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Multidrug resistant Staphylococcus aureus; its incidence and antibiotic sensitivity pattern.

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ABSTRACT… Objective: To determine incidence of MRSA in our community and its antibiotic susceptibility pattern. **Study Design:** Cross Sectional study. **Setting:** Department of Pathology, Sahara Medical College Narowal. **Period:** January 2020 to June 2020. **Material & Methods:** Bacterial isolates were taken from the samples of blood, pus and other body fluids sent to the hospital laboratory to determine culture and sensitivity pattern. Those samples positive for staphylococcus aureus were included in the study for further examination. MRSA were detected using conventional technique (catalase, coagulase and DNase methods) and confirmed by phenotypic and molecular characterization techniques (cefoxitin and oxacillin sensitivity, mec-A gene and 16S rRNA genes sequencing methods). Their antibiotic sensitivity was determined using standard Kirby Bauer disc diffusion technique. Chi square test was applied on collected data using SPSS-24. **Results:** Total 158 bacterial isolates containing staphylococcus aureus were included in the study. MRSA (multi drug resistant staphylococcus aureus) was detected in 33.5% samples and MSSA (multi drug sensitive staphylococcus aureus) was detected in 66.5% samples. Frequency of MSSA was more than MRSA. Male gender was more affected (60%) than female (40%). All bacterial isolates containing staphylococcus aureus were resistant to oxacillin and cefoxitin. MRSA isolates were sensitive to vancomycin, linezolid, teicoplanin, fosfomycin and fusidic acid, rifampicin, clindamycin, minocyclin and chloramphenicol, while resistant to penicillin, azithromycin, ciprofloxacin, erythromycin and co-trimoxazole. **Conclusion:** MRSA is detected by conventional technique followed by phenotypic and molecular characterization methods for confirmation. Methicillin sensitive staphylococcus aureus was detected more commonly than methicillin resistant staphylococcus aureus in our study sample. Proper culture and antibiotic sensitivity pattern is necessary to deal with MRSA.

Key words: Antibiotic Sensitivity Pattern, Kirby Bauer Method, Multidrug Resistant Staphylococcus Aureus, Mec-A Gene, Phenotyping.

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INTRODUCTION

Staphylococcus aureus infection is very common in developing and under developed countries and due to treatment without proper antibiotics it has become resistant to many antibiotics hence difficult to deal with. Initially penicillin was used widely for its treatment that is no longer effective now as its resistance has been developed in staphylococcus aureus. Infections due to MRSA is a huge burden on health system due to its increased morbidity and mortality rate.[1] MRSA infections of skin and soft tissue are much common in young adults. It has high mortality rate as its resistance is being reported against quinolones, trimethoprim-sulfamethoxazole, clindamycin, tetracycline and macrolides.[2] Options of antimicr obial drugs is being narrowed against staphylococcus aureus due to increasing antibiotic resistance. Vancomycin is a drug of choice for such infections but due to its toxicity and compromised penetration in lungs, bones and CNS that's why it is drug of last choice. Appearance of vancomycin resistant species of S. aureus worldwide is a serious issue.[3-4] Staphylococcus aureus has two major classes one is multidrug resistance staphylococcus aureus (MRSA) and methicillin sensitive staphylococcus aureus. Frequency of MSSA is more than MRSA in clinical biopsy specimens.

MRSA is usually found in[5] Staphylococcus aureus is the most virulent species causing community acquired and nosocomial infections both. This is the most common bacteria recovered from skin, soft tissue and blood infections as well as common cause of surgical site infection and pneumonia. Susceptibility of staphylococcus aureus to beta lactam antibiotics has decreased worldwide hence giving rise to MRSA species.[6,7] MRSA was initially found in 1960, causing fatal infections such as necrotizing fasciitis, osteomyelitis, endocarditis, severe pneumonia, toxic shoch syndrome and sepsis etc.[8] Vancomycin has been used widely against MRSA worldwide, but now resistance is increasingly being reported against vancomycin by MRSA so increasing crises.[9] About 28% of healthy individuals have their anterior nares colonized by staphylococcus aureus, while in Pakistan 13.6% sanitary workers, 2.1% administrative workers and 5.3% health workers in Iran contain MRSA in their nasal carriage.[9,10] With the passage of time MRSA strains are increasing creating serious health issues by increasing treatment cost, prolong hospital stay, increasing morbidity and mortality rate especially among those having co-morbidities.[11] Burden of this infection is more in developing and underdeveloped countries as compared to developed countries where it has low prevalence.[12]

There is insufficient data regarding MRSA prevalence and antibiotic susceptibility pattern of MRSA among Pakistani people, so this study was conducted so that it may help us in estimation risk of MRSA infections and selection of proper antibiotic for treatment. This may reduce huge disease burden on our health system, decreasing morbidity and mortality rate caused by this MRSA infections.

MATERIAL & METHODS

It is a cross sectional study conducted at Pathology Department of Sahara Medical College Narowal. Study was completed in six months duration from January to June 2020. Convenient sampling technique was used. Bacterial isolates were taken from the samples of body fluids, wound or pus swabs requested for bacterial culture and sensitivity pattern. These Isolates were subjected to bacterial culture using CLSI 2014 criteria. Those showing staphylococcus aureus bacteria were included in the study and subjected to further biochemical profiling by Gram staining, DNase, Coagulase and Catalase using mannitol salt agar as selective media. Isolated bacterial strains were stored at -80 degree centigrade in 20% glycerol. After confirmation of staphylococcus aureus they were analyzed for MRSA and MSSA by phenotypic methods by which bacterial susceptibility was tested against cefoxitin and oxacillin on Muller Hilton agar (media). Antibiotic sensitivity pattern was determined by Kirby Bauerdisc diffusion method using Muller Hinton agar plates against penicillin, amoxicillin, cefoxitin, cefaclor, ceftriaxone, cephalexin, vancomycin, azithromycin, linezolid, ciprofloxacin, fosfomycin, clindamycin, teicoplanin, fusidic acid, co-trimoxazole, chloramphenicol and oxacillin. Further confirmation of MRSA was done by molecular method using specific sequencing of mec-A gene. Consent was taken from ethical review committee of the institution for conducting study (725-ERC-SMC). All data was analyzed in SPSS-24. Frequencies and percentages were calculated for qualitative variables and means standard deviation was calculated for quantitative variables. Chi square test was applied on the data.

RESULTS

Staphylococcus aureus was detected in 158 isolates. Age range of patients was 5-75 years with mean age 34.67±6.4 years. Methicillin resistant staphylococcus aureus was found in 53(33.4%) samples and methicillin sensitive staphylococcus aureus was found in 105(66.6%) samples. MRSA was detected in 28(52.8%) male and 25(47.2%) female patients. Bacterial isolates positive for MRSA included 18(34%) blood samples, 25(47.2%) pus samples and 10(18.8%) samples taken from other body fluids Table-I.

MSSA was detected in 56(53.3%) male and 49(46.7%) female cases and bacterial isolates were taken from pus in 55(52.4%) samples, from blood in 29(27.6%) samples ad from other body fluids in 22(20.9%) samples Table-II.

Age (years)	Gender		Biopsy Specimens			P-Value
	Male	Female	Pus	Blood	Other Body Fluids	
<20	4 (7.5%)	3 (5.6%)	3 (5.6%)	2 (3.8%)	2 (3.8%)	<0.01
21-40	6 (11.3%)	4 (7.5%)	5 (5.6%)	4 (7.5%)	1 (1.9%)	
41-60	12 (22.6%)	9 (17%)	10 (18.8%)	8 (15.1%)	3 (5.6%)	
>60	6 (11.3%)	9 (17%)	7 (13.2%)	4 (7.5%)	4 (7.5%)	
Total	28 (52.8%)	25 (47.2%)	25 (47.2%)	18 (34%)	10 (18.8%)	

Table-I. Frequency of bacterial isolates containing MRSA in different age groups and biopsy samples. (n=53)

Age (years)	Gender		Biopsy Specimens			P-Value
	Male	Female	Pus	Blood	Other Body Fluids	
<20	7 (6.7%)	4 (3.8%)	6 (5.7%)	3 (2.8%)	2 (2.8%)	<0.01
21-40	16 (15.2%)	12 (11.4%)	15 (14.3%)	8 (7.6%)	5 (4.7%)	
41-60	13 (12.3%)	15 (14.3%)	14 (13.3%)	8 (7.6%)	6 (5.7%)	
>60	20 (19%)	18 (17.1%)	20 (19%)	10 (9.5%)	8 (7.6%)	
Total	56 (53.3%)	49 (46.7%)	55 (52.4%)	29 (27.6%)	21 (20%)	

Table-II. Frequency of bacterial isolates containing MSSA in different age groups and biopsy samples. (n=105)

All 53 bacterial isolates positive for MRSA were sensitive to Vancomycin while 52(98.1%) were sensitive to linezolid followed by 45(84.9%) rifampicin, 41(77.3%) clindamycin and 43(81.1%) samples responded to chloramphenicol, maximum resistance was showed to penicillin (100%) followed by azithromycin (92.4%), Ciprofloxacin (90.5%) erythromycin (88.6%), tetracyclin (66%) and Fusidic acid (41.5%) Figure-2.

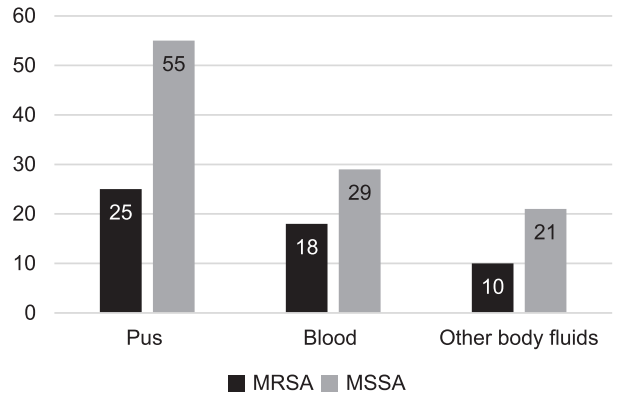

Figure-1. Frequency of MRSA and MSSA in various biopsy samples in study group. (n=158)

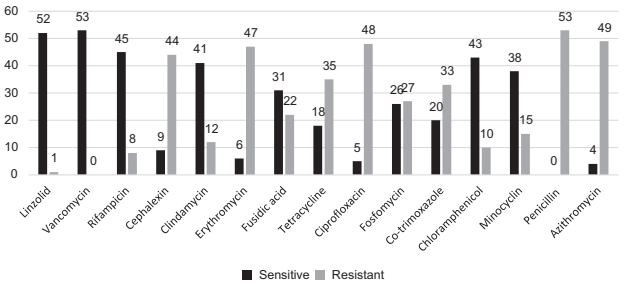

Figure-2. Antibiotic susceptibility pattern among MRSA in study group. (n=53)

DISCUSSION

MRSA spread has increased all over the world in last 20 years. Its prevalence is very high in different countries like in Latin American countries it is >80%, and in Australia its prevalence has increased from 12% to 19%.[13,14] Its prevalence is also increasing in Asian countries such as in India situation is more critical as its infection rate with MRSA is 41±80%.[15] Its mean prevalence is decreasing in many European countries like Canada and America, but still has high prevalence in other countries of world with 15-45% prevalence rate.[15] Due to misuse of antibiotics without

determining culture and sensitivity pattern MRSA prevalence in Pakistan has been determined as 34% according to recent study.[16] This is similar to our results reporting 33.4% prevalence of MRSA. Few previous studies have reported prevalence from 42% to 51%.[17] In our study MRSA was found in 33.4% and MSSA in 66.6%. Majority of patients infected with MRSA and MSSA were males. This is similar to a study conducted in India in which 70% males were infected.[18]

In our study MRSA was frequent in pus taken from wound samples (47.2%). A study conducted in Hyderabad, Pakistan reported 75% samples positive for MRSA.[19] We should improve detection methods for pathogens to avoid misuse of antibiotics and to decrease antibiotic resistance. These methods should be as much improved so that we detect pathogens in early infection. Phenotypic method applied in this study used oxacillin and cefoxitin disc susceptibility and results calculated were comparable to a study conducted in India by Kumar et al.[20] MRSA isolated by these methods are confirmed for having mecA gene by identifying specific DNA sequence.[21] Previously a similar study conducted in Lahore, Pakistan reported complete resistance of MRSA against penicillin and cefoxitin, while high resistance to macrolides (94.4%) and quinolones (91.6%), while high susceptibility for vancomycin and linezolid. These results are similar to our results in which high susceptibility was found against vancomycin (100%) and linezolid (98%) followed by clindamycin (81%) and rifampicin (77.3%).[4] While another study conducted in Saudi Arabia showed slight different results reporting 60% resistance against penicillin while high susceptibility for clindamycin (85%).[22]

CONCLUSION

In our hospital setups conventional and phenotypic molecular techniques are much useful for the detection of MRSA. Antibiotic susceptibility pattern should be used widely before starting any antibiotic treatment to avoid development of antibiotic resistance. We found MRSA high resistance against penicillin and azithromycin while high sensitivity to vancomycin and linezolid.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

No.	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Saeeda Nabat ul Hassan	Topic selection and data collection, Abstract and recording.	
2	Khushbu Farva	Data analysis, Data collection, Data analysis.	
3	Ghulam Asghar Bhutta	Data analysis, Data collection, Found additional literature for information, Data composing.