JOURNAL Of Medical Science And Clinical Research

IGM Publication   An Official Publication Of IGM Publication

Prevalence and Antibiogram of Methicillin Resistant *Staphylococcus aureus* Isolates in a Tertiary Care Centre of Western Uttar Pradesh

Authors
Vijay Prakash Singh[1]*, Devesh Sharma[2], Ashok Kumar Dhanvijay[3]
[1,2]Assistant Professor, [3]Director and Professor, Department of Microbiology,
K.D. Medical College Hospital & Research Centre, Mathura, Uttar Pradesh, India -281406
*Corresponding Author
Dr Vijay Prakash Singh
Room No.-15, Staff Quarters, K.D. Medical College Hospital & Research Centre,
P.O-Akbarpur, District- Mathura, Uttar Pradesh, India -281406
Phone No: +91-9837039416, Email: *vpsingh82@yahoo.com*

Abstract
Methicillin resistant Staphylococcus aureus (MRSA) infections have emerged as one of the most common healthcare acquired infections in the present era. In this study, we report the prevalence and antibiotic susceptibility pattern of MRSA isolates from various clinical samples in a tertiary care hospital.
Various clinical specimens from both outpatients as well as admitted patients were cultured on the suitable culture media and Staphylococcus aureus was identified using standard tests like catalase test, slide and tube coagulase test. All Staphylococcus aureus isolates were tested for their antibiogram pattern by Kirby - Bauer disc diffusion method and MRSA were identified amongst them following CLSI guidelines. The D-test was performed on all Staphylococcus aureus isolates to identify erythromycin induced clindamycin resistance.
The prevalence of MRSA in our study was found to be 60.87%. Linezolid and vancomycin proved to be effective against 96.4% and 100% of MRSA isolates respectively.. Antibiotics with highest resistance against MRSA isolates were penicillin, quinolones (ciprofloxacin, levofloxacin) and ampicillin. Also, 32.14% of MRSA isolates and 22.22% of MSSA isolates showed erythromycin induced clindamycin resistance in this study. Regular surveillance of hospital associated infections along with strict infection control measures, monitoring antibiotic susceptibility pattern of all MRSA isolates and formulation of definite antibiotic policy should be implemented in the hospitals as control measures against MRSA infections.
Keywords: *Staphylococcus aureus, MRSA, cefoxitin, linezolid, vancomycin, Kirby-Bauer disc diffusion method*.

Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) infections are one of the most common healthcare acquired infections in the present era.

Since 1961, when they were firstly reported, MRSA strains have been progressively causing increased mortality, morbidity and health care costs[1]. They cause infections such as post operative wound

infections, catheter associated urinary tract infections, ventilator associated pneumonia, osteomyelitis and central line associated blood stream infections[2]. Though previously reported predominantly as hospital acquired, methicillin resistance is now being increasingly reported from the community. Earlier, physicians used to prescribe vancomycin and other glycopeptide antibiotics for the treatment of staphylococcal infections. But with the advent of VRSA (vancomycin resistant Staphylococcus aureus), it is prudent for the physicians to order for the proper culture and sensitivity test of the appropriate specimens for early detection and treatment of MRSA[3]. Early detection and antibiogram of MRSA isolates would help the physicians to formulate an empirical therapy for MRSA isolates in a particular geographical area. These studies can be used to minimize the irrational use of vancomycin instead of other available effective antibiotics.

Hence the present study is being undertaken to find the prevalence and antibiogram pattern of MRSA isolates in patients attending our centre.

Material and Method

The present study was a prospective study conducted for a period of 6 months from October 2017 to March 2018 on patients attending the out-patient departments (OPDs) and inpatient admissions to a tertiary care teaching hospital attached to a medical college in western Uttar Pradesh, India after getting the clearance from institutional ethical committee.

During the study period, various clinical samples like urine, pus/wound swabs, body fluids, sputum and blood were received and processed in the microbiology laboratory. The specimens were cultured on blood agar and MacConkey agar plates and then incubated aerobically at 37°C for 48 hours. After the required incubation period, suspected Staphylococcus colonies were gram stained to note their morphological characteristics under the microscope. Gram positive cocci were identified as Staphylococcus aureus with the help of standard tests like catalase, slide and tube coagulase[4].

Following identification of Staphylococcus aureus, antibiotic susceptibility testing was performed by Kirby–Bauer disc diffusion method for the following antibiotics:

Ampicillin (10μg), Amoxicillin + clavulanic acid (50/10μg), cefoxitin(30μg), linezolid(30μg), tetracycline (30μg), cotrimoxazole(25μg), cefuroxime (30μg), ciprofloxacin (5 μg), doxycycline (10μg), levofloxacin (5 μg), chloramphenicol (30 μg), clindamycin (2 μg), gentamicin (10 μg), erythromycin (15 μg), penicillin (10 units), and vancomycin (30 μg).

Test for methicillin resistance was performed by Kirby–Bauer disc diffusion method using cefoxitin (30μg) disc according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. A lawn culture of the test isolate was incubated on Mueller Hinton agar with 2% sodium chloride and cefoxitin disc (30 μg) at 37 degree C for 24 hours. A zone size ≤ 22 mm indicated resistance to cefoxitin and was reported as MRSA[5].

D test: The test was done on a Mueller–Hinton agar plate inoculated with a lawn culture of 0.5 McFarland bacterial suspension. The erythromycin (15 μgm) disc and clindamycin (2 μgm) disc was then placed at a distance of 15 mm (edge-to-edge). Following overnight incubation at 37 degree C , flattening of the zone in the area between the two discs giving a D-shaped zone around clindamycin disc indicated inducible clindamycin resistance.

Staphylococcus aureus ATCC 25923 was used as the control strain for antibiotic susceptibility testing. The data obtained was analysed using percentages, normal distribution and contingency tests.

Results

During a period of 6 months, a total of 1638 clinical samples were processed in the microbiology laboratory. Out of all the samples processed, 92 (5.62%) yielded Staphylococcus aureus. Among the 92 staphylococcal isolates, 56 (60.87%) were MRSA and 36 (39.13%) were methicillin sensitive Staphylococcus aureus (MSSA). Thus, prevalence of MRSA isolates in this study was found to be 60.87%.

Out of the various types of clinical samples, urine yielded maximum load (70 %) of MRSA followed by pus/wound swab, sputum, body fluids and finally blood samples. The MRSA distribution for each type of clinical sample is shown in Table-I.

Percentage antibiotic sensitivity patterns are demonstrated in Figure-I. Among MSSA isolates, both linezolid and vancomycin were found to be 100% sensitive. Other antibiotics with high sensitivity rate were doxycycline (88.8%), tetracycline (72.2%), cefuroxime (69.4%) and clindamycin (66.6%). Antibiotics with highest resistance rate against MSSA isolates were ampicillin and levofloxacin.

Vancomycin proved to be effective against 100% of MRSA isolates. Other antibiotics which had decent sensitivity against MRSA isolates were linezolid (96.4%), tetracycline (64.2%) and doxycycline (62.5%). Antibiotics with highest resistance against MRSA isolates were penicillin, quinolones (ciprofloxacin, levofloxacin) and ampicillin. Overall, MRSA isolates were found to have high antibiotic resistance as compared to MSSA isolates.

D-test for detecting erythromycin induced clindamycin resistance was found to be positive in 18 (32.14%) MRSA isolates and 8 (22.22%) MSSA isolates.

Table I: Distribution of MRSA from various clinical samples

Clinical sample	Total (n= 1638)	*S. aureus* (n=92)	MRSA (n=56)	Percentage of MRSA (%)
Urine	480	20	14	70
Pus/aspirate/swab	250	44	28	63.6
Sputum	424	10	6	60
Body fluids	148	4	2	50
Blood	336	14	6	42.8

Figure I: Percentage sensitivity pattern of MRSA and MSSA isolates
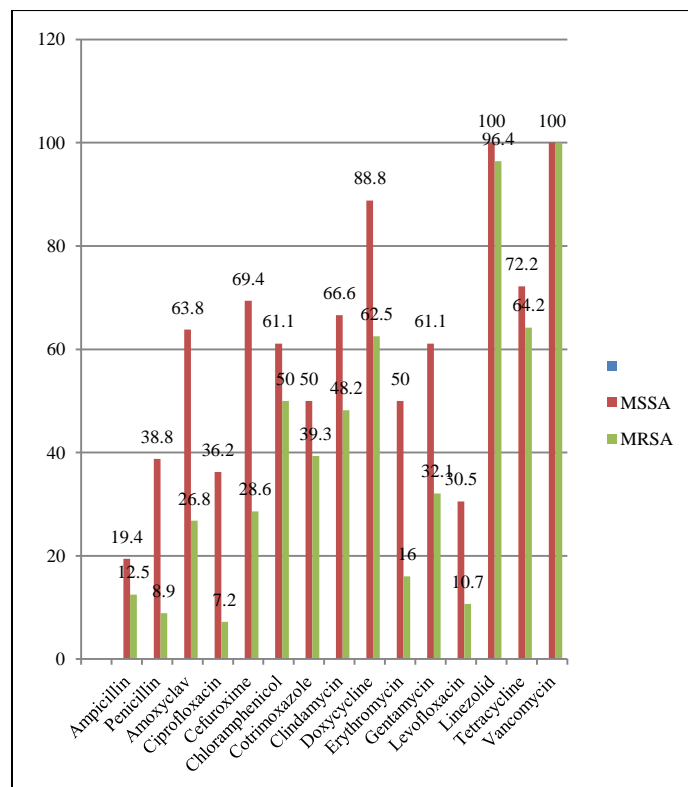
Discussion

The prevalence of MRSA in our six month long study was found to be 60.87%. Previously, various other studies have also reported such high prevalence from Gulbarga (56.7% MRSA)[6], Varanasi (59.3% MRSA)[7] and Patna (58% MRSA)[8]. High prevalence of MRSA in our study may be due to various factors. This hospital is located in a rural area of western Uttar Pradesh near the border areas of Haryana and Rajasthan. It caters mainly to the rural population of the above mentioned 3 states. In rural population of this geographical region, there is a lack of awareness and education regarding health and hygiene. Also, the practice of getting treatment from local quacks is quite prevalent in this region. Injudicious use of antibiotics by the local quacks might be a major contributing factor in the high prevalence of MRSA.

The highest isolation rate of MRSA isolates in our study was from urine (70%) followed by pus/wound swabs (63.6%) and sputum (60%). Majority of the urinary samples that yielded MRSA were found to be from inpatient wards & intensive care units (ICU). Hence they are hospital-acquired in nature.

Infections due to MRSA strains are difficult to treat as they exhibit multi-drug resistance. Due to the production of beta lactamases, they are usually resistant to penicillin and other beta-lactam antibiotics. In this study as well, 61.2% of MSSA and 91.1% of MRSA were found to be resistant to penicillin respectively. High resistance of MRSA to penicillin is in accordance with various other previous studies.[9,10,11]

In our study, resistance to quinolones (ciprofloxacin) was also found to be very high. The resistance rate for ciprofloxacin was 63.8% for MSSA and 92.8% for MRSA respectively. This correlates to the finding of various other previous studies which have also reported a high incidence of ciprofloxacin resistance.[12,13]

The sensitivity of MRSA isolates to linezolid and vancomycin was found to be 96.4% and 100% respectively and these drugs were found to be the most effective drugs in treatment of MRSA infections. Other previous studies have also reported high sensitivity of these drugs against MRSA isolates.[14,15] However, these are expensive as well as the high end drugs . Hence they cannot be used routinely for the treatment of MRSA infections as their increased use causes drug resistance to spread further.

However, appropriate antibiotic-susceptibility testing may guide the clinicians to choose other suitable antibiotics for MRSA isolates so that vancomycin may be spared for the treatment of only severe, last-resort cases. In this study tetracycline, doxycycline, chloramphenicol and clindamycin were found to be useful in treating MRSA with 64.2%, 62.5%, 50%, and 48.2% of MRSA isolates being sensitive to them, respectively.

Among MRSA isolates, 32.14% showed erythromycin induced clindamycin resistance while 22.22% of MSSA isolates showed erythromycin induced clindamycin resistance. Other studies have also reported high rate of erythromycin induced clindamycin resistance in MRSA isolates as compared to MSSA isolates in concordance with our study.[16,17,18]

Conclusion

This study has found an alarmingly high prevalence of MRSA which is a matter of concern for healthcare workers in management of infections. The possible predisposing factors for increased prevalence of MRSA in this region may be prolonged hospital stays, injudicious use of antibiotics and lack of awareness in public regarding antibiotic abuse and resistance.

Vancomycin and linezolid showed the highest susceptibility against MRSA isolates. Hence these drugs may be used as the drug of choice for treating multidrug resistant MRSA infections in life threatening conditions. However, empirical use of these antibiotics may lead to their resistance. Therefore, physicians should explore the treatment options of MRSA with alternate antibiotics instead of vancomycin and linezolid. Our study has revealed tetracycline, doxycycline, chloramphenicol and clindamycin to be such suitable alternatives in a majority of cases.

To conclude, various measures need to be taken for reducing the healthcare burden due to MRSA infections. Regular surveillance of hospital associated infections, strict infection control measures, monitoring antibiotic sensitivity pattern of all MRSA isolates and formulation of definite antibiotic policy should be implemented in the hospitals as control measures against MRSA infections.

Conflict of interest: None

References

1. Maple PAC, Hamilton- Miller JMT, Brumfitt W. Worldwide antibiotic resistance in methicillin resistant Staphylococcus aureus. Lancet 1989;1:537-540.
2. Boucher H, Miller LG, Razonable RR. Serious Infections Caused by Methicillin-Resistant *Staphylococcus aureus*, *Clinical Infectious Diseases*, Vol 51, Issue Supplement_2, 15 September 2010, S183–S197.

3. Lowy FD. Staphylococcus aureus infections. N Engl J Med. 1998 Aug 20; 339(8):520-32.

4. Cookson B. Staphylococcus aureus. In: Emmerson M, Kibbler C, Hawkey P eds. Principles of Clinical Bacteriology. Oxford: John Wiley, 1997; 109–30.

5. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. Clinical and Laboratory Standards Institute, Wayne, PA 2012.

6. Chandrashekar D K et al. Prevalence of *Methicillin Resistant Staphylococcus aureus* in a tertiary care hospital in Gulbarga, Karnataka, J. of Pharmaceutical and Biomedical Sciences. June 2012,19(19)1-3.

7. Arora S, Devi P, Arora U, Devi B. Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in a tertiary care hospital in Northern India. J Lab Physicians. 2010 Jul-Dec;2(2):78-81.

8. Prasad RR, Shree V. Antimicrobial Susceptibility Pattern among Methicillin-Resistant *Staphylococcus Aureus* (MRSA) Isolates in Bihar. International J of Science and Research.Aug.2015;4(8):111-115.

9. Bhujwala RA, Mohapatra LN. Bacteriophage pattern of antibiotic resistance of Staphylococcus aureus. Ind J Pathol and Bacteriol, (1972) 15:66-72.

10. Kulkarni SV, Jahagirdar, BL and Sengupta, SR: Phage typing of staphylococci and its relation to penicillin products and antibiotic resistance. Proceeding of 3rd All India Annual Congress of Medical Microbiologist, Bombay, 1979, pp 25-28.

11. Majumdar D, Bordoloi JS, Phukan AC, Mahanta J. Antimicrobial susceptibility pattern among methicillin resistant staphylococcus isolates in Assam. Indian J Med Microbiol 2001;19:138-40.

12. Udaya Shankar C, Harish BN, Umesh Kumar PM, Navaneeth BV. Prevalence of methicillin resistant Staphylococcus aureus in JIPMER hospital. Indian J Med Microbiol 1997;15:137-138.

13. Saikia L, Nath R Choudhary B, Sarkar M. Prevalence and antimicrobial susceptibility pattern of methicillin resistant Staphylococcus aureus in Assam. Indian J Crit Care Med 2009;13:156-8.

14. Dar JA, Thoker MA, Khan JA, Ali A, Khan MA, Rizwan M, Bhat KH, Dar MJ, Ahmed N, Ahmad S. Molecular epidemiology of clinical and carrier strains of methicillin resistant Staphylococcus aureus (MRSA) in the hospital settings of north India. Ann Clin Microbiol Antimicrob. 2006 Sep 14; 5 :22.

15. Dixit NS, Kothari D, Chandrakar KS. Prevalence of Methicillin Resistant Staphylococcus Aurous In Surgical Units of a Tertiary Care Hospital. International J of Innovative Research in Med. Science (IJIRMS) ;Vol 02 ;Issue 07 ;2017,1076-79.

16. Yilmaz G, Aydin K, Iskender S, Caylan R, Koksal I. Detection and prevalence of inducible clindamycin resistance in staphylococci. J Med Microbiol. 2007; 56:342–5.

17. Prabhu K, Rao S, and Rao V. Inducible Clindamycin Resistance in Staphylococcus aureus Isolated from Clinical Samples. J Lab Physicians. 2011 Jan- Jun; 3(1): 25–27.

18. Gupta V, Datta P, Rani H, Chander J. Inducible clindamycin resistance in *Staphylococcus aureus*: A study from North India. J Postgrad Med. 2009;55:176–9.