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PREVALENCE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERNS IN PUS SAMPLES IN HOSPITAL OF MMIMSR, MULLANA, AMBALA.

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Abstract:

Background: MRSA is one of the most important pathogen implicated in minor skin and soft tissue infections including abscess, cellulitis, necrotizing fasciitis to chronic bone infections and post-surgical wound infections. The magnitude of *Staphylococcus* infection and emergence of its antibiotic resistant has increased day by day due to irresponsible and over use of antibiotics, contamination during operative procedures, prolong stay in hospital, lack of antisepsis measures etc.

Methods: 200 *S.aureus* isolated from pus samples of patients who attended in OPD and admitted in IPD during 2019-2020. *S.aureus* was isolated from pus samples which were identified by direct microscopy, biochemical tests and antibiotic susceptibility testing and Methicillin resistance was detected by disc diffusion tests according to CLSI guidelines.

Results: Out of 200 *Staphylococcus aureus* isolated from pus samples, the rate of incidence of *S.aureus* was 60.5% in males and 39.5% in female and predominance of MRSA isolates was 51% and MSSA isolates was

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49%. Abscesses was most prevalent in MRSA isolates. The incidence of postoperative wounds was high in male than female. Vancomycin and linezolid were most effective drugs against *Staphylococcus aureus* isolates with 100% sensitivity. Ampicillin and Ciprofloxacin were the least effective drugs against MRSA.

Conclusion: Periodic surveillance on rate of postoperative wound and its associated factors, infection control, patient management and antimicrobial susceptibility should be done. Patient isolation and strict enforcement of hand washing should be done to prevent colonization and infection in order to deduce the spread of MRSA infection.

Keywords: Methicillin; Resistant *Staphylococcus aureus;* Antibiotic

INTRODUCTION

The human skin and delicate tissue contaminations brought about by microbial pathogens because of injury, burns and surgeries bring about the creation of discharge which contained dead WBCs, debris and necrotic tissues. Pyogenic contaminations are described by local and systemic aggravation typically with discharge development which might be either endogenous or exogenous and polymicrobial or monomicrobial. The most widely recognized living beings experienced from discharge are Gram positive cocci.[1]

S. aureus inherit a class of traditional flora that present in anterior nare of the nose and regarding 1/4th human population are the carrier of those organism. *S. aureus* can cause a scope of sicknesses, from minor skin contaminations, for example, pimples , impetigo, bubbles, cellulites, folliculitis, carbuncles, singed skin disorder, and abscesses, to perilous illnesses, for example, pneumonia, meningitis, osteomyelitis, endocarditis, bacteremia and sepsis.[2]

Prior to the presentation of antibiotic drug within the late Forties, cocci septicaemia was connected with a really high death rate. Penicillin significantly improved the forecast of this contamination. Be that as it may, penicillin restricting strains were found in some time and became ineffective reciprocally within the hospital and community settings. The advancement of betalactamase penicillins, for example, methicillin and oxacillin in the mid 1960s indeed changed the treatment of *Staphylococcal* contaminations. Inside a period of the usage of methicillin, methicillin resistant *Staphylococcus aureus* (MRSA) strains were represented worldwide and all through the accompanying barely any decades, MRSA has landed at pandemic degrees.[3]

In the middle Nineteen Sixties, Methicillin Resistant *Staphylococcus aureus* (MRSA) created as a health acquired microorganism. From that point forward, it has been progressively revealed from hospital in nations around the globe. Today, infection with MRSA is a typically hospital-acquired as well as community-acquired infection experienced especially in developing nations [4].

PBP is generally present on the surface of *S. aureus* coded by mec A which is responsible to make it resistance. Since b-lactam antibiotics cannot bind to PBP2a, there's synthesis of the peptidoglycan layer and plasma membrane synthesis [5].

Over 1999–2005, it was demonstrated that MRSA related hospitalizations multiplied from 127,036 to 278,203. Another report additionally showed that, in 2005, there were an expected 94,360 obtrusive MRSA

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contaminations in the US of which 18,000 patients lost their lives, an extent which is higher than HIV/AIDS related fatalities [6].

The degree of HA-MRSA in the created nations has extended from 2% to 30%. Also, half of *S. aureus* in various states of India are methicillin resistant showing critical healing test. MRSA now till date endemic in India.[7]

In 1953, Edmund had discovered a drug named vancomycin, isolated from *Streptomyces orientalis* which was used for treating MRSA contaminations. Nowadays its resistance pattern had been increased day by day. First clinical isolate of VRSA was accounted for from United States in 2002.[8]

Since the treatment alternatives are restricted, and therefore the generality rate changes in numerous geologic regions, it's essential to grasp the predominance and susceptibleness profile of MRSA thus important restorative and unwellness management safeguards might be taken. Subsequently this examination was done to decide the pace of colonization of MRSA and antimicrobial susceptibility among clinical MRSA strains and to know the presence of MRSA among samples.[9]

Material and Methods

Sample collection

The present study was conducted on 200 *Staphylococcus aureus* isolates from pus samples collected from the hospital and were processed as per routine standard microbiological procedures in the department of microbiology MMIMSR, Mullana, 2019-2020.

Identification of *S. aureus*

Gram staining was done to observe pus cells and gram positive cocci arranged in clusters and any other relevant structure. Pus sample was inoculated on blood agar and macconkey agar and incubated for 18-24 hours. Culture characteristics were observed and organism was identified with standard biochemical testing protocol such as catalase positive, coagulase positive, beta hemolysis on blood agar, Voges prausker test positive etc.

Detection of methicillin resistance

All the isolates of *Staphylococcus aureus* was subjected for detection of methicillin resistance by cefoxitin phenotypic method. A standard strain of *Staphylococcus aureus* ATCC 25923 was used as control for cefoxitin method.

- Phenotypic method for the detection of MRSA isolates
  Cefoxitin disc diffusion method:

All the isolates were tested with cefoxitin disc diffusion (30µg). A bacterial suspension was prepared and adjusted to 0.5 McFarland for each strain. Plates were incubated at 35°C for 18 hrs. Zone diameters was measured and interpreted according to CLSI (2015) criteria

≤ 21 mm mecA positive (Methicillin resistant)
≥22 mm mecA negative (Methicillin sensitive)

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Antimicrobial susceptibility testing [10]

Antibiotic susceptibility test was done by the Kirby Bauer Disc diffusion method on Muller Hinton agar and interpretation was made according to CLSI (2015) guidelines. *S. aureus* ATCC 25923 was used as controls for the antibiotic susceptibility test.

MRSA strains was subjected to the following antimicrobial agents: cotrimoxazole (25 µg), erythromycin (15 µg), clindamycin (10 µg), ciprofloxacin (30 µg), levofloxacin, cefoxitin(30µg), amikacin (10 µg), linezolid (30 µg), vancomycin (30 µg), clindamycin(10 µg), linezoid(30µg) , azithromycin ,quinpristin (15µg) will be tested.

MSSA strains was subjected to the following antimicrobial agents: ampicillin (10 µg), cephalexin (30 µg) and amoxicillin/ clavulanic acid (30 µg). cotrimoxazole (25 µg), erythromycin (15 µg), clindamycin (10 µg), ciprofloxacin (30 µg), netilmicin (30 µg), amikacin (10 µg) , linezolid (30 µg), vancomycin (30 µg) will be tested.

RESULTS

In this present study, we reported the prevalence of MRSA was 51% and MSSA was 49% respectively. **TABLE NO. I**

Socio-demographic characteristics of patients with MRSA infections: In present study, number of males was 121 which accounted for 60.5% while number of female was 79(39.5%). The prevalence of *Staphylococcus aureus* in the age group 21-40 years was 67(33.5%), 41-60 years was 69(34.5%), 0-20 years was 32(16%) and >60 years was 32(16%). Similarly, 55% of *Staphylococcus aureus* were isolated from the patients of urban which was greater in number in comparison to 90(45%) *S. aureus* isolated from the patients of rural. In present study, 102(51%) isolates of *S. aureus* were obtained from IPD and 98(49%) isolates were collected from OPD. TABLE NO. II

Antibiotic Sensitivity Patterns of *Staphylococcus aureus* isolates and its strains MRSA and MSSA

The antimicrobial susceptibility patterns of 200 *S. aureus* isolates was determined against 19 antimicrobial agents as presented in Table III. The majority (>60%) of the isolates were resistant to the following antibiotics: ampicillin, amoxyclauvulanic acid, erythromycin, gentamicin and cotrimoxazole. *S. aureus* isolates showed <50% of resistance against vancomycin, linezolid, tigecycline, quinpristin, netilmicin and clindamycin. The most effective drugs against both MRSA and MSSA isolates with 100% sensitivity were vancomycin and linezolid. Ampicillin and Ciprofloxacin were the least effective drugs against MRSA isolates with 35.71% and 25.49% sensitivity respectively. **TABLE III**

Frequency of *Staphylococcus aureus* and MRSA isolates in clinical presentations

In this present study, the prevalence of MRSA isolates in abscesses was 26.47% followed by 16.66% in cellulitis, 5.8% in osteomyelitis, 3.92% in perforation, 2.94% in vestibulitis ,2.94% in buccal infection, 2.94% in necrotising fasciitis, 1.96% in diabetic foot , 1.96% in ear infection , 1.96% in Ludwig angina ,1.96% in breast discharge, 0.98% in puerperal sepsis, 0.98% in inguinal hernia and 0.98% in umbilical secretion. **Table No. IV**

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Analysis of characteristics related with isolation of MRSA in postoperative wounds

The present study illustrated that the predominance of MRSA and MSSA isolates in postoperative wounds were 37.5% and 62.5% respectively. Out of 8 patients, the number of males was 6 and that of females were 2. It also illustrated that out of total postoperative wounds, 50% were inguinal hernia followed by similar rate (12.5%) of iliac abscess, proximal humerus, laryngectomy and necrotizing fasciitis. TABLE NO. V

TABLE NO. I Frequency of MRSA and MSSA isolates included in this study

Total No. of Sample	**MRSA**	**MSSA**
200	102(51%)	98(49%)

Table II. Socio-demographic characteristics of patients with surgical site infection

		No of positive patient	MRSA (%)
Gender	Male	121(60.5%)	57(55.88%)
	Female	79(39.5%)	45(44.11%)
Age	<20	32(16%)	—
	20-40	67(33.5%)	—
	40-60	69(34.5%)	—
	>60	32(16%)	—
Residential status	Rural	110(55%)	—
	Urban	90(45%)	—
Ward	IPD	102(51%)	59(57.84%)
	OPD	98(49%)	43(42.15%)

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TABLE III Antibiotic Sensitivity Patterns of *Staphylococcus aureus* isolates and its strains MRSA and MSSA

Antibiobtics	**Sensitive [S]**	**Resistant[R]**	**MSSA(N=98)[S]**	**MRSA(N=102)[S]**
Cefoxitin	98(49%)	102(51%)		
Ciprofloxacin	84(42%)	116(58%)	58(59.18%)	26(25.49%)
Levofloxacin	103(51.5%)	97(48.5%)	63(64.28%)	40(39.21%)
Ofloxacin	96(48%)	104(52%)	61(62.24%)	35(34.31%)
Erythromycin	115(57.5%)	85(42.5%)	70(71.42%)	45(44.11%)
Azithromycin	153(76.5%)	47(23.5%)	90(91.83%)	63(61.76%)
Clindamycin	153(76.5%)	47(23.5%)	89(90.81%)	64(62.74%)
Linezolid	200(100%)	_____	98(100%)	102(100%)
Vancomycin	200(100%)	_____	98(100%)	102(100%)
Chlorompenicol	105(52.5%)	95(47.5%)	66(67.34%)	39(38.23%)
Gentamycin	99(49.5%)	101(50.5%)	63(64.28%)	36(35.29%)
Cotrimaxozole	141(70.5%)	59(29.5%)	74(75.55%)	67(65.68%)
Amikacin	142(71%)	58(29%)	88(89.79%)	54(52.94%)
Netilmicin	178(89%)	22(11%)	91(92.85%)	87(88.77%)
Ampicillin	35(17.5%)	165(82.5%)	35(35.71%)	NA
Amoxyclavulanic Acid	93(46.5%)	107(53.5%)	93(94.89%)	NA
Tigecycline	198(99%)	2(1%)	96(97.95%)	102(100%)
Cephalexin	93(46.5%)	107(53.5%)	93(94.89%)	NA
Quinpristin/ Dalfopristin	187(93.5%)	13(6.5%)	94(95.91%)	93(91.17%)

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Table No. IV Frequency of *Staphylococcus aureus* and MRSA isolates in clinical presentations

Clinical Presentation		**Number of MRSA(102)**
Cellulitis		17(16.66%)
Abscess		27(26.47%)
Diabetic Foot		2(1.96%)
Necrotizing Fascitis		3(2.94%)
Inguinal Hernia		1(0.98%)
Osteomyelitis		6(5.8%)
Bone Infection		1(0.98%)
Buccal Infection		3(2.94%)
Ear Infection		2(1.96%)
Breast Discharge		2(1.96%)
Umbilical Secretion		1(0.98%)
Post Operative Wound		3(2.94%)
Puerperal Sepsis		1(0.98%)
Ludwig Angina		2(1.96%)
Left Emphysematous Lung		1(0.98%)
Paraspinal Abscess		1(0.98%)
Postauricular Swelling		1(0.98%)
Infected Sabaceous Cyst		1(0.98%)
Vestibulitis	Nasal	2(1.96%)
	Vaginal	1(0.98%)
Perforation	Peritonitis	1(0.98%)
	Gallbladder	1(0.98%)
Perforation	Intestinal	1(0.98%)
	Ileum	1(0.98%)

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TABLE NO. V **Analysis of characteristics related with isolation of MRSA in postoperative wounds**

No. of Male	No. of Female	Number of Postoperative Wound(n=8)					MRSA	MSSA
		Iliac Abscess	Proximal Humerus	Inguinal Hernia	Laryngectomy	Necrotizing fasciitis		
6 75%	2 25%	1 12.5%	1 12.5%	4 50%	1 12.5%	1 12.5%	3 37.5%	5 62.5%

DISCUSSION

The present study was carried out on 200 *Staphylococcus aureus* isolates received in the department of microbiology of MMIMSR, Mullana, Ambala for the prevalence of Methicillin Resistance in *Staphylococccus aureus* by using Cefoxitin disc diffusion technique.

The present study showed that there was a prevalence of 102(51%) MRSA and 98(49%) MSSA isolates in pus samples. According to the study conducted by Mohammed Dawood & Sulafa El-Samarrai, 2018, 51.98% were MRSA and 47.3% were MSSA. In another study which was conducted by Mekviwattanawong et al., 2006, there was a predominance of 41.5% MRSA and 58.5% MSSA. The reason might be because of some factors like use of antibiotics incorrectly, unskilled practitioners, reduced hospital sanitary conditions, insufficient inspection could be responsible for extend of resistant bacteria. **(TABLE NO.I)**

In present study, number of males was 121 which accounted for 60.5% while number of female was 79 with 39.5%. Predominance of male was also showed by study done by Khanam et al., 2018 which accounted for 56% male and 43.86% female. The reason behind the elevated rate of *Staphylococcus* infections among males could be due to their outdoor occupation, more prone for injuries and due to exposure to contaminated environment.

In present study, 102(51%) isolates of *S. aureus* were obtained from inpatient department and 98(49%) isolates were collected from outpatient department. von Specht et al., 2014 reported 77 (55.8%) IPD and 77(44.2%) OPD .Similar findings were observed in Jaiswal et al., 2016 and Hospital, 2015. The reasons behind the high

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predominance rate in IPD could be due to stay in hospital for long time, poor sanitation, poor hygiene and conditions of ward like bed making, changing of clothes, sneezing, nose picking etc **.**

The present study demonstrates the prevalence of *Staphylococcus aureus* in the age group 21-40 years was 67(33.5%), 41-60 years was 69(34.5%), 0-20 years was 32(16%) and >60 years was 32(16%). According to study done by Devi & Saikumar, 2017 revealed 23(36.50%) *S. aureus* were isolated from 41-60 age group and 16(25.39%) from 21-40. Another study performed by Alebachew et al., 2012, 59(51.8%) i.e maximum number of *S. aureus* were isolated from 20-45 years and 46(40.4%) from <20 age group. The reason behind this might due to low immune capacity and occurrence of co-morbidities for example hypertension and diabetes. According to the present study, 110(55%) *Staphylococcus aureus* were isolated from the patients of urban which was greater in number in comparison to 90(45%) *S. aureus* isolates from the patients of rural. From the study of Kumar Hanumanthu & B, 2017, 54% isolates of *S. aureus* were from the patients of rural and 47% *S. aureus* isolates from the patients of urban. The reason might be due to inappropriate health care facilities, lack of awareness on sanitation, inadequate excreta disposal facilities, poor hygiene, poor living conditions and unsafe food.

In present study, 65(32.5%) isolates of *Staphylococcus aureus* were obtained from farmer whereas 58(29%) isolates from employer, 47(23.5%) isolates from housewife and 30(15%) isolates were obtained from student. According to study of Kahsay et al., 2014 97(52.7%) isolates of *Staphylococcus* were observed in pus samples of farmer followed by 53(28.8%) isolates from housewife, 23(12.5%) isolates from employee whereas least number of isolates of *S. aureus* were found in pus samples of students. The reason might be due to daily exposure to harsh environment of farmer's work. They were exposed to a number of biological, physical and chemical factors like pesticides, insecticides including dust containing both organic and inorganic toxins which hamper their health.**(TABLE NO. II)**

The antimicrobial susceptibility patterns of 200 S. aureus isolates was determined against 19 antimicrobial agents as presented in Table III. The majority (>60%) of the isolates were resistant to the following antibiotics: ampicillin, amoxyclauvulanic acid, erythromycin, gentamicin and cotrimoxazole. S. aureus isolates showed <50% of resistance against vancomycin, linezolid, tigecycline, quinpristin, netilmicin and clindamycin. The most effective drugs against both MRSA and MSSA isolates with 100% sensitivity were vancomycin and linezolid. Ampicillin and Ciprofloxacin were the least effective drugs against MRSA isolates with 35.71% and 25.49% sensitivity respectively. According to the study carried by Thangavel et al., 2017, *S. aureus* isolates were susceptible to vancomycin(100%), linezolid (100%), gentamycin (53.33%), ampicillin (13.33%) and by Rijal et al., 2017 showed vancomycin(100%), linezolid (100%), clindamycin(67.72%), erythromycin (54.37%), ciprofloxacin (44.17%) and ampicillin (17.48%)

In another study conducted by Rajput et al., 2019, isolates of MRSA and MSSA were 100% sensitive to Linezolid and Vancomycin. Similarly, isolates of MSSA were 42.38% sensitive to erythromycin followed by 14.28% cotrimoxazole and 19.04% amoxyclavulanic acid whereas MRSA isolates showed 38.1% sensitivity rate to amikacin and 4.76% to amoxyclavulanic acid. **(TABLE NO III)**

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The present study demonstrated that abscesses was most prevalent in MRSA isolates which accounted for 26.47% followed by 16.66% in cellulitis, 5.8% in osteomyelitis, 3.92% in perforation, 2.94% in necrotizing fasciitis, 1.96% in diabetic foot. According to the study conducted by Frazee et al., 2005, the predominance of MRSA in cellulitis was 16.66%, 46.67% in abscess. According to the study carried out by Miller et al., 2015 1.6% in osteomyelitis, 1.16% in necrotizing fasciitis. In a study done by Nirmala & Sengodan, 2017 the case of MRSA in diabetic foot infection in patients was high. **(TABLE NO. IV)**

The present study showed that predominance of MRSA isolates was 37.5% in postoperative wounds. Out of 8 patients, 75% were males and 25% were females. According to study carried out by Hospital, 2015, 68.8% were male and 31.11% were female patients. This study also demonstrates the rate of type of postoperative wounds with maximum case of (50%) inguinal hernia followed by related rate (12.5%) of necrotizing fasciitis, iliac abscess, laryngectomy and proximal humerus. In a study done by Thombare & Joshi, 2019 showed case of 27.1 % inguinal hernia whereas Mawalla et al., 2011 reported 5.6% inguinal hernia. The reason might be due to pre-morbid sickness, longer duration of the operation, deficient pre-operative preparation. Also the high incidence of inguinal hernia was high in male might be due to movement of testicle down the inguinal canal. **(TABLE NO V)**

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