medical Sciences & Research (IIMS&R) lucknow were screened for colonization of methicillin – resistant *staphylococcus aureus*. Study was conducted from to January 2019 to June 2019.

Statistical analysis-The data was analysed by using Chi-square test for categorical variables.

Ethical Clearance –Research project was approved by Institutional research & ethical committee for execution.

Specimen collection: Specimen of hospitalized patients from their various sources was taken and cultured as per set guidelines. Nasal and hand swabs were collected from health care workers as under aseptic condition. Clinical samples with MRSA growth received from wards of IIMS&R were subject for bacteriological examination MRSA Clinical isolates labelled as multidrug resistant and originating from Pus, Blood, HVS and Urine of critically ill patients of plastic surgery, medical oncology, radiotherapy, dialysis section, SICU and post-operative ward were included in this study.

Gram stain- Specimens were directly examined by gram stain.

Microscopy-Microscopic examination was done by making a smear from the colony and examining under oil immersion after the gram staining.

Culture-The specimens were inoculated onto Blood agar, MacConkey agar or CLED agar. The culture plates were examining after overnight incubation at 37°C for 18-24hr, the relative numbers and types of colonies was noted and further tests was done for their identification and determination.

Identification of isolates:- After culture the bacteria is obtained from pure culture and then subculture, identification of isolates was done on the basis of colony morphology, catalase, coagulase test and other biochemical tests.

Antibiotic Sensitivity Testing- The antimicrobial susceptibility testing was done by Kirby Bauer's Disk Diffusion Method on Mueller Hinton Agar and interpreted as per CLSI. 2018 , Clinical Laboratory Standard Institution guidelines (9)

The antibiotic disks was used according to bacterial isolate .Susceptibility of staphylococcus aureus isolates to cefoxitin (30) was determined by modified Kirby-Bauer disc diffusion method following CLSI guidelines.Cefoxitin is a surrogate marker for oxacillin resistance. The strains of S. aureus found to be resistant to cefoxitin were eported as MRSA.

RESULTS AND DISCUSSION:

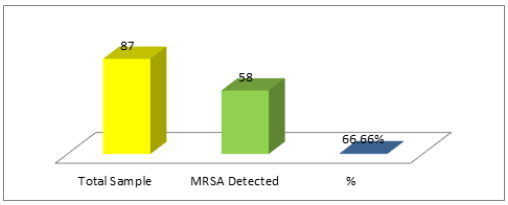
Overall antimicrobial sensitivity pattern in *Staphylococcus aureus* in various samples showed 100 % sensitivity to vancomycin, teicoplanin, linezolid, amikacin, tobramycin ,followed by tetracycline and doxycycline which had both 69.77%, clindamycin 65.12%, gentamycin and cotrimoxazole which had both 62.79% and other antibiotics showed lowest sensitivity in which erythromycin 18.60%, followed by levofloxacin 17.44%, nitrofurantion 12.79%, cefoxitin 10.47% and ciprofloxacin 9.30% respectively.

Sensitivity *of staphylococcus aureus* in blood sample in which vancomycin (VA) showed highest sensitivity (100%), followed by teicoplanin and doxycycline Sensitivity of *staphylococcus aureus* in urine sample in which Norfloxacin showed highest sensitivity (100%), followed by Nitrofurantion, Vancomycin and Teicoplanin. Sensitivity of *Staphylococcus aureus* in pus sample in which Vancomycin showed highest sensitivity 100%, followed by Teicoplanin and Linezolid.

Table 1: Prevalence of MRSA in clinical samples is isolated from various samples

Total Sample	Clinical MRSA	Percentage
87	58	66.66%

Fig1: Prevalence of MRSA in clinical samples is isolated from various samples

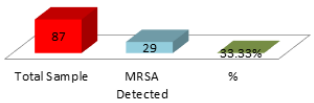


In our study, the prevalence of MRSA in clinical samples was 66.7% and in carrier samples prevalence of MRSA was 65.6%. Antibiotic sensitivity once done for both types of clinical and carrier sample of staphylococcus aureus showed phenotypic relatedness in around 1.12 % of cases i.e., in four nasal swabs and rest of 98.8% cases of carrier MRSA had different antibiotic sensitivity pattern[10].

Table 2: Prevalence of MRSA in carrier sample isolated from Hand and Nasal swab

TotalSample	CarrierSample	Percentage
87	29	33.33%

Fig 2 : Prevalence of MRSA in carrier sample isolated from Hand and Nasal swab

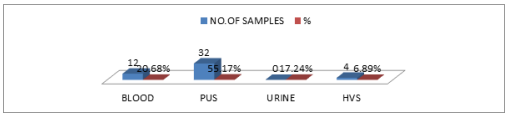


Sensitivity of *Staphylococcus aureus* in nasal swab in which Vancomycin showed highest sensitivity 100%, followed by Teicoplanin and Linezolid. *Staphylococci,* especially *S. aureus* are the most common cause of nosocomial as well as community-acquired infections.

Table no 3: Distribution of clinical samples according to specimen:

Clinical specimes (n=58)	No. of samples	%
Blood	12	20.68%
PUS	32	55.17%
Urine	1O	17.24%
HVS	4	6.89%

Fig 3: Distribution of clinical samples according to specimen

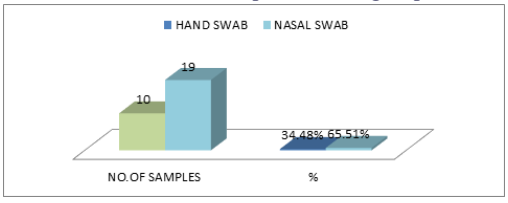


Out of 58, the highest percentage was found in pus 55.17% followed by blood 20.68%, urine 17.24% and HVS 6.89%.

Table No. 4: Distribution of carrier samples according to specimen

Carrier specimen (N=29)	No. of samples	%
Hand swab	10	34.48%
Nasal swab	19	65.51%

Fig4 : Distribution of carrier samples according to specimen



Out of 29, the highest percentage was found in nasal swab 65.51% followed by hand swab 34.48%.

In our study, the prevalence of MRSA in clinical samples was 66.7% and in carrier samples prevalence of MRSA was 65.6%. According to a study by Javid A Dar et al., in 2006, It was shown that 35.1% of Staphylococcus aureus and 22.5% of coagulase-negative staphylococcal isolates were resistant to methicillin. Highest percentage of MRSA (35.5%) was found in pus specimens (n = 151). The multiple drug resistance of all MRSA (n = 180) and Methicillin resistant Coagulase-negative *Staphylococcus aureus* (MRCNS) (n = 76) isolates was detected. In case of both methicillin-resistant as well as methicillin-sensitive *Saphylococcal* isolates zero resistance was found to vancomycin whereas highest resistance was found to penicillin G followed by ampicillin[11].

Antibiotic sensitivity once done for both types of clinical and carrier sample *of staphylococcus aureus* showed phenotypic relatedness in around 1.12 % of cases i.e., in four nasal swabs and rest of 98.8% cases of carrier MRSA had different antibiotic sensitivity pattern thereby showing that there is not much cross infection rate at IIMS&R and reason being good infection control practices being employed at IIMS&R like proper cleaning, proper waste management ,proper disinfection, state of art ICU care and on top of all a good hand hygiene being practiced by the staff.

CONCLUSION

Our study shows that if good infection control practices will be employed in hospitals ,health care and clinical institutions like proper cleaning, proper waste management, proper disinfection ,state of art ICU care and on top of all a good hand hygiene being practiced by the staff**,** then the rate of colonization and transmission of MRSA will decrease by much higher rate.

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