Original Article

Prevalence of methicillin resistant Staphylococcus aureus in various clinical specimen at Kist Medical College and Teaching Hospital

Sweekrity Neupane,Bijendra Raj Raghubanshi,Ruchee Manandhar, Rajni Iama

Department of Microbiology, KIST Medical College, Imadol, Lalitpur

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Corresponding

Sweekrity Neupane
Department of Microbiology ,
KIST Medical College and Teaching Hospital
Imadole, Lalitpur, Nepal.
Email: sweekritinep@gmail.com

Abstract

Introduction: Antimicrobial resistance is a global threat and there is increase in antibiotic resistance in S.aureus. Multidrug resistance developed in S. aureus has been associated with an increase in morbidity and mortality of the patients in the hospital. The main objective of this study was to detect MRSA using phenotypic methods and to determine their antibiogram.

Methods: Various specimens received from September 2020 to september 2021 in Kist Medical College-Teaching Hospital were processed and all S. aureus isolates were included in the study. The isolates were identified by standard laboratory procedure. Cefoxitin was used to detect MRSA by the disk diffusion test. The antibiotic susceptibility pattern of all isolates was determined by modified Kirby Bauer disc diffusion method.

Results: A total of 5,198 samples (blood 1683, pus 130, swab 328, body fluid 849, urine 2208) from patients attending the Kist hospital for treatment were collected. From the total 5,198 samples 80 were confirmed as Staphylococcus aureus. Out of 80 S.aureus isolated 49 (61.3%) were found to be MRSA. All the S.aureus isolated was sensitive to vancomycin. Among 49 MRSA isolates 33 (67.34%) were MDR and among 31 MSSA isolates 4 (12.9%) were MDR.

Conclusion: This study showed a high prevalence of MRSA in tertiary care hospital. Regular surveillance of healthcare-associated infection and monitoring of antibiotic sensitivity pattern is mandatory to reduce MRSA prevalence in hospital. Present study shows that vancomycin remains the drug of choice for MRSA infection.

Keywords: *MRSA, Staphylococcus aureus, Vancomycin*

Introduction

Staphylococcus aureus is among the most successful human pathogens. Since the 1960s, S. aureus strains have emerged resistant to the penicillinase-stable penicillins. This resistance is the result of a supplemental penicillin binding protein (PBP 2a) encoded by the chromosomal mecA gene. These strains are termed methicillin resistant S. aureus (MRSA) and are resistant to all beta-lactam agents. MRSA causes progressive, potential fatal diseases including life-threatening pneumonia, necrotizing fasciitis, endocarditis, osteomyelitis, severe sepsis, and toxinoses such as toxic shock syndrome. The most common risk factors of being colonized or acquiring an MRSA infection are; presence of an open wound and/or other dermatology disease, presence of invasive devices (e.g. catheter), prolonged use of antibiotics, prolonged hospital stay, several admissions to hospital over a short period of time.

Antimicrobial resistance is a global threat and there is increase in antibiotic resistance in S.aureus. MRSA has emerged as an important human pathogen. Various studies from different parts of the world show an increase in the number of Methicillin resistant Staphylococcus aureus (MRSA). MRSA is one of the commonest causes of hospital-acquired infections throughout the world[1]. European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) in January 2008 and had constructed significant antimicrobial

categories for each bacterium.[2] They have defined different levels of antibiotics non susceptibility as: Multidrug resistant (MDR) is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. Extensively drug resistant (XDR) is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories). Pandrug resistant (PDR) is defined as non-susceptibility to all agents in all antimicrobial categories.

Methicillin resistant S. aureus is typically multidrug resistant and treatment options are limited to few antibiotics like teicoplanin and vancomycin. Detection of the resistance pattern is therefore an important support tool to antibiotic treatment guidelines and susceptibility surveillance of S. aureus. This data will be helpful to collate the current state of resistance pattern in Nepal. It will also help to devise treatment protocol for MRSA infections.

Methods

This is a retrospective cross sectional study and conducted at Kist medical college and teaching hospital. Ethical clearance was obtained from KISTMCTH Institutional Review Committee with IRC NO –078/079/56. Staphylococcus aureus isolated in microbiology lab from various clinical specimen collected from September 2020 to September 2021 is the study population. Inclusion Criteria included Staphylococcus aureus isolated from various clinical specimens (blood, bodyfluid, pus, wound swab, sputum, urine). Exclusion Criteria included the specimen without demographic data. All samples (blood, sputum, urine, pus and bodyfluids) of patients who attended the hospital for treatment were collected from various departments for culture after the treating clinician requested them. Collected samples were received at the microbiology laboratory for microbiological tests. Standard laboratory procedure and technique was followed for sample collection.

Identification of S.aureus

Samples were inoculated into McConkey's agar, blood agar and chocolate agar. Blood was inoculated in Brain Heart Infusion broth and sub culture on 24 and 72 hour on BA and McConkey's agar. Selective media for S. aureus was not used. The gram stained smear of the suspected colonies was observed under an oil immersion lens. Gram positive cocci in clusters were subjected to further biochemical tests. Staphylococcus aureus was identified by standard microbiological technique.[3] Different biochemical tests such as catalase, coagulase were performed. Gram positive cocci (GPC) in clusters, which were also catalase positive, were subjected to a slide coagulase test. Gram positive cocci and both catalase and coagulase positive, were considered as S. aureus. Those which were negative by the slide coagulase test were further subjected to a tube coagulase test and were considered S. aureus if were positive for both the catalase and tube coagulase tests. However, only first isolate was included in the study if the same patient had other samples (blood, pus, body fluid and sputum) positive for S. aureus with same antibiogram.

Antibiotic susceptibility testing of S. aureus

Antibiotic susceptibility tests of the S. aureus were performed by a modifed Kirby–Bauer disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2012) on Mueller–Hinton agar.[4] Antibiotic disks (Hi Media Laboratories, Pvt. Limited, India) such as cefoxitin (30 µg), ciprofoxacin (5 µg), erythromycin (15 µg), co-trimoxazole (25 µg), gentamicin (10 µg), amikacin (30 µg), clindamycin (2 µg), cloxacillin (5ug) and vancomycin (30 µg) were used for antibiotic susceptibility tests.

Identification of MRSA

Cefoxitin disc (30 µg) was used for identification of MRSA. Methicillin-resistant S. aureus isolates carry the mecA gene, which confers resistance to all beta-lactam antibiotics, including cephalosporins and carbapenems. Apart from using molecular methods to detect the mecA gene directly, the most accurate phenotypic test for the presence of the mecA gene in S. aureus is the cefoxitin disk diffusion test. According to the Clinical and Laboratory Standards Institute (CLSI), a zone of growth inhibition of ≥22 mm around the cefoxitin disk rules out MRSA; a zone size <22 mm indicates that the mecA gene is present and the isolate should be reported as MRSA.[5]

Statistical analysis

Then data was first entered in MS excel and later data analysis was done in SPSS vs. 26. Data were summarized in frequency distribution table presenting both in number and percentages.

Results

A total of 5,198 samples (blood 1683, pus 130, swab 328, body fluid 849, urine 2208) from patients attending the Kist hospital for treatment were collected. From the total 5,198 samples 80 were confirmed as Staphylococcus aureus. Staphylococcus aureus were further tested for MRSA by using cefoxitin disc. The drug profile was tested for 10 different antibiotics.

Out of 80 S.aureus isolated 49 (61.3%) were found to

be MRSA (Fig 1). Higher number of MRSA was found in females (28) compared to males (21). Maximum number of MRSA (25) was seen among age-group >30. Among various clinical specimen MRSA(16) was mostly isolated from pus sample (Fig 2). Antibiotic sensitivity pattern of S.aureus showed a higher degree of resistance to many antibiotics such as ampicillin (88.8%), erythromycin (65%), clindamycin (58.8%) and cotrimoxazole (60%). All the S.aureus isolated was sensitive to vancomycin (table 1). Multidrug resistant (MDR) is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. Among 49 MRSA isolates 33 (67.34%) were MDR and among 31 MSSA isolates 4 (12.9%) were MDR (table 2).

Figure 1. Prevalence of MRSA

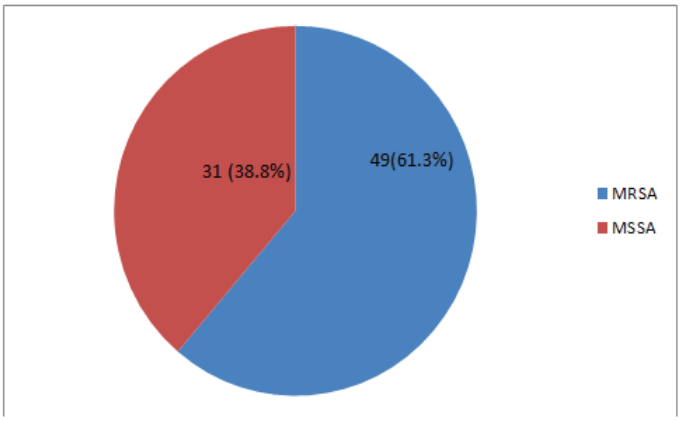

Figure 2. Distribution of S. aureus in clinical samples

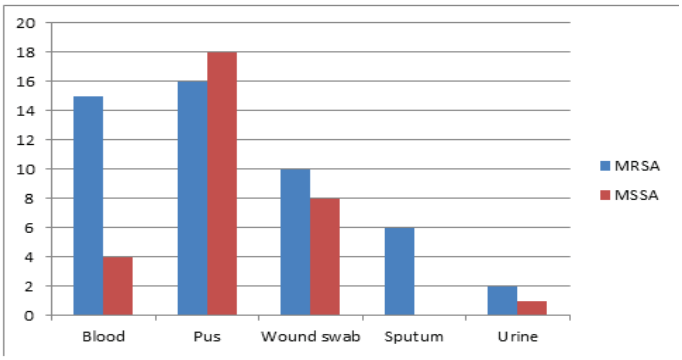

Table 1. Antibiotic susceptibility pattern of S. aureus

Antibiotic susceptibility pattern of (S.aureus (n=80			
Antibiotics	**Sensitive**	**Intermediate**	**Resistant**
Ampicillin	9 (11.3%)		71 (88.8%)
Cefoxitin	31 (38.8%)		49 (61.3%)
Cloxacillin	61 (76.3%)	19 (1.3%)	18 (22.5%)
Cotrimoxazole	30 (37.5%)	2 (2.5%)	(60%) 48
Clindamycin	33 (41.3%)		47 (58.8%)
Erythromycin	28 (35%)		52 (65%)
Gentamicin	55 (68.8%)	3 (3.8%)	22 (27.5%)
Amikacin	62 (77.5%)	4 (5%)	14 (17.5%)
Vancomycin	80 (100%)		

Table 2. MDR pattern among S. aureus isolates

Isolate	**MDR**	**Non- MDR**	**Total**
MSSA	4 (12.9%)	27 (87.1%)	31 (100%)
MRSA	33(67.34%)	16 (32.66%)	49 (100%)

Discussion

The development of multidrug resistance by S. aureus is a public health concern. It has added to the burden of patient by prolonging hospital stay. Methicillin resistant S. aureus has been associated with an increase in morbidity and mortality of the patients in the hospital. According to data published by WHO in 2014, it showed greater than 80% of Staphylococcus aureus infections having MRSA.[6]

The present study showed the prevalence of MRSA to be 61.3%. Findings in our study are consistent with the other studies previously conducted from Nepal. In study conducted at CMS teaching hospital, Chitwan overall prevalence of MRSA was 68.0%.[7] In a similar study conducted by Rijal et al in pokhara MRSA was isolated at the rate 75.5% from clinical samples.[8] However, varying prevalence of MRSA has been reported from different parts of Nepal such as 26.1% in Dharan[9] and 57.1% in Birgunj.[10] The high prevalence of MRSA in this study might be due to readily available and irrational use of antibiotics by the patient without specific laboratory test.

Present study shows maximum number of S.aureus and MRSA isolation from pus among all clinical specimens. This might be due to S.aureus being the most common organism causing pyogenic infection. Other study also showed higher S. aureus from pus sample.[9,11]

Drug resistance in S. aureus is mediated by complex genetic arrays such as the staphylococcal chromosomal cassette mec elements for methicillin. Other resistances against antibiotics like fluoroquinolones, linezolid and daptomycin have developed through spontaneous mutations and positive selection. Detection of the resistance pattern is, therefore, an important support tool for antibiotic treatment guidelines and susceptibility surveillance of S. aureus in places where susceptibility testing is not a routine. In our study S.aureus showed a higher degree of resistance to many antibiotics such

as ampicillin (88.8%), erythromycin (65%), clindamycin (58.8%) and cotrimoxazole (60%). The findings in this study have been consistent with the findings from studies conducted in other parts of Nepal.[9,12] Our study showed that all MRSA isolates were sensitive to vancomycin. This is in consistent with various other studies.[13,14,15,16] These findings suggest that vancomycin is the drug of choice for treating MRSA infections.

Present study shows alarmingly high rate of MDR strain among MRSA isolates (67.34%). Other Studies conducted in eastern and western part of Nepal previously have reported MDR- MRSA to be as high as 65-78%.[(14,17)] In similar study in India showed the isolation of MDR-MRSAas high as 77% 23.[18]

Conclusion

Our study showed higher prevalence of MRSA among clinical specimens, especially pus. The disk diffusion test by cefoxitin for MRSA is simple and cost effective method and can be routinely utilized in resource limited settings to identify these isolates. The prevalence of multidrug resistance was higher among MRSA compared to MSSA. This shows high burden of multidrug resistant organisms. Thus, multi drug resistance is challenging health status of people from this area. In this study, all isolates were sensitive to vancomycin. Thus vancomycin should be considered as a last resort for treatment of MRSA infections. Regular surveillance of MRSA and monitoring of antibiotic sensitivity pattern is mandatory to reduce MDR prevalence in the hospital. Antibiotic stewardship guidelines should be strictly followed in hospital to reduce MDR MRSA.

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