ORIGINAL ARTICLE

Prevalence and Susceptibility Pattern of Methicillin Resistant Staphylococcus aureus (MRSA)

Abdul Bari Khan, Uzma Mussarat

ABSTRACT

Objective: To determine the prevalence and susceptibility pattern of MRSA isolated at a single tertiary care hospital at Rawalpindi and to compare MRSA susceptibility pattern with MSSA (Methicillin sensitive staphylococcus aureus).

Study Design: Descriptive cross sectional data based study.

Place and Duration of Study: The study was conducted at department of microbiology, Pakistan Railways Hospital Rawalpindi, from January 2012 to March 2014.

Materials and Methods: Culture reports data were retrospectively collected from microbiology laboratory. The antibiotic susceptibility patterns of all staphylococcal strains were determined by modified Kirby Bauer antibiotic sensitivity method. The data was analyzed on the basis of antimicrobial susceptibility pattern, location of the patient (OPD, ward patients) and specimen type (wound swab, pus, HVS & effusions).

Results: A total of 167 isolates were used in the study. Among these isolates 55 (33%) were MRSA and 112 (67%) were MSSA. The majority of S.aureus isolates were obtained from patients with skin and soft tissue infections. All (100%) strains of MRSA isolated during study period were found to be sensitive to Vancomycin, and linezolid and 95% to Teicoplanin, as well as they showed higher susceptibility against chloramphenicol (88%),Fusidic acid (70%) and Rifampin (48%)while MSSA showed higher susceptibility to Gentamicin (92%),Erythromycin (86%) and Ciprofloxacin (71%) as compared to MRSA.

Conclusion: This study showed a high prevalence of MRSA in this tertiary care hospital of Rawalpindi. Present study conclusively shows that Vancomycin, Linezolid and Teicoplanin remain the first choice of treatment for MRSA infections. Still alternative antibiotics like chloramphenicol, Fusidic acid, and Rifampin are available to maintain and reserve the efficacy of Vancomycin, Teicoplanin and Linezolid in treating life threatening illnesses.

Keywords: *Antimicrobial Susceptibility, MRSA, MSSA, Prevalence, Staphylococcus aureus.*

Introduction

Staphylococcus aureus remains a compelling human pathogen as it is one of the most significant pathogen known for nosocomial as well as community acquired infections.[1,2] MRSA infections have been associated with increase in morbidity and mortality of patients in hospitals. Knowledge of susceptibility pattern of the antimicrobials commonly recommended for such patients has gained significance for appropriate management of patients with staphylococcal infections in general and post-operative surgical site infections in particular.

Methicillin resistance in S.aureus was reported in October 1960. Resistance to multiple antibiotics among the staphylococci isolates in hospitals has been recognized as one of the major challenges in hospital infection control.[3] Staphylococcus aureus is a major pathogen that causes a wide range of diseases, including wound infections, carbuncles and boils, nosocomial pneumonia, endocarditis, osteomyelitis, toxic-shock syndrome, food poisoning and septicemia.[4]

During the past 15 years, the appearance and world-wide spread of many MRSA clones have caused major therapeutic problems in different hospitals all over the world leading to drainage of considerable resources to control their spread. MRSA now accounts for more than 60% of S.aureus isolates in United States hospital ICUs.[5]

It is considered that over use and misuse of antibiotics has accelerated the progression of MRSA that has led to the emergence of strains which have systematically acquired multiple resistance genes.[6] In the early 1940s almost all S.aureus strains were susceptible to penicillin. In 1944 the first report of penicillin-resistant S.aureus appeared, and today virtually all strains of S.aureus are resistant to natural penicillins, aminopenicillins and antipseudomonal penicillins. Keeping all this in view, this study was aimed to determine the prevalence and sensitivity

Department of Pathology
Islamic International Medical College
Riphah International University, Islamabad

Correspondence:
Prof. Col (Retd) Dr. Abdul Bari khan
Professor of Pathology (Microbiology)
Islamic International Medical College
Riphah International University, Islamabad
E-mail: abdul.barikhan@riphah.edu.pk

Received: June 02, 2015; Accepted: August 19, 2015

pattern of staphylococcal isolates both MRSA and MSSA at Pakistan Railways Hospital, Rawalpindi. Moreover the efficacy of low priced antimicrobials has been assessed to prevent drug resistance against most commonly used antimicrobials against S.aureus infections. It is expected to help the clinicians to understand emerging trends of drug resistance among clinical isolates of S.aureus and provide a platform to initiate epidemiological studies for staphylococcal infections.

Materials and Methods

A descriptive cross sectional data based study was carried out from January 2012 to March 2014. No statistical test applied for data analysis. Clinical specimens received in microbiology laboratory of Pakistan Railways Hospital were processed and all S.aureus isolates were included in the study. The strategy for specimen collection included patient's location in hospitals (outdoor patients/ hospitalized patients), source (wound swab/pus, HVS or urine) of the isolate and the antibiotic susceptibility profiles. One hundred and sixty seven (167) isolates of S.aureus were obtained from samples received for culture and sensitivity from different departments of the hospital during the study period. The inclusion criterion of study data was to take sample from a patient once, no duplicate result from the same patient was considered.

In Microbiology Laboratory, the samples were cultured on Blood agar, MacConkey agar and Mannitol salt agar for 24-48 hours. The antibiotic susceptibility testing was performed by the Kirby Bauer's disc diffusion technique using Clinical and Laboratory Standards Institute (CLSI) recommendations. The characterization of MRSA was done by using Cefoxitin (30 µg) disc and following the interpretation criteria of Clinical and Laboratory Standards Institute (CLSI).[7] Antimicrobial sensitivity testing discs of Oxoid diagnostics were used by the participating laboratory of Pakistan Railways hospital that include Erythromycin (15 µg), Cefoxitin (30 µg), Minocycline (30µg), Ampicillin (10 µg), Chloramphenicol (30 µg), Linezolid (30 µg), Vancomycin (30 µg), Teicoplanin (30 µg), Fusidic acid (5 µg) and Rifampin (5 µg) to assess the sensitivity pattern of these isolates.

The other antibiotics tested include Gentamicin (10 µg), Co-trimoxazole i.e combination of Sulphamethoxazole and Trimethoprim (300 µg), Ciprofloxacin (5 µg), and Cephradin (30 µg). Inoculum was prepared by making a direct saline suspension of isolated colonies selected from an 18 to 24 hours old blood agar plate. Turbidity of the suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland standard and six to eight discs were applied on a 100mmMueller Hinton agar plate as per CLSI guidelines. S.aureus ATCC 25923 was used as the quality control strain for disc diffusion.

Results

Out of the total 167 staphylococcus aureus, 55 (33%) were found to be MRSA and 112 (67%) were MSSA. Among these 55 MRSA isolates, the source from OPD patients were 48% in 2012, 20% in 2013 and 23% in 2014, whereas from indoor patients the values were 52%, 80% and 77% in 2012, 2013 and 2014 respectively. The distribution of these MRSA isolates among outdoor and indoor patients is shown in table I.

Table I: Year wise Prevalence rate of MRSA in OPD Patients and Indoor Patients

	2012	2013	2014 (Jan-March)	Grand Total
Category of patients	No. of MRSA isolates n=27	No. of MRSA isolates n= 15	No. of MRSA isolates n=13	No. of MRSA isolates n=55
OPD patients	13 (48%)	3 (20%)	3 (23%)	19(35%)
Indoor patients	14 (52%)	12 (80%)	10 (77%)	36(65%)

It was found that 45(81%) of these isolates were from pus swabs/pus, followed by HVS 6 (11%) and from catheter tip/urine samples that were 4(8%). The prevalence rate of MRSA in the study period on yearly basis &in different categories of samples is shown in table II.

The antimicrobial susceptibility & resistance pattern of MRSA & MSSA isolated during January 2012 to March 2014 is shown in Table III and IV. Out of the total of MRSA strains isolated during study period 81% were found to be resistant to Co-trimoxazole (combination of Sulphamethoxazole & Trimethoprim), 62% to Gentamicin, 81% to Minocycline, 54% to Erythromycin and 52 % to Rifampin. However, all (100%) MRSA strains were

Table II: Year wise distribution of MRSA from different sources

	2012	2013	2014 (Jan-March)	Grand Total
Specimen	No. of MRSA isolates n=27	No. of MRSA isolates n=15	No. of MRSA isolates n =13	No. of MRSA isolates n=55
Wound/ pus	22 (82%)	13 (87%)	10 (77%)	45 (81%)
Urine	3 (11%)	-	1 (8%)	4 (8%)
HVS	2 (7%)	2 (13%)	2 (15%)	6 (11%)

found sensitive to Vancomycin, and Linezolid, 95% to Teicoplanin and 88% to Chloramphenicol.

Antibiotics like Tetracycline (88% resistance) and

Table III: susceptibility pattern of MRSA against different antibiotics

	2012	2013	2014 (Jan-March)	Grand total
Antibiotics	**Sensitive (%)**	**Sensitive (%)**	**Sensitive (%)**	**Sensitive (%)**
Vancomycin	100	100	100	100
Linezolid	100	100	100	100
Teicoplanin	85	100	100	95
Chloramphenicol	93	100	71	88
Fusidic Acid	67	57	85	70
Rifampin	60	50	33	48
Erythromycin	58	43	36	46
Gentamycin	38	25	50	38
Cotrimoxazole	13	44	0	19
Minocycline	38	20	0	19

Table IV: Comparison of Susceptibility pattern of all MRSA& MSSA in study period (Jan 2012 – March 2014)

Antibiotics	MRSA Sensitive (%) n=55	MRSA Resistant (%) n=55	MSSA Sensitive (%) n=112	MSSA Resistant (%) n=112
Linezolid	100	0%	N/A*	-
Vancomycin	100	0%	100%	0%
Teicoplanin	95	5%	N/A*	-
Chloramphenicol	88	12%	N/A*	-
Fusidic Acid	70	30%	N/A*	-
Rifampin	48	52%	N/A*	-
Erythromycin	46	54%	86%	14%
Gentamycin	38	62%	92%	8%
Ciprofloxacin	22%	78%	71%	29%
Minocycline	19	81%	N/A*	-
Cotrimoxazole	19	81%	31%	69%
Cephradin	N/A*	-	100%	0%
Ampicillin	0%	100	28%	72%

*N/A: Not applicable

Ampicillin (72% resistance) were found to be ineffective against MSSA too. Rest of the antibiotic showed less than 30% resistance towards the isolated MSSA.

Discussion

Increasing emergence of MRSA is a global problem and its prevalence is ever increasing with time. Finding of a prevalence rate of MRSA amounting to 33% in the present study is in close proximity to findings of 31.9% of MRSA in an Australian study involving 32 laboratories from all states and territories of Australia.[8] While a study done in India also revealed prevalence of MRSA varying between20- 25 per cent in western part of India[9] to 50 per cent in South India[10] with prevalence rate as high as 31.1% in clinical samples. Moreover the present data is also close to a study conducted in Nepal where Pandey et.al found at Kathmandu Medical College, Teaching Hospital that 29% of S. aureus isolates were resistant to methicillin.[11]

In the present study another significant observation was the increased emergence of MRSA isolates during first three months of 2014. This increase in MRSA prevalence could be attributed to many explanations like infection control measures, antibiotic prophylaxis and treatments used in each ward/hospital and, not less importantly, the clonal and often epidemic nature of these microorganisms.[12] The limitation of our study is that details of patients presenting in OPD and having MRSA infection are not known whether they were referred from other hospitals/ clinics or they got MRSA infection due to selective pressure of post-operative antibiotics, when they were discharged from our hospital. In such a situation one cannot comment that these patients were infected with MRSA at home or during their stay at hospital.

This study also showed that all MRSA isolates were significantly less sensitive against routinely used anti staphylococcal antibiotics as compared to MSSA isolates. However, significant difference was observed in case of Gentamycin, Erythromycin, and Ciprofloxacin. This antimicrobial susceptibility pattern of MRSA and MSSA isolates against antimicrobial agents has been summarized in Table IV. More than 50% of MRSA isolates were resistant to Gentamycin, Rifampin, Minocycline, Co-trimoxazole (Sulphamethoxazole + trimethoprim), Ciprofloxacin and erythromycin. Least amount of resistance was observed in Vancomycin (0%), Linezolid (0%) and Teicoplanin (5%) and last but not the least Chloramphenicol (12%) & Fusidic acid (30%). β

lactam antibiotics like penicillin (100% resistance) and co-trimoxazole were found to be ineffective against MSSA too. However Erythromycin and Gentamycin showed less than 20% resistance towards the isolated MSSA. This sensitivity pattern exposes the options of using antibiotics like Teicoplanin, Chloramphenicol, Fusidic acid and to some extent Rifampin as well for treating MRSA cases. This susceptibility pattern of drugs matches with a study conducted by Faiza et al in Agha Khan University which showed overall variable susceptibility pattern with high resistance rates to Cotrimoxazole (59%) and Rifampicin (50%) were observed. Resistance to Chloramphenicol (10%) and Fusidic acid (9%) was low.[13]

A US based study has revealed an increase during the ten years spanning between 1999-2008.They have also found a relatively increasing trend in community acquired cases as well.[14] While another nationwide study of US hospitals revealed about 369000 infections by MRSA in US hospitals in 2005.[15] However different countries may have different statistics at various hospitals in different regions. This depends upon many factors like characteristics, size of hospital and antibiotic use policy etc.

Mubbisher et al reported 44%MRSA isolates in two hospitals of District Kohat, Khyber Pakhtunkhwa province, Pakistan.[16] This higher percentage could be attributed to increased use of antimicrobials and higher rate of surgical procedures in that particular hospitals of Kohat. Mehta et.al,[17] in their study on control of MRSA in a tertiary care center in India, had reported an isolation rate of 33% from pus and wound swabs in 1998. Whereas, in 2009; it goes up to 40% as shown by Sangeeta Joshi et.al.[18] In the present study,81% of the total MRSAs were isolated from pus (as shown in Table II), this finding is again very close to a study conducted at Karachi by Fayyaz et.al who reported this figure to be 85.6% MRSA from pus in their study.[19]

Rifampicin is an oral antimicrobial agent with good tissue penetration. This agent could be used to treat MRSA infections in our setting but the problem is that Pakistan is a country where infections with Mycobacterium tuberculosis (TB) is common, so increased usage of Rifampicin is not advisable as a routine to treat MRSA infections because of potential development of resistance in TB. However, its use is justified in less complicated cases, where it can be used in combination of other antibiotics.

The side effect with Chloramphenicol treatment may occur such as bone marrow suppression or idiosyncratic aplastic anemia. This complication is manifested by high dose (4 g/day), prolonged therapy, and markedly elevated levels of Chloramphenicol in serum (20 mg/ml) and is reversible. Keeping in view the low cost and oral preparation of Chloramphenicol coupled with very high rate of in vitro efficacy makes this antimicrobial an ideal choice to treat wide variety of infections caused by MRSA. It is also imperious that since this compound has shown very promising results against MRSA isolates, the availability of this antibiotic must be ensured in the market for the benefit of patients.

This study has revealed a reasonable susceptibility of MRSA against Rifampin and Fusidic acid as well. Rifampin has excellent oral bioavailability and tissue penetration and activity in bio films. Rifampin has potent intrinsic anti staphylococcal activity and is not used alone due to rapid emergence of resistance. Clinical studies have suggested benefits of addition of Rifampin to Fluoroquinolone regimens for treatment of S. aureus and MRSA bone and joint infections, especially device-associated infections and chronic osteomyelitis.[20]

Vancomycin and Linezolid remains the first choice of treatment for MRSA infection worldwide. Still there is possibility of developing toxicities of Linezolid after more than 2 weeks that include anemia and thrombocytopenia, thus hematologic parameters must be monitored. Other serious toxicities reported with prolonged Linezolid therapy include lactic acidosis syndromes, optic neuritis, and peripheral neuropathy.[21] In one study, 80% of 66 patients with chronic S.aureus osteomyelitis were cured after prolonged courses of Linezolid (mean 13 weeks), but treatment-limiting toxicities occurred in one third of patients.[22] Thus, Linezolid is not an ideal agent for very prolonged treatment courses or chronic suppressive therapy.

To preserve its value, use of Vancomycin should be limited to those cases where it is clearly needed. Our study proves the sensitivity of Linezolid, Teicoplanin, Chloramphenicol, Fusidic acid and Rifampin against MRSA that would be beneficial to control emerging resistance with Vancomycin.

Conclusion

In this study, Vancomycin, Linezolid and Teicoplanin are the antibiotics found to give almost uniform sensitivity that is 100%. Therefore, it is construed that these antibiotics remain the mainstay for treatment of MRSA infections. However, still there is choice for clinicians to select Chloramphenicol, Fusidic acid and Rifampicin to treat less serious MRSA infections as these drugs are effective not only in combination but also economical. The use of these antibiotics will preserve the efficacy of Vancomycin, Linezolid and Teicoplanin against serious infections with MRSA.

This study provides a guideline to epidemiologists to understand the nature of MRSA isolates in this tertiary care hospital. It will be helpful to make policies by infection control committee to revise their strategies to combat the emerging infections in their hospitals i.e. cost effectiveness of antibiotics. It is recommended that there is need of clinical studies involving use of Chloramphenicol under medical and laboratory supervision. In addition clinical trials of using combinations of Rifampicin and Fusidic acid with other anti MRSA antibiotics are also recommended.

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