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Detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA) using Modified Conventional Cefoxitin-based Media as an Alternative Screening

Sri Amelia[1]*, Muhammad Fakhrur Rozi[2] and Ridwan Balatif[3]

[1]Department of Microbiology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.
[2]Department of Internal Medicine, Faculty of Medicine Universitas Gadjah Mada, Daerah Istimewa Yogyakarta, Indonesia.
[3]Faculty of Medicine Universitas Sumatera Utara, Medan, Indonesia.

Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) can cause infection with higher morbidity and mortality. In the limited-resource setting, the availability of rapid detection is scarce and may not be available; therefore, prompt detection using the alternative method is indispensable. To compare the detection rate of the modified-conventional method with chromogenic media against mucocutaneous clinical swab, a total of 80 *S. aureus* isolates from previous studies were cultivated and re-cultured into routine media such as blood agar (BA) and mannitol-salt agar (MSA) between June and September 2018. It directly inoculated from plain blood agar that had been incubated previous day before; it further underwent inoculation to other media, such as chromogenic media (CHROMagar), blood agar and mannitol salt agar that had been supplemented with cefoxitin (CFOX) powder manually. The sensitivity MSA-CFOX and BA-CFOX, respectively, was 96.88%. On the other hand, the sensitivity of CHROMagar and MHA-CFOX was 90.62%. The specificity of each MHA-CFOX, MSA-CFOX, and CHROMagar is 87.5% as well as 93.75% for BA-CFOX. The study has demonstrated better performance of modified-conventional method compared to the other media. Hence, the application of modified-media should not be delayed to facilitate the findings of MRSA among hospitalized patients.

Keywords: Antibiotic Resistance, Cefoxitin Based Media, MRSA, *Staphylococcus aureus*

***Correspondence:** sriamelia@usu.ac.id

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INTRODUCTION

Staphylococcus aureus (*S. aureus*), a major commensal human pathogen, can produce severe forms of life-threatening infections.[1] The epidemiological trends have ultimately transformed for decades. Emergence of certain virulence factors and bacterial resistant strain such as Methicillin-resistant *Staphylococcus aureus* (MRSA) has been acknowledged as the causative factors promoting higher hospital expenditures ranging from $100 million to $30 billion annually.[2,3] The prevalence of MRSA is higher among developed nations which is 25-70% among *S. aureus* isolates.[4] In Indonesia, it was predicted that MRSA infection rates are as high as 28%, with a low prevalence of MRSA nasal carriers in the community.[5,6]

Staphylococcal cassette chromosome mec *(SCCmec*), a mobile genetic element (MGE), has been responsible for the pandemic of *S. aureus* resistant to antibiotics. *SCCmec* acts as a methicillin-resistance determinant through *mecA* gene activation and its homologs (*mecB, mecC, mecD*).[7] The activated-gene will encode low-affinity penicillin-binding protein (PBP) to several penicillin-class antibiotics. PBP2a has been hypothesized to have some unique properties, such as a low rate of acylation and low affinity for beta-lactam antibiotics thus producing a more virulent type of infection.[8]

Infectious disease surveillance plays an important role as a tool for monitoring the health of general population. The objectives of surveillance program are to describe the burden and epidemiology of the disease, monitor trends and identify new outbreaks or pathogens.[9] Active surveillance require an active search for patients who are likely to develop a bacterial colonization. This requires the involvement of various experts trained in infection control in wards, intensive care units (ICUs) and microbiology laboratories. Detection of antimicrobial resistance in a pathogen such as MRSA is part of active surveillance. With this approach, patients carrying resistant bacteria such as MRSA can be detected immediately and thus ultimately prevent the transmission of infectious diseases.[10]

MRSA detection approach are only available in two primary forms, which was agar-based, and molecular-based examination. The agar-based method is a daily diagnostic method in hospital setting. However agar-based method requires sufficient, and sometimes lengthy time to ensure its accuracy. Conventional media, such as Mueller-Hinton susceptibility testing, will need 2-3 days. The other agar with broad availability in all microbiology facilities is mannitol-salt and blood agar which has a longer useable period. Nevertheless, chromogenic media can be used to shorten turnaround time (TAT) for MRSA detection, but it is not manufactured by the national industry and has a short expiry date. Many advanced procedures are questionable because of its high price and high difficulty.[11] Therefore, the culture-based method is still the most reliable and efficient method.[12]

Many laboratories in developing countries need an alternative to provide faster and more efficient procedures without sacrificing several critical indicators, including accuracy and other diagnostic variables.[13] Cefoxitin supplementation, both in disc and powder form, when supplemented into mannitol salt agar (MSA) or blood agar (BA) would be another resource-efficient method in alleviating the scarcity of diagnostic method options in resource limited- settings.[14] Methicillin resistance can be detected by employing diffusion circle test using β-lactam substance such as oxacillin or cefoxitin. However, variety of PBP2a expressions could create multitude of results and would affect oxacillin effect. On the other hand, the use of cefoxitin is very sensitive and specific as *mec-A* mediated marker to recognize methicillin resistance.[15] Detection of antimicrobial resistance can also be done by using chromogenic agar media. The use of chromogenic agar media has been used since 20 years ago to detect resistance in several microbes such as MRSA, vancomycin-resistant *Enterococcus* (VRE) and carbapenemase-producing Gram-negative bacteria. However, the use of chromogenic media has the disadvantage that it is relatively expensive and not very specific.[16]

Our study aimed to demonstrate the potency of multiple method to increase MRSA detection rate. Our findings might be beneficial

since no alternative method has been offered to alleviate the diagnostic burden in developing countries. This study also aims to compare the detection ability of MRSA using modified conventional methods and chromogenic media.

MATERIALS AND METHODS

Bacterial isolates

A total of 80 *S. aureus* isolates from our previous study[17] were cultivated and re-cultured into routine media such as blood agar and mannitol-salt agar (MSA) between June and September 2018. A total of 40 isolates (50%) were MRSA bacteria. The study used cefoxitin doses that had been determined through a preliminary study; it demonstrated that 6 mg/L is the minimum dosage to inhibit MSSA in MSA. The screening results of four media were counted using an online calculator provided by MedCalc.[18] *Staphylococcus aureus* colonies were preserved under a glycerol-based cryopreservation technique for PCR examination.[19]

Detection method

Mannitol-salt agar (Oxoid, Basingstoke, United Kingdom) was generated and then froze until below 5°C. Subsequently, a 6 mg/L of cefoxitin powder (Oxoid, Basingstoke, United Kingdom) will be added, called MSA-CFOX. On the other hand, bacterial suspension was prepared in sterile saline to match 0.5 McFarland standards, and the streaking were performed by using a calibrated loop (10µL). This technique was performed onto the media. After 24 hours of incubation, two observers interpreted grown-colonies that appeared on the agar. Yellowish transformation of MSA-CFOX was described as positive for presumptive resistance. Similarly, the preparation for blood agar (Oxoid, Basingstoke, United Kingdom) supplemented with cefoxitin powder (BA-CFOX) was conducted in accordance to the manufacturer's instruction as well as standard susceptibility testing using cefoxitin disc and Muller-Hinton agar (MHA).

ChromID MRSA agar is provided by *Biomerieux*, France, as prepared culture agar. The inoculation of bacterial suspension using a calibrated loop (10µL) was handled to match McFarland 0.5. The interpretation was conducted based on the manufacturer's guides; positive MRSA required one or more green and slightly elevated colonies after 24 hours of incubation period at 37°C, for further reassurance any suspected MRSA colonies underwent further examination includes gram staining and latex agglutination test (Pastorex Staph-Plus, Bio-Rad, France).

RESULTS

The interpretation of media was performed by two microbiologists independently. Statistical analysis for the kappa agreement was calculated to determine the similarity of interpretation between two different interpreters. Substantial agreement (kappa 0.773 95% CI 0.634-0.913) during the observation of chromogenic and blood agar supplemented with cefoxitin powder has been noted. Kappa agreement for MSA-CFOX were almost perfect between both observers

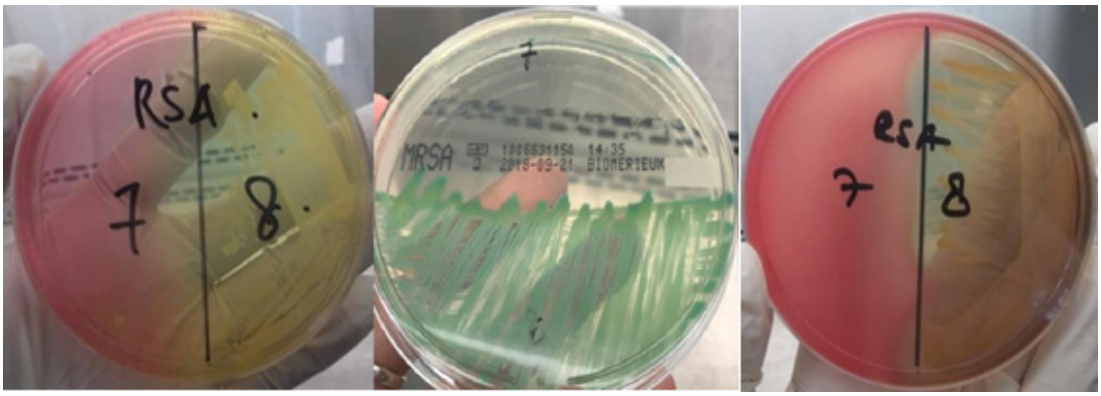

Figure. A) Mannitol-salt agar with cefoxitin powder (MSA-CFOX); B) Chromogenic Agar; C) Blood agar with cefoxitin powder (BA-CFOX)

Table. Comparison of screening test performance of media for detection MSSA and MRSA

Indicator	MHA-CFOX (95% CI)	MSA-CFOX (95% CI)	BA-CFOX (95% CI)	ChromID (95% CI)
Sensitivity (%)	90.62 (74.98-98.02)	96.88 (83.78-99.92)	96.88 (83.78-99.92)	90.62 (74.98-98.02)
Specificity (%)	87.50 (74.75-95.27)	87.50 (74.75-95.27)	93.75 (82.80-98.69)	87.50 (74.75-95.27)
PPV[a] (%)	82.86 (69.40-91.15)	83.78 (70.91-91.63)	91.88 (77.52-96.87)	82.86 (69.40-91.15)
NPV[b] (%)	93.33 (82.59-97.64)	97.67 (85.88-99.66)	97.83 (86.72-99.68)	93.33 (82.59-97.64)

[a]Positive predictive value; [b]Negative predictive value

(kappa 0.923 95% CI 0.838- 1.000). Colonies would appear as the yellowish transformation of media color for resistant colonies. (Figure).

All detection media contained *Staphylococcus aureus* isolates were treated equally, including 24 hours of incubation. The panel of diagnostic value is depicted in Table. Both MSA-CFOX and BA-CFOX have the highest and similar sensitivity compared to the other media. Meanwhile, the specificity of the alternative methods (MSA-CFOX) and chromogenic media showed no considerable difference. In addition, three samples have been tested falsely susceptible or MSSA using MHA-CFOX and chromogenic media, while only one isolate were falsely identified by using BA-CFOX and MSA-CFOX. Four isolates showed atypical colonies in BA-CFOX, which on further identification demonstrated *Bacillus subtilis* growth.

DISCUSSION

In this study, the superiority of modified-conventional method as the alternative option to detect MRSA was apparent thus increasing cefoxitin supplementation could boost the diagnostic value of each media. The sensitivity of BA-CFOX and MSA-CFOX with 6 mg/L of cefoxitin powder reached 96.88%. Meanwhile, chromogenic media and MHA-cefoxitin disc (MHA-CFOX) produced similar results. In the developing region, alternative methods could appear as the options to detect MRSA since the availability of molecular techniques, including phenotypic and genotypic approaches, are less likely to be available due to inadequate resources.[20]

This study also applied cefoxitin as the antibiotic determinant for MRSA. Based on CLSI recommendation, cefoxitin has been encouraged as there is clear visibility surrounding the disc on the agar that could not be misinterpreted compared to oxacillin diffusion test.[21] Furthermore, media containing aztreonam, ceftizoxime, cephamycins, cefoxitin, and oxacillin have been tested in several studies, which demonstrated that cefoxitin is much more superior for MRSA detection.[13,22,23] In current literature, the administration of cefoxitin were shown to bolster detection rate results as it hypothetically induces *mecRI* expression.[19]

The essential ingredient of the media must be supportive to bacterial growth; for instance, the combination of 1% mannitol and 7.5% NaCl can act as a selective medium in promoting *Staphylococcus* sp. growth; it consists of salt which inhibit non-staphylococcal bacterial growth. It also contains phenol red, an acid indicator for fermentation process of mannitol produced by staphylococcal species.[24] Nevertheless, several staphylococcal species are also mannitol positive such as *S. capitis, S. cohnii, S. sciuri*, and other species, by combining the examination with biochemical and coagulase test, microbiologist could avoid false-positive results.[25,26] Nevertheless, there is drawback associated with its permissive properties for ubiquitous bacterial growth, which still needs further elucidation. A few MRSA strains, such as incomplete hemolytic phenotype (SIHP), have been found to express more beta hemolysin concurrent with its unusual virulence activity.[27] In Indonesia, laboratory diagnosis in with novel chromogenic media were uncommon even though it have been shown to reduce the time

of "diagnosis to the management" gap in several investigations, and it has been widely used for screening media in western countries.[28]

The application of MSA with cefoxitin powder supplementation has been investigated in several studies. Han et al. obtained 76.5% sensitivity and 99.6% specificity, respectively, for MSA-CFOX compared to 90.2% and 99.3% while using chromogenic media, but the author suggests that incubation period extension to 48 hours and coagulase test could prevent false-positive results.[29] Similarly, the superiority of MSA-CFOX was reported by Smyth and Kahlmeter with high sensitivity and specificity against oxacillin (100% vs. 90.7% and 100% vs. 96.0%).[30] Furthermore, one study has also successfully demonstrated minimum difference following 24 hours of incubation in four screening media, such as MSA-CFOX, MRSASelect, MRSA ID, and CHROMagar (Sensitivity of 92.9%, 94.9%, 90.9%, 91.9% respectively).[31] Meanwhile, chromogenic media was demonstrated as a feasible detection test; one study revealed specificity and sensitivity value of 100% for 24 hours as well as 48 hours of incubation using pureblood culture.[32] The use of multiple screening methods could increase accuracy especially while employed as conjunction with other types of diagnostic methods.[33]

In this study, *B. subtilis* contamination was found on blood agar media. This can happen due to lack of vigilance during research or due to the inherent weakness of blood agar. Our study has some limitations that need further investigation, particularly to externally validate its function as the screening tools. Pure culture with high inoculum had been suggested to better determine the performance of the modified-conventional method against chromogenic media as well as the conventional method which was not used in our study.

CONCLUSION

The study demonstrated that cefoxitin supplementation could become a new detection approach in combination with other existing methods. Nevertheless, there must be further studies to evaluate its accuracy among the general population.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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