

RESEARCH ARTICLE

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

Experimental Study on Microbiology Laboratorium of Medicine Faculty of UNISSULLA

Hana Tiyas Mustikawati^{1*}, Ridha Wahyutomo², Qathrunnada Djam'an³

¹ Medical Faculty of Sultan Agung Islamic University

² Microbiology Departement of Medical Faculty of Sultan Agung Islamic University

³ Pharmacology Departement of Medical Faculty of Sultan Agung Islamic University
Medical Faculty of Sultan Agung Islamic University, Jl. Kaligawe KM 4 Semarang 50012
Ph. (024) 6583584 fax. (024) 6594366, *Email: HanaTiyas_mustikawati@yahoo.com

ABSTRAK

Pendahuluan: Deteksi fenotipik MRSA masih menjadi masalah sejak pertama kali MRSA ditemukan pada tahun 1962. Beberapa penelitian menyebutkan dalam mendeteksi MRSA dapat menggunakan metode difusi uji disk cefoxitin maupun oxacilin. **Tujuan:** mengetahui adakah perbedaan uji disk cefoxitin dan uji disk oxacilin untuk deteksi MRSA.

Metode: eksperimental laboratorium dengan rancangan uji diagnostik. Penelitian dilaksanakan di laboratorium FK UNISSULLA, menggunakan 24 cawan petri: 12 cawan petri dengan bakteri MRSA (*Meticillin Resisten Staphylococcus aureus*) dan 12 lainnya dengan bakteri MSSA (*Meticillin Sensitive Staphylococcus aureus*). Hasil yang didapat, dikategorikan sensitif ataupun resisten berdasarkan standar CLSI (*Clinical Laboratory Standards Institute*). Uji hipotesis menggunakan uji fisher, dengan signifikansi < 0,05.

Hasil: Deteksi MRSA dengan menggunakan disk cefoxitin menghasilkan 12 sampel resisten dan tidak ada yang sensitif, pada disk oxacilin menunjukkan 9 sampel resisten dan 3 sampel sensitif. Deteksi MSSA dengan menggunakan disk cefoxitin tidak ada yang resisten dan 12 sampel sensitif, pada disk oxacilin menunjukkan tidak ada yang resisten dan 12 sampel sensitif. Uji diagnostik dilakukan dengan CEBM statistic calculator didapatkan sampel MRSA dengan disk cefoxitin nilai Sensitifitas 96,2%, Spesifisitas 96,2%, PPV (*positive predictive value*) 96,2%, NPV (*negative predictive value*) 96,2%. Disk oxacilin didapatkan nilai Sensitifitas 73,1%, Spesifisitas 96,2%, PPV 95,0%, NPV 73,8%. Hasil uji fisher untuk disk cefoxitin dan oxacilin didapatkan nilai $p=0,000$, artinya ada perbedaan uji disk cefoxitin dengan uji disk oxacilin untuk deteksi MRSA.

Kesimpulan: metode difusi pada uji disk cefoxitin lebih baik dari uji disk oxacillin dalam mendeteksi MRSA.

Kata Kunci: MRSA, MSSA, difusi, PPV, NPV.

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 oxacilin disk test can be used to detect MRSA. **Objective:** knowing if there is difference between cefoxitin disk test and oxacilin 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*). specimens 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: specimens MRSA detection using cefoxitin disk resulted 12 resistant specimens and no sensitive specimens. The oxacilin disk resulted 9 resistant specimens and 3 sensitive specimens. MSSA detection using cefoxitin disk resulted no resistant specimens and 12 sensitive specimens, oxacilin disk resulted no resistant specimens and 12 sensitive specimens. Diagnostic test was done by CEBM statistic calculator. The sensitivity and specificity value of MRSA sampels using cefoxitin disk were 96,2% & 96,2%, PPV (*positive predictive value*) 96,2%, NPV (*negative predictive value*) with was 96,2%. While the oxacilin disk, the sensitivity was 73,1%, specificity 96,2%, PPV 95,0%, NPV 73,8%. The result of fisher test for cefoxitin disk and oxacilin disk was $p=0.000$ meant there was difference between cefoxitin disk test and oxacilin 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 Meticylin Resistant *Staphylococcus aureus* (MRSA) (Juuti, 2004). Those *Staphylococcus aureus* are resistant towards antibiotics meticylin because its ability to produce β -laktamase enzyme. This enzyme is able to eliminate antibacterial power especially in penicillin groups such as meticylin, oxacilin, penicilin 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 meticylin 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 oxacilin 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 studied on MRSA detection by dilution resulted that cefoxitin had the sensitivity and specificity value of 99,7% dan 100%. Oxacilin, which is on the same antibiotic group with meticylin, is cheaper and easily accessible (Van Leeuwen WB, 2003; David Velasco et al., 2004). The

sensitivity of the oxacilin can be applied on other penicillinase-stable penicillin Oxacilin zone are often hazy and commonly misinterpreted as the result of oxacilin 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 oxacilin 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 oxacilin 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 Resistent staphylococcus aureus* (MRSA) and *Methicillin Sensitif staphylococcus aureus* (MSSA) bacteria collected from Microbiology Laboratory of Rumah Sakit Umum Dr. Karyadi Semarang with density level of 0,5 Mc Farland ($1,5 \times 10^8$ / 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 oxacilin 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 1. Cross tabulation of cefoxitin disk test

| | | Bacteria | | Total | p |
|-----------|----------------|----------|-------|--------|-------|
| | | MRSA | MSSA | | |
| Resistent | Count | 12 | 0 | 12 | 0.000 |
| | Expected Count | 6.0 | 6.0 | 12.0 | |
| | % of Total | 50.0% | .0% | 50.0% | |
| Sensitive | Count | 0 | 12 | 12 | |
| | Expected Count | 6.0 | 6.0 | 12.0 | |
| | % of Total | .0% | 50.0% | 50.0% | |
| Total | Count | 12 | 12 | 24 | |
| | Expected Count | 12.0 | 12.0 | 24.0 | |
| | % of Total | 50.0% | 50.0% | 100.0% | |

Table 2. Cross tabulation of oxacilin disc

| | | Bakteri | | Total | p |
|-----------|----------------|---------|-------|--------|-------|
| | | MRSA | MSSA | | |
| Resistent | Count | 9 | 0 | 9 | 0.000 |
| | Expected Count | 4.5 | 4.5 | 9.0 | |
| | % of Total | 37.5% | 0.0% | 37.5% | |
| Sensitive | Count | 3 | 12 | 15 | |
| | Expected Count | 7.5 | 7.5 | 15.0 | |
| | % of Total | 12.5% | 50.0% | 62.5% | |
| Total | Count | 12 | 12 | 24 | |
| | Expected Count | 12.0 | 12.0 | 24.0 | |
| | % of Total | 50.0% | 50.0% | 100.0% | |

RESULT

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 on *mecA* 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 penicillin group which are stable towards penicillinase, such as cloxasilin, dicloxacilin, methicillin, flukloxasilin dan nafcillin. 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 tolerancy due to failure in bacteria autolysine 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).

Mustikawati, et al.

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 oxacillin MRSA detection. Suggestions for further research are higher number of sample, same specimens materials, and comparison based on age and duration of the infection.

REFERENCES

- Broekema, N.M., Van, T.T., Monson, T.A., Marshall, S.A., Warshauer, D.M., 2009, *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. Jan;47(1):217-9.
- Brown, D.F.J., Edwards, D.I., Hawkey, P.M., Morrison, D., Ridgway, G.L., Towner, K.J., Wren, M.W.D., 2005, *Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant Staphylococcus aureus (MRSA)*. J Antimicrob Chemother, 56:1000– 1018.
- Clarence, J., Fernandes, 1., Lorna, A., Fernandes, 1., Peter Collignon2., 2005, *Cefoxitin resistance as a surrogate marker for the detection of methicillin-resistant Staphylococcus aureus*.
- CLSI. 2006. *Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A9, ninth ed. CLSI, Wayne, PA.*
- CLSI. 2007. *Performance standards for antimicrobial susceptibility testing. CLSI Approved standard M100-S17, clinical and laboratory standards institute,*

Wayne, PA

- Cohen, M. L., Edward S. W., and Falkow, S., 2008, *Common R-Plasmids in Staphylococcus aureus and Staphylococcus epidermidis During a Nosocomial Staphylococcus aureus Outbreak*.
- Farmakologi dan terapi, 2007, departemen farmakologi dan terapeuik fakultas kedokteran universitas Indonesia, Edisi Kelima. Jakarta; balai penerbit FKUI. 664-683
- Felten, A., B. Grandry, P. H. Lagrange, and I. Casin, 2002, *Evaluation of three techniques for detection of low-level methicillin-resistant Staphylococcus aureus (MRSA): a disk diffusion method with cefoxitin and moxalactam, the Vitek 2 system, and the MRSA-screen latex agglutination test*. J. Clin. Microbiol. 40:2766-2771.
- Juuti, K., 2004, *Surface protein PIs of methicillin-resistant Staphylococcus aureus role in adhesion, invasion and pathogenesis, and evolutionary aspects*. [Disertation]. Helsinki: Department of Biological and Environmental Sciences Faculty of Biosciences. p. 61-63
- Kenneth H. Rand and Herbert J. Houck., 2004. *Synergy of Daptomycin with Oxacillin and Other β -Lactams against Methicillin-Resistant Staphylococcus aureus*. Antimicrob Agents Chemother. 48: 2871-2875
- Madhusudhan, N.S., Deepa, S., Shoba, D.N., 2011, *correlation of cefoxitin disc diffusion test and oxacillin disc diffusion test fordetecting mec a mediated oxacillin resistant staphylococcus aureus*. Journal of Pharmaceutical and Biomedical Sciences
- Mimica, M. J., E. N. Berezin, R. L. B. Carvalho, L., A. P. Safadi, E. Schneider, and H. H. Caiaffa-Filho, 2007. *Detection of methicillin resistance in Staphylococcus aureus isolated from pediatric patients: is the cefoxitin disk diffusion test accurate enough?* Braz. J. Infect. Dis. 11:415-417.
- Mulholland, Adegbola. *Bacterial infections - A major cause of death among children in Africa*. NEJM 2005; 352:75-7.
- Navy Environmental Health Center, 2005, *Guidelines for the Management of Community Acquired Methicillin-Resistant Staphylococcus aureus (CA-MRSA) Infections in the US Navy and Marine Corps*. http://www-nehc.med.navy.mil/Downloads/prevmed/CPG_MRSA_20050516_final.pdf.

- Pottumarthy, S., T. R. Fritsche, and R. N. Jones, 2005, *Evaluation of alternative disk diffusion methods for detecting mecA-mediated oxacillin resistance in an international collection of staphylococci: validation report from the SENTRY antimicrobial surveillance program*. *Diagn. Microbiol. Infect. Dis.* 51:57-62.
- Swenson, J. M., D. Lonsway, S. McAllister, A. Thompson, L. Jevitt, W. Zhu, and J. B. Patel, 2007, *Detection of mecA-mediated resistance using reference and commercial testing methods in a collection of Staphylococcus aureus expressing borderline oxacillin MICs*. *Diagn. Microbiol. Infect. Dis.* 58:33-39.
- Difference on Cefoxitin and Oxacillin Disk Test on in Vitro ...*
- Van Leeuwen WB, 2003, *Molecular approaches for the epidemiological characterization of S. aureus strain*, p 55-95. In Fluit Ad C, and Franz-Josef Schitz (editors), *MRSA: Current perspectives*. Caister Academic Press, Norfolk England.
- Velasco, D., del Mar Tomas, M., Cartelle, M., Beceiro, A., Perez, A., Molina, F., Moure, R., Villanueva, R. & Bou, G. (2004). *Evaluation of different methods for detecting methicillin (oxacillin) resistance in Staphylococcus aureus*. *J Antimicrob Chemother* 55, 379–382.