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Original Research Article.

Association of mec-A and Panton Valentine Leukocidin Gene with Methicillin Resistance in S*taphylococcus aureus*

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ABSTRACT

Background: MRSA is a major nosocomial pathogen that causes severe morbidity and mortality worldwide. Methicillin resistance is caused by the presence of *mec*A gene, which encodes an additional 78 kDa low-affinity penicillin binding protein (PBP)-2a or PBP2' which has a low affinity for β-lactam antibiotics, whereas absence of the gene from *Staphylococcal* strain indicates methicillin susceptibility. Cefoxitin is a potent inducer of the *mec*A regulatory system. The PVL gene has been detected commonly in skin associated community acquired infection.

Aims and Objectives: To isolate methicillin resistant Staphylococcus aureus among various clinical specimens and study the association of mecA and PVL gene in MRSA.

Materials and Methods: Processing of specimens (urine,pus,blood,fluids etc) was done according to standard bacteriological procedures and identification of bacterial isolate was done by colony morphology & biochemical test and further subjected to antibiotic susceptibility testing and to Polymerase chain reaction for detection of mec A gene and PVL gene.

Results: Testing with cefoxitin disk diffusion method identified 300 isolates as MRSA were compared to mec-A gene detection by PCR, 02 cefoxitin resistant isolates were not having mec-A gene. PVL gene was present in 110 isolates out of 138 community acquired MRSA isolates, total 143 isolates were positive for mec-A gene and PVL gene.

Keywords: mec A gene, PVL gene, MRSA.

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INTRODUCTION

The staphylococci are gram positive coccus, which divide in more than one plane to form grape-like clusters. They are preferentially colonize the human body. The staphylococcus aureus was discovered as a cause of wound sepsis by Professor Sir Alexander Ogston. It is responsible for causing a variety of human infections, which may range from minor skin diseases like furuncles, impetigo, carbuncles to life threatening infections like pneumonia, meningitis, endocarditis, osteomyelitis and toxic shock syndrome. It is the second largest cause of hospital-acquired pneumonia. It can cause meningitis, which is usually as a result of infections after brain surgery or a consequence of S.aureus infection in the blood.

It also causes toxin induced food poisoning, invasive infection. Despite the introduction of effective antimicrobial agents and improvements in hygiene, Staphylococci have persisted as important causes of both community acquired and hospital acquired infections, with substantial morbidity and mortality.

When penicillin was first introduced in 1943, no resistance was reported initially. However due to selection pressure exerted by the use of penicillin, resistance rapidly emerged to this antibiotic and by 1960 up to 80% strains of *S.aureus* were penicillin resistant. Resistance to penicillin is mediated by production of penicillinase, an enzyme which inactivates penicillins by opening up the β-lactam ring. Penicillinase resistant penicillins (methicillin, oxacillin, cloxacillin and flucloxacillin) were developed later for treatment of these penicillin resistant strains of *S.aureus*. However, within a few years of their use, resistance to methicillin began emerging. Methicillin resistant *S. aureus* (MRSA) was first described in 1961, one year after introduction of Methicillin.[1] MRSA is now a major nosocomial pathogen that causes severe morbidity and mortality worldwide.[2] MRSA is defined as a strain of *S. aureus* that is resistant to the β-lactam group of antibiotics, that includes penicillins and cephalosporins. The reservoir of Methicillin-Resistant *S. aureus* (MRSA) is infected and colonized

patients, and the major mode of transmission from patient to patient is through the contaminated hands of healthcare workers.[3] This leads to serious endemic and epidemic MRSA infections.[4]

The incidence of MRSA varies according to the region, 25% in Western part of India[5] to 50% in South India.[6] A high prevalence of MRSA (35% in ward and 43% in ICU) was observed from blood culture specimens in a study in Delhi.[7]

Methicillin resistance is caused by the presence of *mec*A gene, which encodes an additional 78 kDa low-affinity penicillin binding protein (PBP)-2a or PBP2' which has a low affinity for β-lactam antibiotics, whereas absence of the gene from *Staphylococcal* strain indicates methicillin susceptibility. Cefoxitin is a potent inducer of the *mec*A regulatory system.[8]

Most *S. aureus* strains responsible for primary skin infections and necrotising pneumonia harbour the PVL determinant.[9,10] The PVL gene has been detected in 93% of isolates associated with furunculosis, 55% of isolates associated with cellulitis, 50% of isolates associated with cutaneous abscess, and 13% of isolates associated with finger-pulp infection, but was absent in isolates associated with superficial folliculitis and impetigo.[9] Highly lethal necrotising pneumonia caused by PVL-positive *S. aureus* has been described in previously healthy young patients.[10] The treatment of such PVL positive MRSA infections is entirely different from that of other staphylococcal infections. Accurate and rapid detection of PVL positive MRSA permits timely implementation of effective antimicrobial therapy.

OBJECTIVES

- Isolation of methicillin resistant Staphylococcus aureus among various clinical specimens.
- To study the association of mecA and PVL gene in Methicillin Resistant *S. aureus.*

MATERIALS AND METHODS

The proposed prospective cross sectional was conducted in the Department of Microbiology, Santosh Medical College, Ghaziabad in collaboration with Department of Medicine, Department of Microbiology and Central Research Laboratory at School of Medical Sciences & Research, Sharda Hospital, Greater Noida. All patients irrespective of age and sex, attending the outpatient departments or admitted in the various wards and ICUs were included in this study. Total 300 MRSA isolates were considered in this study.

Inclusion Criteria

- All MRSA isolates confirmed by Cefoxitin Disc Diffusion test
- All patients who had given their consent for participation in the study.

Exclusion Criteria

- Samples from which MRSA were not isolated.
- Patients with history of oral antibiotic use.

Processing and Specimen Culture: The initial processing of specimens (urine, pus, blood, fluids) was done according to standard bacteriological procedures available.[11] Preliminary identification of bacterial isolate was done by studying colony morphology & biochemical test and further subjected to antibiotic susceptibility testing by Kirby Bauer Disc Diffusion method for Antibiotic Susceptibility testing was performed for all the bacterial isolates according to The Clinical and Laboratory Standards Institute (CLSI) guidelines using Mueller –Hinton's Agar standard media.[12]

Detection of MRSA: by Cefoxitin Disk Diffusion Test and Vitek 2 compact[12] and mecA, PVL gene detection by PCR test

All the phenotypically detected MRSA strains were then subjected to Polymerase chain reaction (PCR) for the detection of mecA & PVL gene. In this study, Genotypic method (PCR) was performed for confirming and comparing it with various phenotypic methods for detection of MRSA.

Extraction of DNA: DNA Extration was done according to procedure given in kit (Qiagen Mini)

Materials:

- Template DNA
- Primers with Probe
- Buffers
- MgCl2
- Taq Polymerase
- dNTPs
- Reaction Mixture.

Table 1: Detail of Primers for PVL gene & mecA gene

Pantone Valentine Leukocidine (PVL)		
Primer	**Orientation**	**Oligonucleotide Sequence**
Luk	Forward	CAGGAGGTAATGGTTCATTT
	Reverse	ATGTCCAGACATTTTACCTAA
MRSA (mec A) Gene		
mecA	Forward	TCCAGATTACAACTTCACCAGG
	Reverse	CCACTTCATATCTTGTAACG

Table 2: Pipetting scheme for PCR Master Mix

MRSA MASTER MIX	1 rxns.	10 rxns.
MRSA Super Mix (R1)	12 µl	120 µl
MRSA Mg. Sol. (R2)	2.5 µl	25 µl
IC-1 (R3) RG	0.5 µl	5 µl
Total	15 µl	150 µl

Statistical Evaluation

All statistical analysis was performed by using SPSS software. The result normally distributed were presented as mean, standard deviation and the result not normally distributed are presented as median. The collected data was analyzed by using IBM-SPSS version 22.0. The appropriate statistical methods were used as per requirement.

OBSERVATIONS AND RESULTS

Total 5258 culture samples for the study were received from patients of all age and sex who attended the hospital's out-patient department or were admitted to the hospital and showed signs & symptoms of infection. Total number 300 methicillin resistant staphylococcus aureus isolates included in the study.

Out of 300 MRSA, 171 MRSA were isolated from in patients' sample and 129 were isolated from outpatient department. In this study 212 MRSA isolates (70.66 %) were from males and 88 isolates (29.33 %) were females.

The MRSA isolates were further classified into hospital acquired MRSA (HA-MRSA) and community acquired MRSA (CA-MRSA). Hospital acquired MRSA infection was defined as occurring in a patient whose MRSA isolate was cultured more than 48 hours after admission to the hospital or who had a history of hospitalization, surgery, dialysis or residence in a long-term health care facility within one months prior to the culture date or who had a indwelling intravenous line, catheter or any other percutaneous medical device present at the time the culture was taken. Patients with none of the above conditions were classified as having community acquired MRSA infection. In our study 162 (54%) of the MRSA isolates were hospital acquired MRSA while 138 (46%) were community acquired MRSA. (Table 3) Maximum number of MRSA isolates were obtained from surgical ward were 110 (35.66%) cases followed by orthopaedic ward 63 (21 %) Gynaec ward 43 (14.33) pediatric ward 42 (14%) and medical ward 34 (11.33%). (Figure 1) Testing with cefoxitin disk diffusion method identified 300 isolates as MRSA and compared to mec-A gene detection by PCR, 02 cefoxitin resistant isolates were not having mec-A gene. PVL gene was present in 110 isolates were community acquired MRSA and 33 isolates were from hospital acquired MRSA, all 143 cases were positive for mec-A gene and PVL gene. 02 cefoxitin sensitive isolate were mec-A gene negative (false positive MRSA). The sensitivity of cefoxitin disk diffusion method was 99.3% specific.

Testing with Vitek-2 Compact method identified 295 isolates as MRSA and compared to mec-A gene detection by PCR, 03 isolates were not detected by Vitek-2 Compact method but was having mec-A gene. PVL gene was present in 110 isolates were community acquired MRSA and 33 isolates were from hospital acquired MRSA, all 143 cases were positive for mec-A gene and PVL gene.(Table 4,5)

Figure 1: Distribution of MRSA in various wards

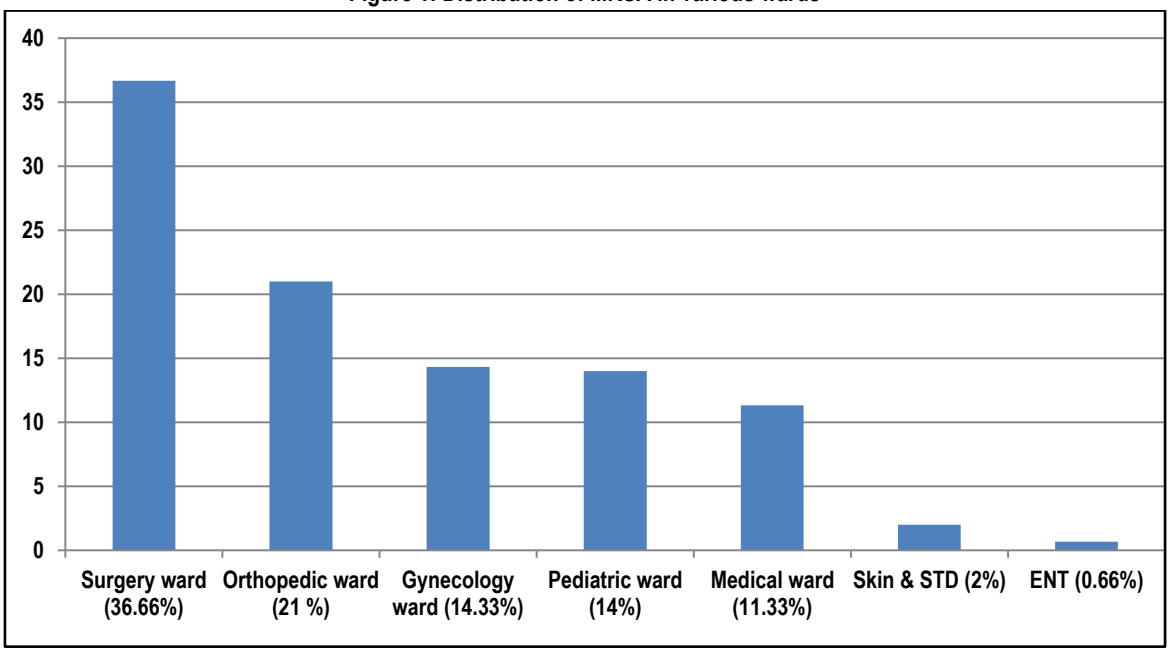

Table 3: Distribution of Hospital acquired and Community acquired MRSA

	Number	%
Hospital Acquired MRSA (HA-MRSA)	162	54
Community Acquired MRSA (CA-MRSA)	138	46
Total	300	100

Table 4: Results of Cefoxitin Disc Diffusion method and their correlation with mec-A gene & PVL gene detection

Genotypic Markers	Cefoxitin Disc Diffusion (30µg) (n=300)	
	Resistance	%
mec-A(+) (298)	297	99%
PVL (+) (143)	143	100%
Mec-A & PVL (143)	143	100%

Table 5: Results of Vitek-2 Compact methods and their correlation with mec-A gene& PVL gene detection

Genotypic Markers	Vitek-2 Compact (300)	
	Resistance	%
mec-A(+) (298)	295	98.3
PVL (+) (143)	142	99.3
Mec-A & PVL (143)	143	100

DISCUSSION

MRSA is the most prominent cause of nosocomial infections caused by a single bacterial pathogen in the US and many parts of the world. It is estimated that about 44% of all hospital-associated infections can be attributed to MRSA.

We have also compared the relative performance of common phenotypic methods available for identification of MRSA against PCR for mec-A gene & PVL gene detection.

In our study the prevalence of MRSA was higher in males (70.67%) as compared to females (29.3%).The highest prevalence was seen in the age group of 51-60 years and MRSA

was isolated in 57 % of cases from inpatient and 43 % cases were from outpatient of hospital, and 54 % isolates were Hospital acquired MRSA and 46 % cases were Community acquired MRSA.(Table 3) and most commonly affected age group was 51-60 years of age group as compare to other study in which the age group is of 21-30 years.[13]

In our study MRSA was isolated in 70.67 % males and 29.33 % of female Similar to our study, another study by Kulshrestha A. et al 2017 from India, reported higher MRSA prevalence in males (73%). The increased rate of MRSA infections among males could be due to their more outdoor activities, in turn exposing them to contaminated environment and also compared to females, accidental injuries are more common among men. Another study performed by Khanal LK. Et al 2010 also noted that the rate was significantly higher among males (75%) than females (25%).[14] MRSA infection rate was higher among patients above 30 years of age. In a study by Jaiswal S. et al 2016 from Pokhra, reported prevalence of MRSA was higher in males (55%) than in females (45%) and the prevalence of MRSA was higher among the age group of 15-45 years indicating MRSA infection is more common in working and old age group.[15] The reason for this may be that younger age group are more involved in outdoor activities thus exposing them to contaminated environment and in older age group it may be due to warning immunity, hormonal abnormalities and co-morbid conditions. In contrast to this, another study by Bhatt CP et al 2014, from Nepal found MRSA prevalence to be higher in females (73%) than male (23%) and maximum number (31.5%) was found in age group 0-10 years.[16] The contrasting results from this study might be due to less sample size evaluated.

In the present study, maximum number of MRSA isolates were obtained from surgical cases (36.66%) followed by orthopaedic (21%) gynaec cases (14.33) pediatric (14%), and medical cases (11.33%) (Figure 1). These findings are similar to those in other studies. In a study by Rajesh TP et al, majority of isolates were from General Surgery department (44%), followed by General Medicine (15%), Orthopedics (8%), Pediatrics and rest from other departments (<5%)[16].

Also, Kulshrestha A. et al, in their study reported that among inpatient department patients' maximum strains were isolated from surgical and orthopedics ward.[17] This might be because the patients in these wards are usually with open wounds and are debilitated. They undergo multiple interventions in the hospital which further increases the risk of MRSA infection due to involvement of multiple persons as well as prolonged stay in the wards. This variation between different wards is due to the differences in compliance in hand hygiene, infection control practices, antibiotic policy and practices of doctors and health care workers.

In our study the Highest number of MRSA were isolated from pus (32.33%) consistent with the suppurative nature of Staphylococcal infections, followed by followed by Urine (14.33%), Throat swab (13%), Nasal swab (12.33 %), Blood (9.66%), Sputum (8.33%), Catheter tips (6.33%), Body Fluids (3.66 %).

Similar to our study, Kulshrestha A. et al, also isolated most MRSA from pus (61%), followed by blood (15%), respiratory secretions (10%), Swabs and body fluids (5%) and least from Urine (4%).[18] This pattern also correlates with other studies in India conducted by Vidya Pai et al., in 2010 and Nitish Kumar Sharma et al., in 2013 and Kaur DC in 2015.[19,20]

Among all antibiotic classes, glycopeptides emerged as the most sensitive class of antibiotic against MRSA. Methicillin Resistant Staphylococcus aureus (MRSA) showed 100% sensitivity to Vancomycin and Teicoplanin, followed by Linezolid (98.67%) and Amikacin (74.67%). Isolates from urine samples showed 41.86% sensitivity to Nitrofurantoin and 44.18% sensitivity to Norfloxacin. The least sensitivity was observed for Ciprofloxacin (13.679%) followed by Levofloxacin (18.34%) and Erythromycin (25.34%). There was 100% resistance to penicillin, followed by Ciprofloxacin (86.33%), Levofloxacin (81.66), Erythromycin (74.66%) and Gentamicin (57.6%). There was no resistance to Vancomycin and Teicoplanin followed by linezolid (1.33%). Among urine samples 41.86% isolates were resistant to Nitrofurantoin.

The main mechanism of resistance is due to acqution of the mec-A gene, which encodes for low-affinity PBP2a. Therefore, presence and absence of mec-A gene indicates methicillin resistance and methicillin susceptibility in staphylococci respectively. PCR for the amplification of the mec-A is presently considered the gold standard for the detecting methicillin resistance in S. aureus.[21] Nucleic acid-amplification techniques offer clear benefits over traditional culture-based assays, in particular, a reduced time to identification and an improved specificity and sensitivity.[22]

In comparison to gold standard method of PCR, many authors have recommended that cefoxitin could be a marker for the detection of methicillin resistance in the settings where PCR is not feasible, as it is a better inducer of mec-A gene and PVL gene by disc diffusion test using cefoxitin gives clearer endpoints. However, Cefoxitin will detect only MRSA with a mec-A mediated resistance mechanism. However, non-mecA mediated methicillin resistance in S. aureus is a rare occurrence, as reported by Sharma S et al.[23]

In the present study we evaluated different phenotypic methods for detection of MRSA taking PCR for mec-A & PVL gene as the gold standard. Staphylococcus aureus isolates included in this study were subjected to cefoxitin disc diffusion, VITEK-2 compact screen test and mec-A & PVL gene detection.

CONCLUSIONS

Detection of mec-A gene & PVL gene by polymerase chain reaction is considered as the best method to detect MRSA. However, cefoxitin disk diffusion test, which is a simple and cost-effective method has shown high sensitivity and specificity. Thus, it can be used as an accurate surrogate marker in routine testing to detect MRSA.

REFERENCES

1. Maple PAC, Hamilton- Miller JMT, Brumfitt W. Worldwide antibiotic resistance in methicillin resistant Staphylococcus aureus. Lancet 1989;1:537-40.

2. Karthik M, Thilaak P, Priyadharsini I, Marappan S. Panton-Valentine Leukocidin gene positive methicillin resistant Staphylococcus aureus, the community strains causing infections in intensive care unit - High risk of outbreak and preventing strategies. Journal of Medical & Allied Sciences 2016; 6(2): 56-60.

3. Eveillard M, Martin Y, Hidri N, Boussougant Y, Joly-Guillou ML. Carriage of methicillin-resistant Staphylococcus aureus among hospital employees: Prevalence, duration, and transmission to households. Infect Control Hosp Epidemiol. 2004;25: 114–20.

4. Rajaduraipandi K, Mani KR, Panneerselvam K, Mani M, Bhaskar M, Manikandan M. Prevalence and antimicrobial susceptibility pattern of methicillin resistant Staphylococcus aureus: A multicentre study. Indian J Med Microbiol.2006;24:34–8.
5. Patel AK, Patel KK, Patel KR, Shah S, Dileep P. Time trends in the epidemiology of microbial infections at a tertiary care center in west India over last 5 years. J Assoc Physicians India 2010; 58: 37-40.
6. Gopalakrishnan R, Sureshkumar D. Changing trends in antimicrobial susceptibility and hospital acquired infections over an 8 year period in a tertiary care hospital in relation to introduction of an infection control programme. J Assoc Physicians India 2010;58:25-31.
7. Wattal C, Goel N, Oberoi JK, Raveendran R, Datta S, Prasad KJ. Surveillance of multidrug resistant organisms in tertiary care hospital in Delhi, India. J Assoc Physicians India. 2010; 58(Suppl):32–6.
8. Swenson JM, Lonsway D, McAllister S, Thompson A, Jevitt L, Patel JB. Detection of mecA mediated Resistance using Cefoxitin Disc Diffusion (DD) in a Collection of Staphylococcus aureus Expressing Borderline oxacillin MICs. Diagn Microbiol Infect Dis 2007; 58:33-9.
9. Gillet Y, Issartel B, Vanhems P. Association between Staphylococcus aureusstrains gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. Lancet 2002; 359: 753-9.
10. Lina G, Piemont Y, Gamot FG. Involvement of Panton-Valentine leukocidin producing Staphylococcus aureus in primary skin infections and pneumonia. Clin Infect Dis 1999; 29: 1128-32.
11. Mackie & McCartney practical Medical Microbiology, Laboratory strategy in the diagnosis of infectives syndromes, 14th edition, Delhi: Elsevier publication 2006, 53-93.
12. Vitek 2 instrument user manual, BioMerieux 2008- https://www.scribd.com/document/341141470/Biomerieux-Vitek-2-User-manual-pdf
13. Kulshrestha A, Anamika V, Mrithunjay K, et al. A Prospective Study on the Prevalence and Antibiotic Sensitivity Pattern of Methicillin Resistant Staphylococcus aureus isolated from Various Clinical Specimen at a Tertiary care Post Graduate Teaching Institute. Int J. Curr. Microbiol. App. Sci. 2017; 6(3): 1859-69.
14. Khanal LK and Jha BK. Prevalence of Methicillin resistant Staphylococcus aureus (MRSA) among skin infection cases at a hospital in Chitwan. Nepal, Nepal Med Coll J 2010;12:224-8.
15. Jaiswal S., Thapa A., Mali G., et al. A Study of Methicillin Resistant Pattern on Clinical Isolates of Staphylococcus aureus in Tertiary CareHospital of Pokhara.BMR Microbiology2016;2(1):1-8.
16. Bhatt CP, Karki BMS, Baral B et al. Antibiotic susceptibility pattern of staphylococcus aureus and methicillin-resistant staphylococcus aureus in a tertiary care hospital. Journal of Pathology of Nepal, 2014; 4: 548-55.
17. Anamika V, Mrithunjay K, et al. A Prospective Study on the Prevalence and Antibiotic Sensitivity Pattern of Methicillin Resistant Staphylococcus aureus isolated from Various Clinical Specimen at a Tertiary care Post Graduate Teaching Institute. Int J. Curr. Microbiol. App. Sci. 2017; 6(3): 1859-69.
18. Arora S, Devi P, Arora U, et al. Prevalence of Methicillin-resistant Staphylococcus aureus (MRSA) in a tertiary care hospital in northern India. J Lab Physicians 2010;2:78-81.
19. Rajkumar S, Sistla S, Manoharan M, et al. Prevalence and genetic mechanisms of antimicrobial resistant in Staphylococcus species: A multicentre report of the Indian Council of Medical Research Antimicrobial Resistance Surveillance Network. Indian J Med Microbiol 2017;35:53-60.
20. Nitish K, Raina G, Shrikala B, et al. Nosocomia Infections and Drug Susceptibility Patterns in Methicillin Sensitive and Methicillin Resistant Staphylococcus aureus. Clin. Diagn. Res. 2013; 7: 2178-80.
21. Panda RK, Mahapatra A, Mallick B, et al. Evaluation of Genotypic and Phenotypic Methods for Detection of Methicillin Resistant Staphylococcus aureus in a Tertiary care Hospital of Eastern Odisha. Journal of Clinical and Diagnostic Research. 2016;10(2): 19-21.
22. Witt RT, Belkum AV, Leeuwen WB. Molecular diagnostics and genotyping of MRSA: an update. Expert Rev Mol Diagn 2010;10(4):375-80.
23. Sharma S, Srivastava P, Kulshrestha A, et al. Evaluation of different phenotypic methods for the detection of methicillin resistant Staphylococcus aureus and antimicrobial susceptibility pattern of MRSA. Int J Community Med Public Health. 2017; 4(9): 3297-301.

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