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# Phenotypic methods of greater accuracy to detect the mecA gene product for the recognition of MRSA in resource constraint settings

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ABSTRACT

**Objective:** To evaluate the detection of methicillin resistant *Staphylococcus aureus* (MRSA) and analyze the performance of Mastalex MRSA (Mast, UK). **Methods:** Two hundred and ten *Staphylococcus aureus (S. aureus)* strains were isolated from different clinical samples and were tested for methicillin resistance by Oxacillin (1 μg) and Cefoxitin (30 μg) disc diffusion, oxacillin agar screen, and minimum inhibitory concentration of oxacillin and cefoxitin. *S. aureus* isolates were grown on the blood agar and mannitol salt agar with (2 mg/L) and without oxacillin for the analysis of Mastalex MRSA. **Results:** Out of 210 *S. aureus* strains tested, 103 strains were detected as methicillin resistant by Cefoxitin disk diffusion, Cefoxitin minimal inhibitory concentration(MIC) and Mastalex MRSA test. Whereas oxacillin disc diffusion and oxacillin agar screen detected 91 and 97 MRSA respectively. The Cefoxitin MIC test performance was equivalent to Cefoxitin disc diffusion. 103 (100%) strains grown on blood agar without and with oxacillin, and 76 (74%) and 93 (91%) strains grown on mannitol salt agar without and with oxacillin shown positive agglutination with Mastalex MRSA test respectively. **Conclusions:** The cefoxitin disk diffusion/ Mastalex MRSA is very suitable for detection of MRSA and the tests can be an alternative to PCR for detection of MRSA in resource constraint settings. Mastalex test would be particularly useful when confirmation of resistance is urgently required.

## 1. Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) strains emerged soon after the prologue of methicillin into clinical practice[1]. Clinical professionals depend on the laboratory for the reliable and early detection of MRSA in clinical specimens. This has connotations for the treatment of persistent infections, pre-operative prophylaxis, and infection control procedures. Surveillance of MRSA is also dependent on precise laboratory reporting.

Several studies have reported that the Cefoxitin disk diffusion (DD) test is a good alternative method for detection of mecA-gene-mediated methicillin resistance[2-4] .The Clinical and Laboratory Standards Institute (CLSI) guidelines (2006) has recommended 30 μg cefoxitin disc diffusion method for the detection of MRSA[5].

PCR, a gold standard method for detecting the mecA gene will provide results within a few hours, but are not accessible in most laboratories and test expenses are relatively high. A rapid latex agglutination test based on detection of PBP2a has been described[6]. The method employs latex particles coated with monoclonal antibodies to PBP2a, which is extracted from test colonies. With colonies of a wide range of MRSA grown on blood agar, a sensitivity of 98.5-100% and a specificity of 100% have been reported[7-9]. The Mastalex™ MRSA kit (Mast, UK) MRSA screening test is a commercially available, rapid slide latex agglutination test for the detection of PBP2a.

The study aim was to evaluate several phenotypic methods, including a commercial latex agglutination kit the 'Mastalex MRSA' that detects the mecA gene product, for the detection of methicillin resistance in *Staphylococcus aureus (S. aureus)* and to analyze the performance of the Mastalex MRSA screening test, with *S. aureus* isolates grown on blood agar and mannitol salt agar medium with and without oxacillin.

Similar studies have not been performed before in Ethiopia.

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## 2. Materials and methods

Two hundred and ten *S. aureus* strains were isolated from different clinical samples like blood, CSF, pus, sputum and urine.

Samples were processed at School of Medical Laboratory Technology, Jimma University, Jimma, and at School of Medical Laboratory Technology, Haramaya University, Harar, and collected from Jimma hospital, Jimma, Ethiopia and, from Hiwot Fana hospital, Jogol hospital and Regional laboratory, Harar, Ethiopia.

All samples were cultured on blood agar and MacConkey's agar plate. These plates were incubated at 37 ℃ for 24 hours; the isolates of *S. aureus* were identified by colonial morphology on blood agar plates, gram stain characteristics, catalase test, coagulase positive test, mannitol fermentation, and by voges proskauer test. All media and antibiotics used were from Oxoid, Basingstoke, UK.

### 2.1. Disk diffusion test[5]

All the confirmed *S. aureus* strains were subsequently tested for methicillin resistance by Oxacillin (1 μg) and Cefoxitin (30 μg) Disk Diffusion (DD) test as per standard guidelines. A 0.5 Mc Farland standard suspension of the isolate was made and lawn culture done on Mueller Hinton Agar plate. Plates were incubated at 37 ℃ and zone diameters were measured. Zone diameters as recommended by Clinical and Laboratory Standards Institute (CLSI) were read both at 18 h and 24 h. Cefoxitin inhibition zone diameter of ≤19 mm was reported as oxacillin resistant and ≥20 mm was considered as oxacillin sensitive. For oxacillin, the isolates were considered methicillin resistant if the zone of inhibition was 10 mm or less.

### 2.2. Oxacillin agar screen test[10].

All MRSA isolates were spot inoculated onto a Mueller-Hinton agar plate. Supplemented with 6 μg of oxacillin per mL and 4% NaCl by using a cotton swab dipped into a 0.5 McFarland standard suspension of each test isolate. The plates were incubated at 37 ℃ for 24 h. If any growth was detected, the isolate was considered oxacillin resistant.

### 2.3. MIC of oxacillin and cefoxitin[10]

The CLSI broth macro dilution (BMD) reference method was used to determine the MIC of Oxacillin and Cefoxitin. (MIC cut off criteria as recommended by CLSI for Oxacillin less than or equal to 2 μg/mL for susceptible and greater than or equal to 4 μg/mL for resistance. Modified breakpoint criteria for Cefoxitin less than or equal to 4 μg/mL for susceptible and greater than or equal to 8 μg/mL for resistance). Isolates that had MIC value of greater than or equal to 4 μg/mL for Oxacillin and greater than or equal to 8 μg/mL for Cefoxitin were taken as Methicillin resistant.

### 2.4. Analyzing the performance of the Mastalex™ MRSA kit

*S. aureus* isolates were grown on the following media for the analyzing of Mastalex MRSA latex agglutination method: Blood Agar with 5% sheep blood; blood agar containing oxacillin 2 μg/mL; Mannitol Salt Agar containing oxacillin 2 μg/mL. Plates were incubated for 18 h in air at 37 ℃ except for blood agar with oxacillin 2 μg/mL, which was incubated for 18 h at 30 ℃.

### 2.5. Mastalex MRSA latex agglutination method

The Mastalex MRSA method (Mast, UK) was used according to the manufacturer's instructions. Briefly, 10 colonies were suspended in 200 μL 'extraction reagent 1' and heated in a boiling water bath for 3 minutes. Tubes were cooled and 50 μL 'extraction reagent 2' was added. Tubes were centrifuged at 1 500×3 g for 5 minutes. 50 μL of supernatant were mixed with 50 μL sensitized latex suspension and rotated manually for 3 minutes while looking for agglutination. The supernatant was tested simultaneously with a negative control latex suspension. The time at which agglutination was visible by eye was recorded.

Quality control strains-methicillin sensitive *S. aureus* (MSSA) ATCC 25923 and methicillin resistant *S. aureus* (MRSA) ATCC 43300- were used as negative and positive controls, respectively[5].

## 3. Results

Out of 210 (100%) *S. aureus* strains tested for methicillin resistance, 103 (49%) strains were detected as methicillin resistant by Cefoxitin DD, Cefoxitin MIC and Mastalex MRSA test.

Comparison of phenotypic methods for the routine detection of MRSA is tabulated in Table 1. All methods showed 100% specificity.

**Table 1**
Comparison of conventional and non-conventional phenotypic testing methods for MRSA using a Mastalex™ MRSA kit as a reference standard.

| Phenotypic test method | Detected as MRSA | Sensitivity (%) |
|---|---|---|
| Oxacillin DD (1 μg) | 91 | 88.3 |
| Oxacillin agar screen (6 μg) | 97 | 94.2 |
| Cefoxitin DD (30 μg) | 103 | 100.0 |
| Oxacillin MIC test | 100 | 97.0 |
| Cefoxitin MIC test | 103 | 100.0 |
| Mastalex MRSA | 103 | 100.0 |

Oxacillin agar screen method detected 94.2% strains including six strains of *S. aureus* which had oxacillin zone diameters of 11-12 mm. These strains were further tested by Cefoxitin DD method. No difference in zone diameters was seen at 18 hrs and 24 hrs.

Oxacillin and Cefoxitin MIC's results were shown and compared in the Table 2.

The use of Cefoxitin MIC test performance was equivalent to Cefoxitin DD when using modified breakpoints of less

than or equal to 4 μg/mL for susceptible and greater than or equal to 8 μg/mL for resistance.

Out of 103 cases with Mastalex positive results, 68 had zone diameter <14 mm, 32 had 15 mm, 2 had 16 mm and 1 had 17mm; while out of 107 negative cases, 2 had 21 mm, 11 had 22 mm, 46 had 23 mm, and 48 had 24 mm.

**Table 2**

Oxacillin and Cefoxitin MIC's results.

| | Total No. | MIC (μg/mL) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 |
| Oxacillin | 210 | 9 | 30 | 48 | 23 | 1 | 35 | 48 | 16 |
| Cefoxitin | 210 | 0 | 0 | 0 | 75 | 32 | 1 | 35 | 67 |

Mastalex MRSA latex agglutination test results showed $10^3$ (100%) strains grown on blood agar without and with 2 μg/mL oxacillin have positive agglutination in an average time of 47 seconds and 21 seconds respectively, whereas only 76 (74%) and 93 (91%) strains grown on mannitol salt agar without and with 2 μg/mL oxacillin displayed positive agglutination in an average time of 98 and 84 seconds respectively.

Tests with 27 (26.2%) and 10 (9.7%) of the 103 MRSA gave false-negative results with colonies from the MSA media without and with oxacillin respectively. These tests were significantly less reliable than tests on colonies from blood agar.

All agglutination reactions with colonies from blood agar were positive within the 3 minutes specified by the manufacturer. All methicillin-susceptible isolates were mecA negative and did not grow on media containing oxacillin. No methicillin-susceptible isolate, was positive with the Mastalex test. All MRSA were mecA positive.

## 4. Discussion

Detection of mecA gene or its product, penicillin binding proteins (PBP2a), is considered the gold standard for MRSA confirmation[11]. Recent studies indicate that disc diffusion testing using cefoxitin disc is far superior to most of the currently recommended phenotypic methods like oxacillin disc diffusion and oxacillin screen agar testing and is now an accepted method for the detection of MRSA by many reference groups including CLSI[12]. The accurate and early determination of methicillin resistance is of key importance in the prognosis of infections caused by *S. aureus*. In this study, we attempted to evaluate different methods for detection of mecA.

Several studies have been done to investigate the utility of Cefoxitin DD for detection of MRSA[13,14]. In our study, the observations using the CLSI disc diffusion criteria to define resistance showed the sensitivity and specificity were 100% in the 210 strains tested in the study whereas the results of oxacillin DD test and screen agar were not so accurate.

The results of Cefoxitin DD method were better for isolates with Oxacillin MIC between 4-8 μg/mL. Our study also revealed that low level Oxacillin resistance was detected better by Cefoxitin DD test.

Results of cefoxitin disc diffusion test is in concordance with the Mastalex™ MRSA kit. Anand *et al* reported a good correlation between cefoxitin disc diffusion test and PCR[15], a good correlation also reported between Mastalex™ MRSA kit[16,17] /other trade mark rapid latex agglutination MRSA screening method and PCR[7-9].

Detection of the mecA product, PBP2a, was a highly sensitive and specific technique for the detection of methicillin resistance in *S. aureus*[15]. Several workers have corroborated the high sensitivity and specificity of MRSA detection with this method, even in strains with ambiguous and borderline oxacillin resistance[7,17,18].

From a clinical perception, it is important to differentiate isolates that have mecA-positive resistance from the infrequently encountered isolates that have borderline resistance because it may affect therapy. Strains that possess mecA-classic resistance are either heterogenous or homogenous in their expression of resistance. It is the testing of heteroresistant isolates which may appear as susceptible. The presence of resistance in *S. aureus* isolates on an oxacillin screen agar plate generally means that the isolate is mecA positive. Occasionally, however, heteroresistant mecA positive strain is not detected due to low expression of resistance. Oxacillin agar screen generally does not detect borderline resistant strains, when studies have included strains whose resistance is heterogeneous the test has been shown to perform less well. Also, agar dilutions and oxacillin disc diffusion method may be affected by various components of mueller hinton agar, temperature, and duration of incubation[19].

Presence of methicillin, oxacillin or sodium chloride in selective media may affect the reliability of the test with some strains, particularly those which are distinctly heterogeneous and do not grow well on selective media. Addition of oxacillin to mannitol salt agar improved the reliability of tests, false-negative results decreased from 26.2% (without antibiotic) to 9.7% (with antibiotic).

For Mastalex™ MRSA latex kit tests we rotated slide manually, which may give more efficient mixing and hence more rapid agglutination. However, the full 3 minutes of rotation was needed for some strains and this is tedious if done manually.

The mean times to read a positive result were markedly lower for tests on colonies from blood agar than from those on mannitol salt agar. Agglutination times were also reduced on media to which oxacillin was added. This is presumably related to induction of PBP2a production by growth in the presence of oxacillin, leading to a higher concentration of PBP2a in the agglutination reaction.

The blood agar plates with and without oxacillin were incubated at 30 and 37 ℃, respectively, and the lower temperature of incubation of the plates with oxacillin may have contributed to better expression of PBP2a and consequently more rapid agglutination results with colonies from this medium.

Mastalex latex agglutination test for methicillin resistance in *S. aureus* is highly dependable and reasonably rapid if colonies are grown on blood agar, with or without added

oxacillin. Caution is needed when the method is used with colonies grown on mannitol salt agar.

Detecting mecA gene characterization by PCR/PBP2a is recognized as gold standard for detection of MRSA. However, use of PCR assay is generally limited to reference laboratories, especially in developing countries. Thus the cefoxitin disk diffusion method/ Mastalex MRSA screening method is very suitable for detection of MRSA and the test can be an alternative to PCR for detection of MRSA in resource constraint settings.

Our study clearly showed, the substitution of a Cefoxitin DD for an Oxacillin DD test, will result in an easier to read test with greater accuracy for detection of Methicillin resistance in *S. aureus*.

Mastalex latex agglutination test would be particularly useful when confirmation of resistance is urgently required.

## Conflict of interest statement

We declare that we have no conflict of interest.

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