Sains Medika Journal of Medicine and Health

• pISSN: 2085-1545
• eISSN: 2339-093X

http://jurnal.unissula.ac.id/index.php/sainsmedika



## RESEARCH ARTICLE

# Diagnostic Study on Identification Method of *Enterobacteriaceae* Directly from Blood Culture

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**Abstrak:**

**Pendahuluan:** Penyediaan hasil diagnosis yang cepat dari kultur darah penting bagi manajemen klinis sepsis. Metode konvensional yang menjadi metode referensi di laboratorium memerlukan waktu 24 jam lebih lama karena harus melalui tahap isolasi primer. **Tujuan:** menguji keakuratan metode inokulasi langsung dari media kultur darah positif ke tabung uji biokimia tanpa melalui tahap isolasi primer untuk identifikasi Enterobacteriaceae, penyebab kedua terbanyak dari sepsis.

**Metode:** Penelitian dilakukan di Laboratorium Mikrobiologi Fakultas Kedokteran Universitas Diponegoro (FK Undip). Rancangan penelitian adalah uji diagnostik. Sebagai sampel penelitian, kultur darah pada botol Bactec dari RSUP Dr. Kariadi dengan pertumbuhan kuman. Kriteria inklusi adalah hasil pengecatan Gram dari media Bactec berupa kuman bentuk batang Gram negatif, dan sebagai kriteria eksklusi adalah terdapat lebih dari satu macam koloni kuman bentuk batang Gram negatif pada media agar darah dan media agar Mac Conkey dan menunjukkan hasil positif pada pemeriksaan oksidasi. Identifikasi kuman berdasarkan pada tabel uji biokimia Enterobacteriaceae. Data dianalisis menggunakan tabel 2x2.

**Hasil:** Tiga puluh dua sampel masuk dalam penelitian ini, sepuluh sampel (31%) dieksklusi. Dua puluh satu dari dua puluh dua sampel (95%) yang diteliti teridentifikasi secara tepat sampai dengan tingkat genus dengan metode inokulasi langsung.

**Kesimpulan:** Hasil penelitian menunjukkan bahwa metode inokulasi langsung untuk identifikasi Enterobacteriaceae dapat dilakukan, dengan potensi penghematan waktu 24 jam dibandingkan dengan metode konvensional.

**Kata kunci :** Identifikasi langsung, Enterobacteriaceae, uji biokimiawi

***Abstract:***

***Introduction:*** *The provision of rapid diagnosis results from positive blood cultures is important for clinical management of sepsis. Using conventional method as a reference method in laboratory, time needed for bacterial identification are 24 hours longer because it has to deal with primary isolation step.* ***Objectives:*** *This study investigated the accuracy of direct inoculation technique of bacteria from positive blood culture vials to biochemical test tubes without primary isolation step to identify Enterobacteriaceae, second most common causative agent of sepsis.*

***Methods:*** *The study was conducted at the Microbiology Laboratory Medical Faculty of Diponegoro University. This is a diagnostic study. As the study sample, blood cultures in BACTEC bottles from Dr. Kariadi General Hospital Semarang with bacterial growth in it. Inclusion criteria was Gram-negative rod bacteri, staining results from BACTEC blood culture bottles, and as a exclusion criteria, there are more than one colony found on blood agar and Mac Conkey agar and showed positive result in oxidation test. Identification of bacteria based on biochemical table of Enterobacteriaceae. Data were analyzed with a 2x2 table.*

***Results:*** *Thirty two samples included in this study. Ten samples (31%) were excluded. Twenty one from twenty two (95%) study samples accurately identified to the genus level by direct inoculation method.*

*Conclusion: The results showed that the direct inoculation method provides an acceptable genus identification, with a potential saving of 24 hours compared with conventional methods.*

***Keywords:*** *direct identification, Enterobacteriaceae, biochemical test*

## INTRODUCTION

Microbes invasion in the blood, or bacteraemia can lead to serious consequences, including shock, multiple organ failure, disseminated intravascular coagulation (DIC), and death. It is estimated that that 200.000 cases of bacteremia and fungemia occurs annually, with a mortality risk of between 20% -50% (Forbes et al., 2007).

The provision of rapid diagnosis and treatment of bacteremia is one of the important role of the clinical microbiology laboratory (Peterson et al., 2001). Bacteremia mortality risk can be reduced through early appropiate administration of antibiotics (Micek et al., 2005). Identification of the microorganism to genus level will support the interpretation of test results of antibiotic sensitivity. The conventional method used

• pISSN: 2085-1545
• eISSN: 2339-093X



as reference in identification for microorganism takes 48 hours calculated from blood culture tested positive for the proliferation of germs. Twenty-four hours for the primary isolation of Bactec bottle to a solid medium, and the second for 24 hours of incubation colonies which have been inoculated on media and reagents biochemical tests for identification (Ng et al., 2007). Direct innoculation methods can accelerate the identification time to 24 hours` by eliminating the stage of the primary isolation on solid media.

Several studies have successfully accelerate the identification of bacteria, including by direct inoculation of blood culture positive on the test card Vitek 2 and MALDI-TOF, but the necessary tools are expensive and not always available in hospitals (de Cueto et al., 2004; La Scola et al., 2009). The purpose of this study was to test the feasibility of identification methods directly from blood culture with simple biochemical test that is commonly used in microbiology laboratory FK Undip. Germ to be identified is a genus of Enterobacteriaceae, one group of germs Gram-negative rod shape, which is the most common cause of sepsis (Martin et al., 2003).

## METHODS

This study carried out between August and October 2011 at the Laboratory of Microbiology Faculty of Medicine Diponegoro University of Semarang. Blood culture vial was obtained from hospitalized patient of Dr.Kariadi Hospital Semarang. Gram staining was performed on all vials with a positive results after incubattion in in the Bactec 9240 system. Vials with rod shaped Gram-negative only were included in this study. When multiple vials from the same patient were detected, only the initial possitive vial was included in this study.

Blood culture with possitive growth of rod shaped Gram-negative bacteria was sub cultured on to blood agar (Oxoid Dehydrated Culture Media, Blood For Base, CM0055B) and Mac Conkey (Oxoid Dehydrated Culture Media, Mac-Conkey order 3, CM0115B) for primary isolation. 0.5 ml sample of blood culture was diluted with 2.5 ml of sterile 0.9% NaCl. This dilution was then used for direct inoculation into a set of biochemical test media consisting of Triple Sugar Iron order / TSIA (Oxoid Dehydrated Culture Media Triple Sugar Iron order, CM0277B), test indole (Oxoid Dehydrated Culture Media, SIM Medium, CM0435B), methyl red / MR (Oxoid Dehydrated Culture Media, MRVP Medium, CM0043B), Voges-Proskauer / VP (Oxoid Dehydrated Culture Media, MRVP Medium, CM0043B), the use of citrate (Oxoid Dehydrated Culture Media, Simmons Citrate Agar, CM0155B), test the motility (Oxoid Dehydrated Culture Media, SIM Medium, CM0435B), and a test of urea (Oxoid Dehydrated Culture Media, Urea To Base, CM0053B, and Oxoid Supplementary Reagents, Urea 40% Solution, SR0020K). All media were incubated 24 hours at 35°C. Following incubation, the samples (specimens) with more than one colonial morpholotype on blood agar and so Mac Conkey. Samples (isolates?) with possitive test results of oxidase production (BD BBL Oxidase Dry Slide Slides) were excluded from the study. Biochemical test results are read and interpreted according to the table of biochemical test result for Enterobacteriaceae (Barrow and Feltham, 2003).

The conventional method is done by taking the colonies of that Mac Conkey and then cultured in the same biochemical test media with direct inoculation method. The genus identification obtained by direct inoculation method and the conventional method were then compared with a two by two (2x2) table.

## RESULTS

Thirty-two possitive blood culture vial with Gram-negative rods included in this study. Three samples were sub sequently excluded because they had more than one colonial growth on solid media and seven samples showed positive result in examinations oxidation. The remaining twenty two vials were possitive for *Enterobacter spp.* (n = 7), *Klebsiella spp.* (n = 4), S*erratia spp.* (n = 4), *Escherichia spp* (n = 3), *Salmonella spp.* (n = 2), *Proteus spp.* (n = 1), *Yersinia spp.* (n = 1).

**Table 1. Enterobacteriaceae Identification results**

| Genus | Number of identified isolates with conventional methods | Number of identified isolates with direct methods | Number of unidentified isolates with direct methods |
|---|---|---|---|
| *Enterobacter* spp | 7 | 7 | 0 |
| *Klebsiella* spp | 4 | 4 | 0 |
| *Escherichia* spp | 3 | 2 | 1 |
| *Serratia* spp | 4 | 4 | 0 |
| *Salmonella* spp | 2 | 2 | 0 |
| *Proteus* spp | 1 | 1 | 0 |
| *Yersinia* spp | 1 | 1 | 0 |

**Table 2. Direct identification methods 2x2 analysis**

| | | Enterobacteriaceae identification (Conventional method) | |
|---|---|---|---|
| | | Yes | No |
| Enterobacteriaceae identification (Direct method) | Yes | 21 | 0 |
| | No | 1 | 0 |

Direct inoculation method correctly identified twenty-one of twenty twoisolates, with an accuracy of 95%. The cultures were identified as *Escherichia spp.* by the conventional methods, but not by direct inoculation method.

## DISCUSSIONS

The result of this study demonstrate that the direct innoculation method used to identify *Enterobacteriaceae* with the 95% of accuracy. However, the direct inoculation method is only suitable for the identification of common clinical isolates of members of the *Enterobacteriaceae* species. (Ng et al., 2007). In our study, a great number of samples were excluded because besides *Enterobacteriaceae* different bacteria belonging family of oxidase-positive Gram-negative bacilli was also identified (Barrow and Feltham, 2003).

*Enterobacteriaceae* identification satisfactory result is consistent with research that examines Beuving direct method using a BD PHOENIX semi automatic without primary isolation, where accuracy reaches 95.2% (Beuving et al., 2011). Research by the method of direct identification using biochemical tests were performed by Ng even has an accuracy of 98% (Ng et al., 2007). Direct identification is done with Hyplex Blood Screen Wellinghausen Multiplex PCR-ELISA compared to the API to get the 100% sensitivity and specificity between 92.5% and 100% for the genus of *Enterobacteriaceae* (Wellinghausen et al., 2004).

One sample was not identifiable in this study. This occurs because of discrepant result in which methyl red (MR) test results showed negative results by direct inoculation but not with the conventional method. MR test is used to determine whether the bacteria were able to produce a strong acid as the end result of fermentation of glucose. MR indicators show the results of the final pH after rod shaped Gram-negative bacteria ferment glucose, and will give the red color to yellow at pH 4.4 and pH 5.8. Positive results would be obtained if the metabolism of the bacteria using the Embden-Meyerhof pathway produces a strong acid (Garcia, 2010). Another metabolic pathway is through butylene glycol pathway, where the end products of metabolism are acetoin and butanediol, with pH> 6. Most enterobacteriaceae using one of these lines, there is a very rare genus using two metabolism (Garcia, 2010). Cultures was identified as *Escherichia spp.* with conventional methods, with readings in the media Triple Sugar Iron Agar (TSIA) acid/sour, gas positive, H2S negative, and IMViCMU + + - - + -, where as the direct method results IMViCMU yield is + - - - + -

In the table of Cowan and Steel, germs that can give positive results including *Citrobacter spp., Edwardsiella spp., Escherichia spp., Klebsiella spp., Kluyvera spp., Morganella spp, Proteus spp, Providencia spp., Shigella spp., and Yersinia spp.* The entire genus can provide test results the positive MR and negative Voges-Preskauer (VP) test. In the citrate utilization test, Citrobacter spp can be ruled out because ofthe possitive result. In motility test, *Klebsiella spp., Shigella spp.* may be ruled out as a result of negative motility. In the test of urea, *Morganella spp., Proteus spp., Providencia spp. and Yersinia spp.* can be ruled out because it gives a positive test result. Readings on TSIA media is acidic/ sour, gas positive, H2S negative. Edwardsiella spp. can be ruled out because of positive test results H2S. From these results, the possible genus identification includes *Escherichia spp and Kluyvera spp.* (Barrow and Feltham, 2003). *Kluyvera spp.* habitat is water, and can also be found in animals, especially snails (Murray, 2007). Cases of bacteremia with *Kluyvera spp.* cause is rare, but the cases in immuno compromised patients has been reported (Carter and Evans, 2005; Moonah et al., 2010).

The identification of more appropriate outcome is *Escherichia spp,* with differences in test results of MR. The possibility of mixed colonies in a Bactec media is very small, because the culture results with more than one colony are excluded in isolation in solid media. If identified different bacteria, VP test should be positive, because, as has been explained, the germ Enterobacteriaceae using one of the metabolic pathways which is detected with the MR positive test results or test positive VP (Garcia, 2010). The direct identification method, showed both test MR and VP were negative.

MR difference in test results is likely to be caused by the amount of inoculum bacteria that are embedded in the test medium MR with direct inoculation is too small to be able to cause a biochemical reaction (de Cueto et al., 2004). In this study, bacteria culture in the test medium was made without prior centrifugation.

Other studies used centrifugation in serum separator tube to maximize the number of bacteria and reduce the rest of the blood components (Beuving et al., 2011).

The quality of the medium plays a major role in determining the accuracy and genus of organisms due to the use of a simple biochemical test. Control for the quality of the media can be done by making the media compatible with procedures and planting pure isolates were tested in each examination. Efforts to avoid contamination should be concidered (CLSI, 2008).

This present study found that only three samples (9%) had more than one colonial growth of bacteria visible in Mac Conkey media. The finding supports the previous studies on the direct identification method (Christneret al., 2010). The small number of blood cultures with more than one colony of bacteria can support direct inoculation method in clinical implementation.

The limitation of this study is that the biochemical test used in this study can identify *Enterobacteriaceae* to genus level. The addition of media sugars such as maltose and sucrose can be helpful in the determination of species (Barrow and Feltham, 2003). Identification to species level is necessary for epidemiological interest, while for the interpretation of the guidelines for the identification of antibiotic sensitivity can be done only to genus level (Ng et al., 2007).

This study provide data for clinical microbiology laboratory in performing identification of rod shaped Gram-negative *Enterobacteriaceae* at. Planting directly to the media biochemical test performed in conjunction with a primary isolation (subculture) on solid media. The primary isolation remains necessary for determination of bacterium's oxidation test results Results of planting on biochemical test media can be read only if the test result is negative and the result TSIA oxidation legible acid/sour. If all conditions are not met, the identification must be made by conventional methods. To overcome these problems, further research on the direct method is needed to bacterial identification of non fermenter class so that all rod shapped Gram-negative bacteria can be identified by the direct method.

## CONCLUSION

The results suggest that the direct inoculation method for genus identification for *Enterobacteriaceae* is suitable, with potential savings of 24 hours compared with conventional methods.

## ACKNOWLEDGEMENT

This study was supported by UP3 FK UNDIP.

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