International Research Journal of Pharmaceutical and Applied Sciences (IRJPAS)
Available online at **www.irjpas.com**
Int. Res J Pharm. App Sci., 2013; 3(6):13-16

Research Article

PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS ISOLATED AT A TERTIARY CARE INSTITUTE IN NORTH WEST REGION OF RAJASTHAN

Dr. Yogesh Kumar Gupta[1*], Dr. Garima gupta[2], Dr. S.P. Garg[3], Dr. Prem Singh nirwan[4]

[1]Consultant Microbiologist and infection control incharge, S.K. Soni Hospital, Jaipur,Rajasthan, India. [2]Dentist, Damodar dental & research hospital, Gangapur city – 322201, Rajasthan

[3]Professor & Head, Department of Microbiology, Saraswathi Insititue of Medical Sciences National Highway -24, Anawarpur, Pilakhuwa, Hapur, Distt.- Ghaziabad (U.P.) Pincode- 245304

[4]Professor & Head, Department of Microbiology, NIMS Medical College, NIMS University, Shobha Nagar, Jaipur-Delhi Highway (NH-11C),Jaipur – 303121,Rajasthan, India

Corresponding Author: Dr. Yogesh Kumar Gupta; Email: dr_yogeshsms2007@yahoo.com

Abstract: The emergence of *Methicillin-resistant Staphylococcus aureus* (MRSA) has posed a serious therapeutic challenge. We report the prevalence and antibiotic susceptibility pattern of MRSA in the hospitals attached to SPMC, Bikaner . A total of 300 *staphylococcus aureus* strains were been taken for study from various clinical specimens like pus, blood, sputum, throat swab, ear swab, high vaginal swab, CSF, urine, pleural fluid, semen, bile, corneal swab, etc. Routine antibiotic susceptibility testing was performed and interpreted as per standard guidelines. Methicillin resistance was detected using oxacillin disc and oxacillin screen agar method. A total of 107 (35.67%) strains were found to be Methicillin resistant. However, no strain was resistant to vancomycin. Regular surveillance of hospital-associated infection and monitoring of antibiotic sensitivity pattern is required to reduce MRSA prevalence

Key words: MRSA, mec A gene, risk factor

Introduction

Methicillin resistant Staphylococcus aureus *(MRSA)* strains reported just one year after the launch of methicillin in 1961. *MRSA* strains emerged in the last few decades as one of the most important nosocomial pathogens all over the world[1, 2]. Oxacillin and methicillin are semi synthetic penicillin's that are stable to staphylococcal β-lactamase. These drugs were developed specially for the treatment of infections caused by β-lactamase producing *Staphylococcus aureus.* However, resistance to penicillinase resistant penicillin developed soon by acquisition of *mec* A gene which codes for a penicillin binding protein *PBP2a.* This protein participates in cell wall synthesis despite the presence of methicillin type drugs[7]. The mechanism of resistance to methicillin was uncovered in 1981 with the identification of reduced affinity Penicillin Binding Proteins in *MRSA.* The altered protein, Penicillin Binding Protein 2a (PBP2a) retain effective transpeptidase activity while having reduced affinity for penicillin and other available β-lactam antibiotics[3]. The organism is often sub-categorized as Community-Associated MRSA (CA-*MRSA*) or Health Care-Associated MRSA (HA-*MRSA*) although this distinction is complex[4].

The predisposing factors of *MRSA* emergence are[5]

- Prolonged hospital stay
- Carriage of *MRSA* in anterior nares
- Burn and trauma
- Chronic infections
- Patient on dialysis
- Indiscriminate use of antibiotics
- Lack of awareness
- Receipt of antibiotics before coming to the hospital
- Intravascular catheterization
- Hospitalization in intensive care unit.

Serious endemic and epidemic *MRSA* infections occur globally, as infected and colonized patient mediate the dissemination of these isolates and hospital staff assist further transmission[2].

The proportion of *MRSA* has increase worldwide since last two decade. *MRSA* is now responsible for around 30% or more of all serious *Staphylococcus aureus* infections, although there is considerable variability in this figure worldwide[1]. Prevention of Staphylococcus aureus infections is an area of ongoing investigation. No single prevention approach is likely to work alone

Careful hand washing is important for prevention of the spread of many infections, including Staphylococcus aureus. Persons with active Staphylococcus aureus skin infections should take care to cover the area if it is draining, to wash their hands after doing dressing changes, and to properly dispose-off soiled dressings. In the health care setting, use of standard precautions for infection control practices (such as wearing gloves before and after contact with infectious

body tissues and proper hand washing) can reduce the spread of *MRSA*. *MRSA* has been found to live in steam baths; both in the inside seating area and outside bench areas. Cleaning the seating areas with a dilute bleach solution after use appears to be most effective way to disinfect steam baths. Also, a barrier to prevent direct contact with the seat (such as cardboard) may be a way to prevent spread of *MRSA* in the steam bath. More research needs to be done to determine how effective these methods are for preventing illness. A protein-polysaccharide conjugate vaccine has been developed and has shown promise for preventing the most serious infections in one high risk group, dialysis patients. However this has not been tested in otherwise healthy persons. This vaccine has not been approved by the FDA and approval will require further evidence of effectiveness.

The recommended treatment for multi resistant *MRSA* is glycopeptides, particularly vancomycin, teicoplanin, linezolid, daptomycin and/or rifampicin[6]. Clindamycin may be an option for therapy of selected serious *MRSA* infections once sensitivity results are available and inducible clindamycin resistance has been excluded[7].

Material and Methods

The present study has been carried out in Bacteriology laboratory of Microbiology Department of Sardar Patel Medical College, Bikaner (Rajasthan) from January 2009 to March 2010.

A total of 300 *staphylococcus aureus* strains were taken for study from various clinical specimens like pus, blood, sputum, throat swab, ear swab, high vaginal swab, CSF, urine, pleural fluid, semen, bile, corneal swab, etc.

Isolates were plated on nutrient agar plate and blood agar plate and were incubated at 37^0C for 24 hour. The plates were examined and colonies of *staphylococcus aureus* were identified on the basis of colony morphology, gram staining, catalase test, coagulase test (tube & slide coagulase), mannitol fermentation. All the isolates were subjected to the antibiotic sensitivity by Kirby Bauer method. Mueller Hinton agar was used for sensitivity testing. Antibiotics tested were amikacin(30µg), amoxycillin/Clavulanic acid(20/10 µg), cefuroxime(30 µg), clindamycin(2 µg), co-Trimoxazole (Trimethoprim/ Sulphamethoxazole) 1.25/23.75 µg, tetracycline(30 µg), erythromycin(15 µg), ofloxacin(5 µg), gentamycin(10 µg), vancomycin(30 µg). Oxacillin disc (1µg) was put on a separate Mueller Hinton Agar (MHA) (Hi Media, Mumbai) plate supplemented with 4% NaCl. Zone diameters were measured following CLSI criteria.

Oxacillin screen agar test[8,9,10]

All methicillin resistant strains were also confirmed by oxacillin screen agar test. Mueller Hinton agar plate with 4% NaCl and 6µg/ml oxacillin are prepared. 0.5 Mc Farland suspension of isolate is made and streaked on the one quadrant of plate. The plate in incubated at 35^0C for 24 hour. Plates were observed carefully in transmitted light for any growth. Any growth after 24 hour is considered methicillin resistant[11].

Results

Out of 300 Staphylococcus aureus isolates, 107 strains (35.67%) were found to be MRSA and 193 strains (64.33%) were Non MRSA.

Out of 172 Staphylococcus aureus isolated from male patients, 61 (i.e. 35.47%) were found to be MRSA & 128 Staphylococcus aureus isolated from female patients, 46 (i.e. 35.94%) were found to be MRSA. The maximum numbers of MRSA 42 were isolated from pus & wound swab, whereas 18 MRSA were isolated from sputum/throat swab, 17 from urine, 15 from Blood and 15 from others. Isolation rate of MRSA from different clinical specimens is shown in Table 1.

The distribution of methicillin resistant Staphylococcus aureus was maximum in surgery 28.08% (30), followed by paediatrics 17.76% (19), medicine 14.2 % (15), gynecology 13.08% (14) and urology 10.28% (11). Distribution of methicillin resistant Staphylococcus aureus according to various units is shown in Table 2.

81.3 % i.e. 87 strains of methicillin sensitive Staphylococcus aureus were resistance to Erythromycin. Resistance to Cefuroxime Sodium was 60.75 % (i.e. 65 strain), to Amoxycillin/Clavulanic acid 47.66% (i.e. 51 strain), to Co-trimoxazole (Trimethoprim/ Sulphamethoxazole) 39.29 % (i.e. 42 strain), to Amikacin 31.78 % (i.e. 34 strain), to Clindamycin 29.9 % (i.e.32 strain), to Gentamycin 62.62% (i.e. 67 strain), to Tetracycline 13.08 % (i.e. 14 strain) and resistance to Ofloxacin was 6.54 % (i.e.7 strain). No one strain was found to be resistant to Vancomycin. Antibiotic resistance pattern of methicillin resistance Staphylococcus aureus is shown in Table 3.

Discussion

In the present study prevalence of methicillin resistant Staphylococcus aureus was 35.67%, which correlates with the observation of Srinivasan et al (2006)[12], Rajaduraipandi K et al (2006)[2] & Ambumani N et al (2009)[5], where methicillin resistant Staphylococcus aureus isolates were observed in 33.33%, 31.9% & 31% cases respectively. In our study no difference was observed in incidence on gender basis for MRSA isolates. Similar results are also observed in study of Rijal K R et al (2008)[13] and Mcveigh et al (2006)[14]. In the present study highest number of MRSA (39.25%) was isolated from pus & wound swab. Similar results were also obtained by Rajaduraipandi K et al[2] (33.6%) and Anbumani N et al[5] (32.77%).

In the present study the maximum number of MRSA strain were isolated from surgery units (28.04%), followed by paediatrics (17.76%), medicine (14.02%), gynecology (13.08%), urology (10.28%), orthopaedics (3.74%), ENT & burn (2.8% each) and neurosurgery, ICU, dental & TB units (1.87% each).

Present study shows that all the MRSA isolates were susceptible to vancomycin and all other studies show similar results.

Table No. 1: Isolation rate of MRSA from different clinical specimens

S. No.	Clinical specimens	MRSA Strain	Percentage of MRSA
1.	Throat swab/Sputum	18	16.82%
2.	Pus & wound swab	42	39.25%
3.	Blood	15	14.02%
4.	Urine	17	15.89%
5.	Pleural fluid	3	2.8%
6.	Vaginal swab	8	7.48%
7.	Ear swab	2	1.87%
8.	Bile	1	0.93%
9.	Umbilical stump	1	0.93%

Table 2: Distribution of methicillin resistant Staphylococcus aureus according to various units

S. No.	Wards	No. of MRSA (%)
1.	ICU	2 (1.87%)
2.	Neurosurgery	2 (1.87%)
3.	Medicine	15 (14.02%)
4.	Surgery	30 (28.04%)
5.	Paediatrics	19 (17.76%)
6.	Burns	3 (2.8%)
7.	Urology	11 (10.28%)
8.	Gynecology	14 (13.08%)
9.	Orthopedics	4 (3.74%)
10.	Dental	2 (1.87%)
11.	T.B.	2 (1.87%)
12.	ENT	3 (2.8%)
	Total	107 (100%)

Table No.3: Antibiotic resistance pattern of methicillin resistance Staphylococcus aureus (Total 107 isolates)

S.No	Antibiotics	Potency of disc	Number of resistant isolates	Percentage
1.	Amikacin	30 µg	34	31.78 %
2.	Amoxycillin/Clavulanic acid	20/10 µg	51	47.66%
3.	Cefuroxime Sodium	30 µg	65	60.75 %
4.	Clindamycin	2µg	32	29.9%
5.	Co-Trimoxazole (Trimethoprim/ Sulphamethoxazole)	1.25/23.75µg	42	39.29 %
6.	Tetracycline	30 µg	14	13.08 %
7.	Erythromycin	15µg	87	81.3 %
8.	Ofloxacin	5 µg	7	6.54 %
9.	Gentamycin	10 µg	67	62.62%
10.	Vancomycin	30 µg	Nil	0 %

Conclusion

Following are the relevant finding in the study:-

- Methicillin resistant among isolates of Staphylococcus aureus was 35.67%.
- The poly antimicrobial resistance was significantly higher in methicillin resistant Staphylococcus aureus than methicillin sensitive Staphylococcus aureus.

- Among MRSA vancomycin was most sensitive antibiotic followed by ofloxacin, tetracycline, clindamycin and amikacin.
- Among MRSA erythromycin was most resistant antibiotic followed by gentamycin and cefuroxime. High rate of resistant to these antibiotics could be because of poorly directed therapy and over use of antibiotics.
- The risk factor involve in acquisition of methicillin resistant were:- Long term antibiotic, prolong hospital stay, invasive medical devices, old age, poor nutritional status, recent surgery, burn and admission in ICU.
- MRSA emerge as a potent etiological agent in nosocomial infections and they posed a considerable threat for the treating physician in the management of patients with MRSA infection because these strains also exhibit multi drug resistance.
- Their spread in hospitalized patients can be through the environment or the health care workers who are positive nasal carrier of MRSA and who may disseminate the organism to patients and other staff by droplet spread or by direct spread. Even a healthy visitor from the community may also introduce MRSA in the hospital environment if he/she is carrier.
- Proper management of patients with infection by such strains is required so that their emergence as potent nosocomial agent is contained.
- Periodical epidemiological studies must be carried to identify the source of infection and measured must be instituted to cure or eradicate the source of infection.

References

1. Borriello SP. Topleys & Wilsons Microbiology & Microbial infections, 10th ed., **2005**. P.771-832
2. Rajaduraipandi K, Mani K R et al. Prevalence and antimicrobial susceptibility pattern of methicillin resistant Staphylococcus aureus: A multicentre study. *Indian J Med Microbial* **2006**; 24 (1): 34-38
3. Hartman Barry & Tomasz Alexander. Altered Penicillin-Binding Proteins in Methicillin-Resistant Strains of Staphylococcus aureus. Antimicrobial agents & chemotherapy, **1981**: 726-735
4. Methicillin resistant Staphylococcus Aureus. Wikipedia the free encyclopedia. June 1, 2010 Available at http://en.wikipedia.org/wiki/MRSA
5. Anbumani N, Wilson Aruni A, Kalyani J, mallika M. Prevalence of MRSA in a Tertiary referral Hospital in Chennai, South India. *Indian Journal for the Practicing Doctor*; 3(4)(2006-2008 – 2006-2009). Available at http://www.indmedica.com/journals.php?journalid=3&issueid=84&articleid=1140&action=article
6. Wootton M, Howe R. A., Hillman R., Walsh T. R., Bennett P. M. and A. P. A modified population analysis profile (PAP) method to detect hetero-resistance to vancomycin in Staphylococcus aureus in a UK hospital. *J Antimicrob Chemother* **2001**; 47: 399-403
7. Mallick S.K., Basak S., Bose S. Methicillin resistant Staphylococcus Aureus (MRSA)-a chellange to Medical Fraternity. *Indian Medical Gazette*, **2009**; 359-363
8. Clinical and Laboratory Standards Institute (CLSI). M7 – A4, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved standard- Fourth Edition.
9. Clinical and Laboratory Standards Institute (CLSI). M2 – A5: Performance Standards for Antimicrobial Disk Susceptibility Tests – Fifth edition; Approved standard
10. Clinical and Laboratory Standards Institute (CLSI). M100 – S16, Performance Standards for Antimicrobial Susceptibility Testing; Sixteenth International Supplement
11. Vidhani S, Mehndiratta PL, Mathur MD. Study of Methicillin Resistant S. aureus (MRSA) isolates from high risk patients. *Indian J Med Microbial* **2001**; 19 (2): 13-16
12. Srinivasan S, Sheela D, Shashikala, Mathew R, Bazroy J, Kanungo R. Risk factors and associated problems in the management of infections with methicillin resistant Staphylococcus aureus. *Indian J Med Microbial* **2006**; 24 (3): 182-185
13. Rijal K R, Pahari N, Shrestha B K, Nepal A K, Naudel B, Mahato P and Skalko-Basnet N; Prevelance of methicillin resistant Staphylococcus aureus in school children in Pokhara, *Nepal Med Coll J* **2008**;10(3):192-195
14. McVeigh A, FitzGerald S.F., Fenelon L.E. Prevalence of methicillin resistant Staphylococcus aureus infection among Inpatient colonize ,