Comparison of Performance between Mannitol Salt Agar-supplemented Cefoxitin Disc and Chromogenic Media for Methicillin-resistance Staphylococcus Aureus Screening

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Abstract: Methicillin-resistance Staphylococcus aureus (MRSA) and its burden still become a major problem in hospital setting worldwide. Accurate and rapid screening method is needed to prevent transmission We conducted a study to compare the performance between conventional method, modified-conventional method, and chromogenic media to detect MRSA carrier. This cross-sectional study was obtained 80 nasal swabs of medical personnel who worked in Intensive Care Unit. The location of study was in Hasan Sadikin General Hospital and Sentosa Hospital, Bandung, Indonesia between March and July 2009. Meanwhile, incubation and identification process were set in Department of Microbiology, Universitas Padjajaran, Bandung, Indonesia. Modified-conventional method, cefoxitin disc plated at the bottom of Mannitol-Salt media, was made. The result showed that specificity increased (100%, p-value < 0.001, kappa index= 1.00) using modified media and similar result was also found with chromogenic media. While using conventional method alone just produced 98.7% of specificity and 100% of sensitivity (p-value <0.001, kappa index=0.85). Thus, modified-conventional method can be considerable since its detection rate of MRSA similarly found as a reliable method for MRSA screening.

1 INTRODUCTION

Since its findings in Detroit in 1981 among injecting drug users, Methicillin-resistance Staphylococcus aureus (MRSA) remains problematic. MRSA will produce low-affinity penicillin-binding protein 2 (PBP2) encoded by mecA gene, and bacteria still continue its cell-wall synthesis, then it causes using beta-lactam lineage as antimicrobial therapy is meaningless (Schroeder et al., 2017). Furthermore, using highly expensive antibiotics and prolonged hospitalization of a patients infected-MRSA will lead to a higher hospital expenditure (Collins, 2010).

MRSA has become emerging problem causing nosocomial infection, especially in Intensive Care Unit (ICU), anterior nares is its predilection mostly, therefore colonization in this structure plays an important role in MRSA transmission. The transmission generally involves medical health-workers who acquired infection and MRSA colonization in their anterior nares. Transmission will ensue directly if there is contact with infected-individual. Endemically, asymptomatic carrier is presence among hospitalized-patients and commonly caused MRSA bacteraemia especially in critically-ill patients (Marcel, 2008). Carrier identification and isolation (carrier) MRSA are effective method in controlling its incidence. MRSA screening should be performed for both inpatients and all medical personnel working in certain units, especially who works in Intensive Care Unit (ICU), the screening is mandatory since it related to morbidity and mortality of a patients-infected MRSA (Klevens, 2007).

Screening media can be performed using conventional method, in which the isolates will be implanted in blood agar then followed by Staphylococcus aureus identification process up to susceptibility test against methicillin. In addition to blood agar, MRSA identification can also use selective medium for Staphylococcus aureus such as Mannitol Salt agar (MSA), furthermore, as the confirmatory test, susceptibility test by using Muller-Hinton agar (MHA)-supplemented cefoxitin must be performed. MSA, MHA, and oxacillin

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resistant screening agar are commonly used among other solid conventional methods in Europe. However, chromogenic media can be used in several laboratories because it eliminates all the issue related to time-consuming procedure for MRSA screening method and it is also widely used in Europe (Fagan, 2010).

As mentioned above, alternative method is needed for screening MRSA in low-cost laboratory, high cost and short expired date become a limitation using chromogenic media as main screening media for MRSA. Our study was conducted to determine and compare the performance of conventional method, modified-conventional method or MSA-supplemented cefoxitin disc (MSA-CFOX), and chromogenic media.

2 THE MEDIA

This study involved 80 health-workers who worked in Intensive Care Unit (ICU) of Hasan Sadikin General Hospital and Santosa Hospital, Bandung, Indonesia between March and July 2009. This cross-sectional study aimed to determine and compare the performance of modified conventional method and ChromIDTM for screening MRSA. Firstly, samples were obtained from nasal swab of all health-workers who had given their approval. Nasal swabs (BBL CultureSwab Liquid Stuart) were directly sealed inside the reaction tube which already contained transport medium.

Furthermore, the specimens were inoculated into conventional media (blood agar and Muller-Hinton agar), modified-conventional media (MSA-supplemented cefoxitin disc or MSA-CFOX), and chromogenic media (ChromIDTM MRSA). Bacterial inoculation and identification processes were carried out in Microbiology Department, Faculty of Medicine, Universitas Padjajaran, Bandung, Indonesia.

2.1 Modified-Conventional Method or MSA-CFOX

We provided petri dishes with cefoxitin disc placed at the bottom of the media and it was divided into three sections. We prepared initially MSA medium under laboratory roof and directly plated cefoxitin disc before the media solidified. Finally, modified-conventional medium or Mannitol-Salt agar supplemented cefoxitin disc (MSA-CFOX) were ready to be used and incubated for 24 hours at 37°C. Previously, we also assessed whether cefoxitin substance had disseminated all over the compartment of medium by using High-Performance Liquid Chromatography (HPLC) method in School of Pharmacy, Institut Teknologi Bandung (ITB), Bandung, Indonesia.

After 24 hours, the colonies were seen yellow and microscopically the bacteria arranged in groups like grapes, from this simple examination we called this findings as 'suspected' MRSA. For confirmation, the colonies had also to be inoculated into Muller-Hinton Agar (MHA) plate by placing cefoxitin 30 µg disc on it (Kirby Bauer disc diffusion method). Both medium acquired equal handling with clearly established procedure. After 24 hours of incubation, observation and measurement of inhibition zone were performed. Inhibition zone ≤ 22 mm, it is evident for MRSA while if ≥ 22 mm is MSSA.

2.2 Conventional Method

Inoculation into blood agar was performed before it was incubated for 24 hours at 37°C. After incubation, we obtained colony growth on the surface of agar. Evaluation of the colony was performed by microbiologist to determine whether Staphylococcus aureus colonies are positive or not. The colonies were circular, smooth, slightly appeared on surface, glistening, and gray to yellowish brown. Furthermore, the colony was obtained using sterile ose and we fixated it on the object glass for gram staining, catalase test, and coagulase test. MHA medium was used as a part of confirmatory for MRSA detection. Previously, inoculum was incubated in McFarland medium to increase the number of bacteria. We used cefoxitin 30 µg to determine the inhibition zone. Final results would determine whether the presence of MRSA is evident.

2.3 Chromogenic Media

Inoculation was carried out using ChromIDTM MRSA, France for 24 hours at 370 C. It is well explained that the colonies would appear greenish for MRSA colonies.

2.4 Data Analysis and Study Approval

We used fisher exact test for data analysis while data processing was done by calculating the kappa coefficient of agreement (K) between the two methods calculated by 2x2 proportion table. There are three criteria for the kappa limit value, for

example if the kappa ≥ 0.75 is mentioned as high conformity, if kappa between 0.40 and 0.75 is called moderate conformity, whereas ≤ 0.40 is mentioned as less conformity. Study approval was also obtained from ethics commission of medical research of Universitas Padjajaran, Bandung, Indonesia.

3 RESULTS

A total of 80 nasal swabs were examined and inoculated then. Finally, the results were positive for MRSA only in four samples (3.8%), and 15 samples (18.8%) were positive for Methicillin-sensitive *Staphylococcus aureus* (MSSA). The rest, *Streptococcus sp.* (32.5%), *Staphylococcus saphrophyticus* (7.5 %), and *Staphylococcus epidermidis* (37.5%) were also found from samples. After incubation period, we validated three different methods and compared them with multistep procedure mentioned above. We presented the result in Table 1.

Table 1: MRSA identification using three different methods.

Methods	Sensitivity (%)	Specificity (%)	p-value	kappa Index
Conven-tional method	100	98.7	<.001	0.85
MSA-CFOX	100	100	<.001	1.00

Isolates that exhibited MRSA positive were four samples (3.8%), confirmatory test was also done by procedure mentioned above. Recently, some molecular methods are introduced to detect MRSA, detecting mecA gene still become reference tools to demonstrare methicillin resistance, however, because of inadequacy of tools and personnel, this method can hardly be used in most laboratories particularly in developing countries. Therefore, proper and inexpensive screening method is still required for routine procedure (Xu, 2017).

Identification of MRSA is depending on certain factors, such as cost, speed of result, availability of tools and equipment, sensitivity, and specificity. For routine diagnostic purpose, tube coagulase or latex agglutination, and catalase test should be done at the beginning of identification process. Afterward, options for detection methicillin resistance, including disc diffusion, minimum inhibitory concentration (MIC) measurements, chromogenic agar, latex agglutination, rapid screening methods and molecular detection are the methods can be used for the next identification process. Media type, incubation times and temperature are important in determining the results of methicillin sensitivity. In addition, by plating before broth enrichment will increase the sensitivity of the screening method, particularly using cefoxitin but it does not increase the turnaround time (TAT) (Zurita, 2010), (Marlowe and Bankowski, 2011). Therefore, for MRSA identification, BSAC (British Society for Antimicrobial Chemotherapy) recommends dilution or disc diffusion of Columbia or Muller Hinton agar with NaCl (2%) and incubation for 24 hours. Nevertheless, because of its highly sensitive (>98%), chromogenic agar is also commonly used for rapid MRSA screening. Furthermore, FDA-approved method for PCR and chromogenic agar only available for nasal swabs, because of its different result in each sample locations (Nathwani, 2008).

Selection of antibiotics for plating is also essential since it determines the outcome of culture. A study conducted by (Yamada, 2010) tried to compare sensitivity and specificity among media containing certain antibiotics, cefoxitin-based agar media had 100 % sensitivities at 24 hours culture, while lower results were found on the media containing oxacillin or ceftizoxime. Consequently, cefoxitin-based agar also is commonly used as first option antimicrobial containing media for MRSA screening while oxacillin as second option (Smyth and Kahlmeter, 2005).

We conducted the study by using chromogenic media and modified-conventional method, similar results were found between the two-screening method, by adding cefoxitin disc diffusion its increased MRSA detection rate (p-value <0.001, kappa index= 1.00) than conventional method alone. (Han, 2007) evaluated mannitol salt agar (MSA) and chromogenic media (CHROMagar Staph. aureus and CHROMagar MRSA) for Staphylococcus aureus detection, chromogenic media had shown higher sensitivity at 24 hours (90.2 versus 76.5 % at 24 hours, p-value= 0.11), it is profound that the result showed statistically non-significant, while using MSA had higher specificity than chromogenic media at 24 hours, 99.6% and 99.3 % respectively. But the study only compared between two chromogenic-based media for MRSA detection. In contrast, a study conducted by (Patil and Gadagil, 2016) stated that cefoxitin disc diffusion better than oxacillin and chromogenic media, they carried out 200 clinical isolates of Staphylococcus aureus and found 100 % of sensitivity and specificity by using MSA-CFOX,

meanwhile with similar sensitivity, chromogenic media had lower specificity, because of the findings, chromogenic media should be used carefully in detecting MRSA, especially alone.

Reduction in workload and reporting time by using rapid identification like chromogenic media is evident in study conducted by Lagace´-Wiens et al. (2008) but using chromogenic media as screening method is quite challenging since it can only be used for a short period of time because of having short expired date and the price is still high. Therefore, using alternative method for screening MRSA is a basis of this study especially beneficial for low-cost laboratory. Poojay and Bhandarkar (2015) reported lower price could be obtained by using conventional method (MSA, blood agar plate, gram stain, catalase test, coagulase test, and screening test using cefoxitin) than using chromogenic media. Nevertheless, using chromogenic media still saves more than 48 hours instead of its high cost.

Although it was proved in 2009, we also ascertain that the data was still reliable to be compared to recent studies because of inconsistency of the result showed in several studies which compared the detection rate of MRSA using MSA-CFOX and chromogenic media.

4 CONCLUSIONS

To conclude, modified-conventional method, we used MSA-CFOX, becomes promising method for MRSA screening instead of another expensive method. Similar findings, sensitivity and specificity, were evident in our study between using modified–conventional method and chromogenic media. In addition, this study also did not escape the limitation since its MRSA positivity only found in four samples, furthermore larger study is also needed to give the evidence that modified-conventional method is able to become one of choice for MRSA screening. Notwithstanding, even our study was conducted in 2009, we also proved using modified-conventional method is become considerable method to detect MRSA in general population.

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