Difference on Cefoxitin and Oxacillin Disk Test on In Vitro MRSA Detection (Meticillin Resistant Staphylococcus aureus)

Experimental Study on Microbiology Laboratory of Medicine Faculty of UNISSULLA

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ABSTRACT

Background: MRSA phenotypic detection has been a problem since it was found in 1962. Some studies explain that the diffusion method of cefoxitin and oxacillin disk test can be used to detect MRSA. Objective: knowing if there is difference between cefoxitin disk test and oxacillin disk test to detect MRSA.

Method: laboratory experimental research with diagnostic test design. Research was done at the laboratory of medicine faculty of UNISSULLA using 24 watch glasses, 12 with MRSA bacteria (Meticillin Resisten Staphylococcus aureus), and the other 12 with MSSA bacteria (Meticillin sensitive Staphylococcus aureus). The results were classified into sensitive and resistant category based on CLSI standard (Clinical Laboratory Standards Institute).

Hypothesis test using fisher test, with significance level <0.05

Results: MRSA detection using cefoxitin disk resulted 12 resistant specimens and no sensitive specimens. The oxacillin disk resulted 9 resistant specimens and 3 sensitive specimens. MSSA detection using cefoxitin disk resulted no resistant specimens and 12 sensitive specimens, oxacillin disk resulted no resistant specimens and 12 sensitive specimens. The results of fisher test for cefoxitin disk and oxacillin disk test to detect MRSA was p=0.000, meaning there was difference between cefoxitin disk test and oxacillin disk test to detect MRSA.

Conclusion: diffusion method in cefoxitin disk is better than oxacillin disk in MRSA detection.

Keywords: MRSA, MSSA, diffusion, PPV, NPV.
INTRODUCTION

Resistant Staphylococcus aureus towards meticillin antibiotics is known as Metsicillin Resistant Staphylococcus aureus (MRSA) (Juuti, 2004). Those Staphylococcus aureus are resistant towards antibiotics meticillin because its ability to produce β-laktamase enzyme. This enzyme is able to eliminate antibacterial power especially in penicillin groups such as meticillin, oxacillin, penicillin G and ampicillin. (Juuti, 2004). MRSA phenotypic detection has been a problem since found in 1962 (Madhusudhan NS, et al., 2011). MRSA diagnosis is very important. Accuracy and reliability to detect meticillin resistance is the most important key to confirm antibiotic treatment for infected patient and to control MRSA staphylococci around hospital environment (Velasco, et al., 2005). MRSA resistance detection can be conducted by using oxacillin or cefoxitin diffusion method (Van Leeuwen WB, 2003; Broekema NM, et al., 2009).

Infection incidence of MRSA are increasing globally. Percentage of MRSA are quite high in Asia. In Taiwan are 60%, China 20%, Hong Kong 70%, Filipina 5%, and Singapore 60% (Mulholland et al., 2005). Prevalence level in Indonesia during 2006 were of 23,5% (Sulistyaningsih, 2010).

A good diagnostic instrument can be recognized from its high sensitivity, specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV). Study by Madhusudhan NS, et al. in 2011 using 100 MRSA specimens by diffusion method resulted that on detection using cefoxitin disk, 84 resistant. False positive value was 11% and expected positive value of cefoxitin was 86.90% (Jana M. Swenson, et al.). Oxacillin, which is on the same antibiotic group with meticillin, is cheaper and easily accessible (Van Leeuwen WB, 2003; David Velasco et al., 2004). The sensitivity of the oxacillin can be applied on other penicillinase-stable penicillin Oxacillin zone are often hazy and commonly misinterpreted as the result of oxacillin sensitivity (Pottumarthy, S., T. R. Fritsche, dan R. N. Jones, 2005). Cefoxitin can be used as MRSA detection both by diffusion or gel dilution (Clarence J. Fernandes, et al., 2005). Cefoxitin result is easier to be interpreted and more readable (Felten, A., 2002; Mimica, 2007 Pottumarthy, S., T. R. Fritsche, dan R. N. Jones, 2005). Cefoxitin sensitivity on MRSA detection is mediated by mec-A gene (Swenson, J. M., et al., 2007).

Based on oxacillin and cefoxitin disks difference on MRSA detection, a research was conducted. This research aims to differentiate sensitivity, specificity, PPV, and NPV of cefoxitin disk test and oxacillin disk test to detect MRSA by diffusion method.

METHOD

This research is laboratory experiment with specific method diagnostic test. Population of the study are Methicillin Resistant staphylococcus aureus (MRSA) and Methicillin Sensitif staphylococcus aureus (MSSA) bacteria collected from Microbiology Laboratorium of Rumah Sakit Umum Dr. Karyadi Semarang with density level of 0,5 Mc Farland (1,5 x 10^8 / ml) and 0,2 cc of volume embedded into 24 petri dish with muller hinton media.

Specimens used were 12 petri dishes with MRSA bacteria and 12 petri dishes with MSSA bacteria. Each dishes were tested with diffusion method on oxacillin disk and cefoxitin disk and resulted into 48 dishes. The amount of the specimens were counted from total sample formula.

Data analysis by fisher test were conducted to test research hypothesis with significance level of < 0.05.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>MRSA</th>
<th>MSSA</th>
<th>Total</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Resistant</td>
<td>Count</td>
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<td>12</td>
</tr>
<tr>
<td></td>
<td>Expected Count</td>
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<td>6.0</td>
<td>12.0</td>
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<td>% of Total</td>
<td>50.0%</td>
<td>.0%</td>
<td>50.0%</td>
</tr>
<tr>
<td>Sensitive</td>
<td>Count</td>
<td>0</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Expected Count</td>
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<td>% of Total</td>
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<td>Expected Count</td>
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<td>% of Total</td>
<td>50.0%</td>
<td>50.0%</td>
<td>100.0%</td>
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</tbody>
</table>
RESULTS
The result of cefoxitin disk to determine MRSA and MSSA were illustrated in the table below:

Table 1. showed cefoxitin test resulted into 12 MRSA resistant specimens and no MRSA sensitive specimens. While for MSSA there are no resistant specimens. 12 sensitive specimens were tested with fischer hypothesis test and acquired, $p=0.000 (<0.05)$, meaning cefoxitin disk test is significant in MRSA detection.

Table 2. showed oxacilin disk resulted 9 resistant MRSA specimens and 3 sensitive MRSA specimens. While for MSSA there were no resistant specimens and 12 sensitive specimens were using Fisher test to test hypothesis resulted $p=0.000 (<0.05)$. This concluded that oxacilin disk test have significant value on MRSA detection.

Each data were tested diagnostically using Centre for Evidence-Based Medicine (CEBM) statistic calculator. On cefoxitin disk, sensitivity value were 96.2%, specificity 96.2%, PPV (positive predictive value) 96.2%, NPV (negative predictive value) 96.2%. On oxacilin disk sensitivity value were 73.1%, specificity 96.2%, PPV 95.0%, NPV 73.8%.

DISCUSSION
This research resulted that there are differences in sensitivity, specificity, PPV, NPV between oxacilin disk and cefoxitin disk in MRSA detection, Cefoxitin sensitivity (96.2%) were higher compared to oxacilin. (73.1%). Cefoxitin and oxacilin specificity were similar (96.2%). Cefoxitin PPV (96.2%) was higher compared to oxacilin (95.0%). Cefoxitin NPV (96.2%) was higher than oxacilin (73.8%).

This finding similar to previous study conducted by Clarence J. Fernandes, et al., 2005, which stated that sensitivity and specificity of cefoxitin are higher compared to oxacilin. So that cefoxitin can be used for MRSA detection whether with diffusion or dilution method. (Clarence J. Fernandes, et al., 2005). The superiority of cefoxitin on MRSA detection is because cefoxitin act as strong inducer onmecA Gene regulatory system (Swenson JM, et al, 2007). Cefoxitin is easier to interpret and to read (Felten, A., 2002; Mimica, 2007 Pottumarthy, S., T. R. Fritsche, dan R. N. Jones, 2005). MRSA resistance mechanism toward cefoxitin is because its difficulties to be broken by drugs; loss specific penicillin binding protein (PBP); and drugs degradation by betalaktamase (Yati & Gan, 2007).

Oxacilin, which is also on the same antibiotic group with meticillin, is cheaper and accessible (Van Leeuwen WB, 2003; David Velasco et al., 2004). Oxacilin replace metycilin which is no longer available commercially in the US and oxacilin is more possible to detect heteroresistant strain. Vulnerability result of oxacilin can be applied to penicilin group which are stable towards penisilinase, such as cloxasilin, dicloxacillin, methicillin, flukloxasilin dan naficillin. Oxacilin zone are often hazy and commonly misinterpreted as oxacilin sensitivity (Pottumarthy, S., T. R. Fritsche, dan R. N. Jones, 2005). MRSA resistance mechanism to oxacilin antibiotic was caused by betalaktamase enzyme formation; drug tolerance due to failure in bacteria autolycine enzyme; bacteria which do not have celluler walls (mikoplasma), PBP changes or drugs unable to reach PBP (Yati & Gan, 2007).

MSSA detection by using cefoxitin disk as well as oxacilin disc showed that all 24 specimens were sensitive, confirmed by Short-Incubation Automated Instrument Systems (SIAIS). Detection of MRSA by cefoxitin disk showed that all 12 resistant specimens confirmed by SIAIS. But on MRSA detection with oxacilin disc showed that 9 specimens were resistant, while 3 specimens were sensitive confirmed by (SIAIS).

These three different results possibly because oxacilin zone are often hazy so it was misinterpreted as the evidence of oxacilin sensitivity (Pottumarthy, S., T. R. Fritsche, dan R. N. Jones, 2005).
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Limitation of this study was researcher only use disk diffusion test. It would be better if the antibiotic sensitivity test by dilution as antibiotic sensitivity test gold standard is used. Other constrains were specimens material collection from the patients are not similar. For example there was sputum and blood specimens. The differences of the specimens were not effecting the research validity.

CONCLUSION

Based on the research data on difference between cefoxitin disc and oxacilin disc on in vitro MRSA detection using diffusion method, it can concluded that there are significant difference between cefoxitin disc and oxacilin disc. Cefoxitin sensitivity to detect MRSA (96,2%) were higher than oxacilin (73,1%). Cefoxitin specificity to detect MRSA is similar to oxacilin (96,2%). Cefoxitin PPV to detect MRSA (96,2%) is higher than oxacilin disc (95,0%). Cefoxitin NPV to detect MRSA (96,2%) is higher compared to oxacilin disc(78,1%). Diffusion method on cefoxitin disk is better than oxacilin MRSA detection. Suggestions for further research are higher number of sample, same specimens materials, and comparison based on age and duration of the infection.

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