

STUDY ON PREVALENCE OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS; COMPARISON OF FOUR DIAGNOSTIC METHODS FOR DETECTING MRSA- FROM A TERTIARY CARE HOSPITAL, MADURAI

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ABSTRACT

BACKGROUND

Prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in India ranges from 30 to 70% which leads to high mortality, increased economic burden and high treatment failure when compared to MSSA. Rapid and accurate detection of MRSA is essential to take measures for infection control and also to prevent unnecessary use of antibiotics.

MATERIALS AND METHODS

Prospective observational study was carried out in our tertiary care hospital over a period of 6 months (May-October 2017). All *Staphylococcus* grown among 240 various clinical samples received were tested for MRSA by four phenotypic methods - cefoxitin and oxacillin disc diffusion test and MIC oxacillin E test and oxacillin resistant screen agar test (ORSA) with mecA-PCR as gold standard.

RESULTS

A total of 103 out of 200 (51.5%) bacterial isolates were *Staphylococcus aureus*. Pus from diabetic wound yielded high number of growth (42%) followed by blood (36%) which is alarming note. 55.4% were identified as MRSA by PCR; most common presenting group was >50 years (36.8%) followed by infants (28%). Cefoxitin disc method has the highest sensitivity and specificity of 98.2% & 100% followed by E test (94.2% & 95.8%) and ORSA (87.5% & 94.2%). Oxacillin disc diffusion method had the least 79.5% & 92.9%. Most MRSA isolates were multi drug resistant.

CONCLUSION

Cefoxitin disc diffusion when combined with Oxacillin agar or E test will be helpful to detect all MRSA strains including hyper production of betalactamases. As these phenotypic assays are simple and relatively cheap, this can be used as an alternative to PCR in resource constraint settings.

KEYWORDS

MRSA; Prevalence; mecA Gene; Cefoxitin Disc; Phenotypic Tests.

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BACKGROUND

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is an important cause of nosocomial and community associated infections throughout the world.⁽¹⁾ First MRSA was described among nosocomial isolates of *Staphylococcus aureus* in 1961, England which subsequently spread throughout the world.⁽²⁾ In India the prevalence of MRSA ranges from 30 to 70% which leads to high mortality, increase economic burden.^(3,4)

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MRSA treatment will be more problematic as these strains often show resistance to wide range of antibiotics compared to methicillin susceptible isolates.⁽³⁾ Therefore, rapid and accurate detection of MRSA is very essential in order to choose appropriate therapy, to take necessary measures for infection control and also to prevent unnecessary use of glycol-peptides antibiotics.

MRSA strains harbour the mecA gene, which encodes a penicillin binding protein (PBP2a) with low affinity for all β -lactam antibiotics including methicillin which has limited therapeutic option.⁽⁵⁾ Hence methods used to identify MRSA from clinical samples should have high sensitivity and specificity and most importantly the result should be available within a short time. Different phenotypic methods such as oxacillin and cefoxitin disc diffusion test, oxacillin agar screening test, and determination of minimum inhibitory concentration (MIC) for oxacillin and cefoxitin are available in clinical laboratories.⁽⁶⁻⁹⁾ Phenotypic expression is affected by various conditions such as temperature,

osmolarity of the medium and inoculum size that may affect the accuracy of the methods used to detect methicillin resistance.^(9,10) Some strains of *S.aureus* hyper produce beta lactamase known as Borderline Oxacillin Resistant *S.aureus* (BORSA) will be oxacillin resistant, do not possess the usual genetic mechanism for MRSA resistance.⁽⁸⁾

Even though genotypic method which detects the *mecA* gene to identify MRSA among the *S.aureus* isolates is considered to be the gold standard test for detecting MRSA,^(6,7,10) it could not be adopted as a routine method of screening MRSA in small laboratories since it requires costlier and sophisticated equipment. Hence the easily available, non-expensive phenotypic methods are the one needed to identify the MRSA isolates for many labs.

Our study is planned to know the prevalence of MRSA among different clinical samples received in laboratory with the aim to compare the PCR of the *mecA* gene with four phenotypic methods - cefoxitin disc diffusion test, oxacillin disc diffusion test and MIC of oxacillin by E test and screen agar test for detection of MRSA.

MATERIALS AND METHODS

Prospective observational study was carried out in Velammal Medical College Hospital and Research Institute, Madurai, Tamilnadu over a period of 6 months (May -October 2017).

A total of 240 samples received in the lab during the study period were tested for *Staphylococcus aureus* from various clinical samples like blood, pus, urine, sputum and throat swab. Isolates were identified as *S. aureus* by their colony morphology, gram staining, catalase and coagulase tests (both tube and slide coagulase tests).

All the *Staphylococcal* isolates were tested for antibiotic susceptibility by the Kirby-Bauer disc diffusion method. Following antibiotics were selected based on clinical and laboratory standards Institute (CLSI) guideline: Penicillin (10 u), Erythromycin (15 µg), Clindamycin (2 µg), Ciprofloxacin (5 µg), Cotrimoxazole (1.25/23.75 ug), Amikacin (30 µg), Oxacillin (1 µg), Cefoxitin (30 µg) and Linezolid (30 µg), Vancomycin E strip. Antibiotic discs and E strips were bought from Hi-Media diagnostic laboratory, Mumbai.⁽¹¹⁾

Study Design

Phenotype Identification of MRSA:

Phenotypic methods for detection of MRSA strains were carried out according to CLSI guideline as follows:

Cefoxitin Disc Diffusion Test

Cefoxitin (30 µg) disc diffusion method was carried out on Mueller-Hinton agar (MHA) by using a 30 µg cefoxitin disc. The standardised inoculum was streaked on MHA and incubated at 37° C for 24 hrs. An inhibition zone diameter of ≤ 21 mm was reported as methicillin resistant and a diameter of ≥ 22 mm was considered as methicillin sensitive

Oxacillin Disc Diffusion Test

Oxacillin (1 µg) disc diffusion method was carried out on Mueller-Hinton agar supplemented with 4% NaCl. Plates were incubated at 35° C for 24 hrs. The isolates were considered as resistant when the diameter of inhibition zone of oxacillin was ≤10 mm, as intermediate when the diameter was 11-12 mm and as sensitive when the diameter was ≥13 mm.

Oxacillin E Strip Test

In Muller-Hinton agar plates supplemented with 4% NaCl, the test strain was lawn cultured, oxacillin E strip was placed on the medium and incubated for 24 h at 35° C. After incubation, inhibitory concentration studied as zone of inhibition intersected the strip in the form of ellipse and interpreted according to the CLSI criteria. MIC of ≤2µg/ml was considered as sensitive; ≥4µg/ml was considered as resistant.

Oxacillin Resistant Screen Agar Test (ORSA)

Muller-Hinton agar plates containing 4% NaCl, 6 µg/ml of oxacillin and chromogenic component aniline dye were prepared. Agar base were obtained from Himedia, Mumbai. A loopful of 0.5 McFarland's suspension of the isolate was inoculated as a spot on the agar surface and it was incubated at 35° C for 24 h. The plates were observed for colour change from original grey to blue to identify as MRSA.

Genotype Identification of MRSA by PCR

Genotypic identification of *mecA* gene was carried out by conventional Polymerase Chain Reaction (PCR) using thermocycler in Helini Biomolecules, Chennai. DNA was extracted by the rapid cell lysis method using Helini extraction kit- 1.5 mL of an overnight culture of bacteria grown in Mueller-Hinton broth was harvested by centrifuging in a micro centrifuge tube at 10,000 rpm for 5 mins. 400 µL of lysis buffer with 40 µL of proteinase K was added to the pellet and incubated at 70°C for 10 minutes. After adding 100 µL of isopropanol, the entire sample was pipetted into pure fast spin column and centrifuged for 1 min. Flow through was discarded and spin column washed with 500 µL wash buffer 1 & 2 and then 100 µL of pre-warmed buffer EB added and incubated for 2 mins. After the final centrifugation extracted bacterial DNA was collected for PCR assay.

PCR for *mecA* gene was performed using the following primers- Forward (5' GCA ATC GCT AAA GAA CTA AG 3') and Reverse (5'GGG ACC AAC ATA ACC TAA 3') primers in the thermocycler (Eppendorf Master Cycler Gradient thermocycler, Germany) with a final reaction mixture volume of 50µl. Cycling conditions were 94° C-3 mins of initial denaturation followed by 30 cycles of Denaturation at 94° C-1 min, Annealing at 60° C- 1 min, Extension at 72° C- 1 min and a final extension of 72° C- 5mins. The amplicon for *mecA* gene was detected by the band at 220 bp on an agarose gel (2%) with ethidium bromide by electrophoresis.

Statistical Analysis

Data analysis was done using SPSS 16 version. Comparison of all phenotypic methods were carried out taking *mecA* PCR as a gold standard technique, sensitivity and specificity were expressed as percentage using Chi square test. Prevalence, Age, sex and risk factors analysis were expressed as percentage.

RESULTS

Among the 240 samples received during the study period, 200 samples showed growth. Total of 240 isolates were detected from those 200 samples. A total of 103 samples out of 200 (51.5%) yielded the growth of *Staphylococcus aureus* during the study period. Pus sample (40/103= 38.8%) followed by blood sample (37/103=35.9%) yielded the highest number of *S.aureus* growth (Figure 1).

Out of 103 *S.aureus* isolates 57(55.4%) were Methicillin Resistant *S.aureus* (MRSA) and 46 (44.6%) were Methicillin Sensitive *S.aureus* (MSSA) as detected by taking PCR as a gold standard technique. Sample wise analysis of MRSA showed that 29 out of 40 (72.5%) *S.aureus* strains isolated from pus followed by 19 out of 37(51.4%) from blood were MRSA (Table 1).

Age wise distribution analysis revealed that MRSA were most common among the older age group (21/57= 36.8%) followed by infants (28%) (Figure 2).

Among the 57 MRSA isolates 16(28.06%) were associated with risk factors. Analysis of risk factors revealed that 10 patients (17.54%) of MRSA wound infection had diabetes; 3 patients (5.26%) had chronic liver disease and 2 patients (3.5%) had a history of intermittent catheterisation; 1 patient (1.75%) had a history of prolonged self-medication with Quinolone antibiotic.

All the 103 *S.aureus* isolates were subjected to PCR to identify *mecA* gene. Among these 57 (55.4%) were identified as MRSA by PCR.

All the 103 *S.aureus* isolates were also subjected to methicillin susceptibility testing by disc diffusion method by using 30 µg Cefoxitin and 1 µg Oxacillin discs, E-Test (oxacillin), Oxacillin Resistant Screen Agar. Cefoxitin disc diffusion method identified 56 (54.4%) isolates of *S.aureus* as MRSA followed by Oxacillin E-Test identified 54 (52.4%) *S.aureus* strains as MRSA (Table 2).

Comparison of all phenotypic methods were carried out taking PCR as a gold standard technique. Among the 57 PCR positive MRSA strains, Cefoxitin disc identified one isolate as negative (false negative) (Table 3).

Cefoxitin disc method has the highest sensitivity (98.2%) and specificity (100%) and the PPV and NPV are 98.24% and 100% respectively (Table 4).

Most of our MRSA isolates were multi drug resistant (MDR) as 91% resistant to fluoroquinolones, 88% resistant to aminoglycosides, 83% cotrimoxazole (Table 5).

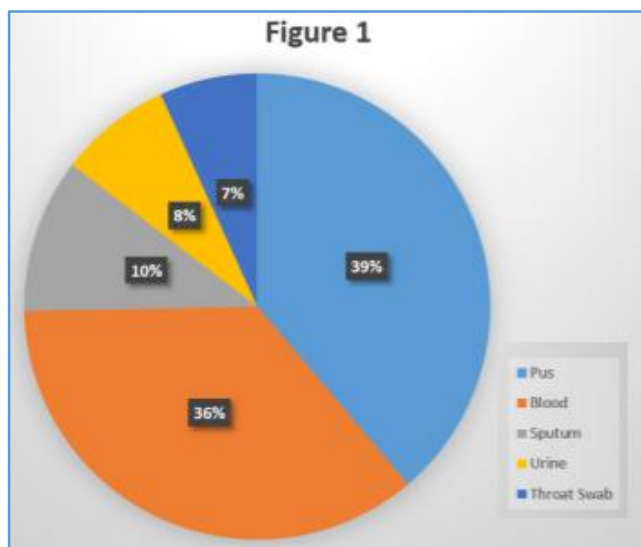


Figure 1. *Staphylococcus aureus* isolates among various clinical samples

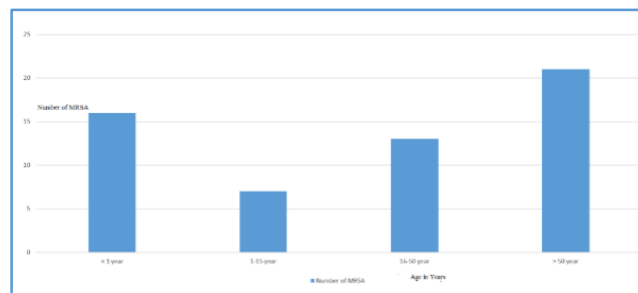


Figure 2. *Staphylococcus aureus* isolates among various age groups

Specimen	<i>S.aureus</i>	MRSA	Percentage
Pus	40	29	72.5%
Blood	37	19	51.4%
Sputum	11	4	36.4%
Throat swab	8	2	25%
Urine	7	3	42.91%
Total	103	57	55.3%

Table 1. Sample wise distribution of Methicillin Resistant *Staphylococcus aureus* (MRSA)

Test (n=103)	MRSA	MSSA
PCR (<i>mecA</i> gene)	57 (55.4%)	46 (44.6%)
Cefoxitin Disc	56 (54.36%)	47 (45.63%)
E-Test	54 (52.42%)	49 (47.57%)
ORSA	51 (49.51%)	52 (50.48%)
Oxacillin Disc	48 (46.6%)	55 (53.39%)

Table 2. Detection of Methicillin Resistant *Staphylococcus aureus* (MRSA) by various phenotypic and genotypic method

Methods	True Positives	False Negatives	False Positives
PCR	57	-	-
Cefoxitin disc	55	1	-
E-Test	49	3	2
ORSA	42	6	3
Oxacillin disc	35	9	4

Table 3. Comparison of PCR for *mecA* gene with phenotypic methods

TEST	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
Cefoxitin disc	98.2%	100%	98.24%	100%
E-Test	94.2%	95.8%	96.42%	94.23%
ORSA	87.5%	94.2%	92.44%	90.22%
Oxacillin disc	79.5%	92.9%	88.30%	82.93%

Table 4. Sensitivity and Specificity of various phenotypic tests

	P	CD	E	CIP	AK	COT	VA	LZ
MRSA	57	32	37	52	50	47	0	0
N=57	(100%)	(56.1%)	(64.9%)	(91.2%)	(87.7%)	(82.5%)	-	-

Table 5. Other antibiotic resistance pattern of MRSA isolates

P-Penicillin (10 u), CD- Clindamycin (2 µg), E-Erythromycin (15 µg), CIP-Ciprofloxacin (5 µg), AK- Amikacin (30 µg), COT- Cotrimoxazole (1.25/23.75 ug), VA- Vancomycin E strip, LZ-Linezolid (30 µg).

DISCUSSION

For the past 50 years, MRSA is probably the most challenging bacterial pathogen causing significant morbidity and mortality that affect patients in hospital as well as in the community.⁽¹²⁾ Methicillin-resistant strains in all over the world have been posing a great difficulty in selecting antimicrobial agents for the management of the infections that leads to treatment failure. Hence, an accurate and rapid detection of methicillin resistance in *Staphylococci* is therefore important, not only for choosing the appropriate antibiotic therapy, but also to control of the endemicity of the MRSA.^(2,3)

In our study, 51.5 % (103/200) of the isolates were *Staphylococcus aureus* which was isolated as the prime organism in all infections except urinary tract infection. Occurrence of *S.aureus* as the prime organism may be due to the presence of a number of enzymes and toxins produced by *S.aureus* which may inhibit the phagocytic and opsonic mechanisms.^(1,12) Less frequent occurrence of *S.aureus* in UTI may be due to genotypic factors of *S.aureus* which may affect the uroepithelial susceptibility to the adherence molecules.⁽¹³⁾

Our study revealed *S.aureus* was the common isolate from pus samples from localised diabetic wound lesions (39%) which was in concordance with the findings of dechen C Tseringet al.,⁽¹⁴⁾ who also reported 42% of isolation from pus samples. Localisation of pus is mainly due to the unique presence of coagulase in *S.aureus* which has the capacity of forming a wall of fibrin clot around the lesion. The challenging thing is nearly 36% of *S.aureus* was isolated from blood which was even higher than the other studies.^(15,16) Bacteraemia due to *S.aureus* has increased dramatically in recent years which may end up in life-threatening complications like infective endocarditis and metastatic infections. This is driving as urgent need for improved strategies to prevent these infections.

In our study 55.4% were MRSA which was much higher than other studies,^(3,17,18) which indicates MRSA is steadily increasing which may be due to the adverse use of antibiotics or poor infection control practices in the environment. Among MRSA, 28.15% were isolated from pus samples of diabetic wound infection which is in concordance with finding of sgangaet al.,⁽¹⁹⁾ reported 30% of MRSA. The increased incidence of MRSA in wound infection may be due to the production on Panton Valentine Leucocidin by MRSA which is shown too associated with tissue necrosis.

Age wise analysis revealed MRSA was commonly present in the extremes of age which is similar to the other study results.^(20,21) Higher incidence in older age group may be due to immunosuppression caused by various factors like diabetes mellitus, chronic hepatitis, anaemia and non-adherence to antibiotic course and infants there may be poor development of immune system which may associated with acquired chemotactic or opsonic defects

Early and accurate determination of Methicillin resistance is of key importance in the prognosis of infections caused by *S.aureus*. Although many phenotypic methods of detection of this resistance have been developed they often show insufficient sensitivity and specificity to ensure appropriate treatment of the MRSA infected patients.^(1,8,13) The sensitivity and specificity of these methods vary depending on the patients carrying them, environmental factors, and the techniques used.

Our study revealed disc diffusion using cefoxitin disc showed higher sensitivity (98.2%) and specificity (100%) which is almost similar to the study results of Pourmand et al.,⁽⁹⁾ who also documented 99.1% sensitivity and 98.1% specificity by cefoxitin disc method. Even Swenson et al., Jain et al.,^(22,23) showed 100% specificity and sensitivity with cefoxitin disc methods. Our findings also emphasize that cefoxitin disc method is close enough to PCR in identifying MRSA.

Oxacillin disc diffusion method had the least sensitivity (79.5%) and specificity (92.9%) followed by ORSA 87.5% sensitivity and 94.2% specificity. E test had better sensitivity and specificity (94.2% & 95.8%) when compare to ORSA and oxacillin disc but inferior to cefoxitin disc diffusion test. This may be due to the fact that detection of MRSA by Cefoxitin based methods will not be affected by temperature variations between 35^o C and 37^o C but Oxacillin method will be affected by temperature variations especially if it is increased above 35^o C and also medium supplementations. Similar factors decrease the sensitivity and specificity of ORSA and E-Test.⁽²⁴⁾

False positivity (resistant to oxacillin but sensitive to cefoxitin and negative for mecA gene) in ORSA methods and E-Test is due to the hyper production of betalactamase which may lead to phenotypic expression of oxacillin resistance do not possess the usual genetic mechanism for such resistance. Probably these strains under antibiotic pressure may evolve into fully resistant isolates subsequently.⁽²⁵⁾

One more problematic fact is most of the MRSA isolates were multi drug resistant (MDR). Isolates have shown 91% resistant to fluoroquinolones, 88% resistant to aminoglycosides, 83% cotrimoxazole and more than 50 % to macrolides. This is an alarming note as only few therapeutic opinions available. So early diagnosis is will be helpful to avoid the unnecessary use of antibiotics.

CONCLUSION

Our study findings reveal that oxacillin disk diffusion method was found to be less sensitive for the detection of MRSA. Cefoxitin disc diffusion followed by Oxacillin E strip was in concordance with the results of PCR for mecA gene. As cefoxitin method is easy to perform, does not require special technique, media preparation, incubation temperature and more cost effective in comparison to other methods this can be used as an alternative to PCR for the detection of MRSA in resource constraint settings. Cefoxitin when combined with Oxacillin agar or E test will be helpful to determine the all MRSA strains including hyper production of betalactamase.

REFERENCES

- [1] Stefani S, Chung DR, Lindsay JA, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA): global epidemiology and harmonisation of typing methods. *Int J Antimicrob Agents* 2012;39(4):273-82.
- [2] Brown DF. Detection of methicillin/oxacillin resistance in staphylococci. *J Antimicrob Chemother* 2001;48 (Suppl 1):65-70.
- [3] Rajaduraiipandi K, Mani KR, Panneerselvam K, et al. Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus*: a multicentre study. *Indian J Med Microbiol* 2006;24(1):34-8.

- [4] Saikia L, Nath R, Choudhury B, et al. Prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* in Assam. *Indian J Crit Care Med* 2009;13(3):156-8.
- [5] Hartman BJ, Tomasz A. Low-affinity penicillin-binding protein associated with beta-lactam resistance in *Staphylococcus aureus*. *J Bacteriol* 1984;158(2):513-6.
- [6] Gradelski E, Valera L, Aleksunes L, et al. Correlation between genotype and phenotypic categorization of *Staphylococci* based on methicillin susceptibility and resistance. *J Clin Microbiol* 2001;39(8):2961-3.
- [7] Koupahi H, Jahromy HS, Rahbar M. Evaluation of different phenotypic and genotypic methods for detection of Methicillin Resistant *Staphylococcus aureus* (MRSA). *Iran J Pathol* 2016;11(4):370-6.
- [8] Farahani A, Mohajeri P, Gholamine B, et al. Comparison of different phenotypic and genotypic methods for the detection of Methicillin-Resistant *Staphylococcus Aureus*. *North Am J Med Sci* 2013;5(11):637-40.
- [9] Pourmand MR, Hassanzadeh S, Mashhadi R, et al. Comparison of four diagnostic methods for detection of methicillin resistant *Staphylococcus aureus*. *Iran J Microbiol* 2014;6(5):341-4.
- [10] Baddour MM, AbuElKheir MM, Fatani AJ. Comparison of *mecA* polymerase chain reaction with phenotypic methods for the detection of methicillin-resistant *Staphylococcus aureus*. *Curr Microbiol* 2007;55(6):473-9.
- [11] Clinical Laboratory Standards Institute. New Antimicrobial Susceptibility Testing Standards - CLSI <http://clsi.org/blog/2015/01/08/clsi-publishes-new-antimicrobial-susceptibility-testing-standards/>
- [12] Vidhani S, Mehndiratta PL, Mathur MD. Study of methicillin resistant *S. aureus* (MRSA) isolates from high risk patients. *Indian J Med Microbiol* 2001;19(2):13-6.
- [13] Mohanasoundaram KM, Lalitha MK. Comparison of phenotypic versus genotypic methods in the detection of methicillin resistance in *Staphylococcus aureus*. *Indian J Med Res* 2008;127(1):78-84.
- [14] Tsering DC, Pal R, Kar S. Methicillin-Resistant *Staphylococcus Aureus*: prevalence and current susceptibility pattern in Sikkim. *J Glob Infect Dis* 2011;3(1):9-13.
- [15] Naber CK. *Staphylococcus aureus* bacteremia: epidemiology, pathophysiology and management strategies. *Clin Infect Dis* 2009;48 (Suppl 4):S231-S7.
- [16] Vijayamohan N, Nair SP. A study of the prevalence of methicillin-resistant *Staphylococcus aureus* in dermatology inpatients. *Indian Dermatol Online J* 2014;5(4):441-5.
- [17] Saxena S, Singh K, Talwar V. Methicillin-resistant *Staphylococcus aureus* prevalence in community in the east Delhi area. *Jpn J Infect Dis* 2003;56(2):54-6.
- [18] Pillai MM, Latha R, Sarkar G. Detection of methicillin resistance in *staphylococcus aureus* by polymerase chain reaction and conventional methods: a comparative study. *J Lab Physicians* 2012;4(2):83-8.
- [19] Sganga G, Tascini C, Sozio E, et al. Early recognition of methicillin-resistant *Staphylococcus aureus* surgical site infections using risk and protective factors identified by a group of Italian surgeons through Delphi method. *World J Emerg Surg* 2017;12:25.
- [20] Madani TA. Epidemiology and clinical features of methicillin-resistant *Staphylococcus aureus* in the University Hospital, Jeddah, Saudi Arabia. *Can J Infect Dis* 2002;13(4):245-50.
- [21] Sangvik M, Olsen RS, Olsen K, et al. Age-and gender-associated *Staphylococcus aureus spa* types found among nasal carriers in a general population: the Tromsø Staph and Skin study. *J Clin Microbiol* 2011;49(12):4213-8.
- [22] Swenson JM, Tenover FC, CDS Group. Results of disk diffusion testing with cefoxitin correlate with presence of *mecA* in *Staphylococcus spp.* *J Clin Microbiol* 2005;43(8):3818-23.
- [23] Jain A, Agarwal A, Verma RK. Cefoxitin disc diffusion test for detection of methicillin-resistant staphylococci. *J Med Microbiol* 2008;57(Pt 8):957-61.
- [24] Broekema NM, Van TT, Monson TA, et al. Comparison of cefoxitin and oxacillin disk diffusion methods for detection of *mecA*-mediated resistance in *Staphylococcus aureus* in a Large-Scale Study. *J Clin Microbiol* 2009;47(1):217-9.
- [25] Maalej SM, Rhimi FM, Fines M, et al. Analysis of Borderline Oxacillin-Resistant *Staphylococcus aureus* (BORSA) strains isolated in Tunisia. *J Clin Microbiol* 2012;50(10):3345-8.