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Original Article

Accuracy and reliability of direct disc diffusion antibiotic susceptibility test from flagged-positive of blood culture

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Abstract

Introduction: Antibiotic susceptibility tests (AST) done on blood cultures are critical for the treatment of patients suspected to be suffering from bloodstream infection. The objective of this study was to evaluate the accuracy and reliability of disc diffusion AST conducted directly (direct AST) from flagged-positive blood cultures, especially for Gram-positive cocci bacteria.

Methodology: This study compared direct AST with conventional AST (broth micro-dilution). The antibiotics studied were piperacillin/tazobactam, gentamicin, ceftazidime, erythromycin, and penicillin. Accuracy was determined by calculating very major, major, and minor errors. The reliability was determined by categorical agreement and weighted Kappa index.

Results: Gram-positive cocci bacteria were grown in pairs of blood culture bottles and tested with the two methods of AST. No very major errors were detected among the five types of antibiotics. Major errors of 2.56% and minor errors of 4.93% were found when testing gentamicin. The major and minor errors when testing erythromycin were 2.85% and 1.23%, respectively. Perfect agreements (categorical agreement: 100%; weighted Kappa index: 1) of the two AST methods were observed with piperacillin/tazobactam, ceftazidime, and penicillin. Almost perfect agreement was found with gentamicin and erythromycin. Categorical agreement results when testing antibiotics gentamicin and erythromycin were 93.83% and 97.53%, respectively. In addition, the weighted-Kappa index when testing these two antibiotics were 0.92 and 0.96, respectively.

Conclusions: The accuracy and reliability of the direct AST was within acceptable limits.

Key words: accuracy; blood culture; direct AST; agreement.

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Introduction

Bloodstream infection (BSI) is a serious and potentially life-threatening condition in healthcare facilities worldwide. A population-based study conducted during the period of 2004–2018 reported that the annual morbidity rate associated with this infection was an average of 216 episodes/100,000 population [1]. Another retrospective study reported that the prevalence of BSI in hospital admission was 1.7% [2]. The prevalence of bacteremia among children < 18 years old who suffered from pneumonia was 2.2% [3]. A study in rural Thailand reported that community onset of *Staphylococcus aureus* (*S. aureus*) bacteremia was 9.3 per 100,000 persons per year, whereas the hospital onset was 0.13/1,000 hospitalizations/year [4].

BSI may sometimes occur due to catheter insertion. The incidence rate in a study conducted in an Indian tertiary hospital was 8.75 per catheter days [5]. A multicenter cohort study reported that the incidence rate of central catheter-related BSI was 3.23 per catheter days [6].

The impact of BSI can range from mild to severe conditions. It could result in longer length of stay, higher risk of intensive care admission, invasive mechanical ventilation, or shock [3]. In addition, this infection may even cause death. A population-based cohort study reported 30-day mortality rates of first time BSI patients in three-time intervals. The 30-day mortality in the time intervals of 1992–1996, 1997–2001, and 2002–2006 were 22.7%, 21.2%, and 20.6%, respectively [7]. The 30-day mortality of 4.3% was reported in another study on BSI caused by methicillin resistant coagulase negative *Staphylococcus* [8]. The 1-month fatality rate of BSI in 2004 was reported as 13.0%, which over time declined slightly to 12.6% in 2018 [1].

Earlier detection of BSI, followed by earlier appropriate antimicrobial treatment are considered to positively contribute to controlling disease progression. Accordingly, in order to obtain earlier results of the

antibiotic susceptibility test (AST), some researchers developed direct disc diffusion AST, hereinafter referred to as direct AST. This test is conducted directly from the broth of the blood culture bottles with evidence of bacterial growth, and without previously undergoing sub-culture. Culture broth is directly inoculated in Mueller Hinton agar and subsequently the antibiotic discs are placed directly on it [9–11]. This study aimed to evaluate the accuracy and reliability of direct AST of Gram-positive cocci bacteria which was conducted on flagged-positive specimens of blood culture.

Methodology

This cross-sectional study was designed to evaluate the accuracy and reliability of direct AST, as compared with conventional AST of blood cultures which requires prior sub-culture. This study was approved by the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada Yogyakarta (number KE/FK/1284/EC/2020), and conducted in the Clinical Laboratory of Dr. Sardjito Public Hospital, in Yogyakarta, Indonesia.

The blood cultures that indicated positive signs of bacterial growth in two bottles were taken out from the incubator. After gentle shaking of the bottles, smears were made on an object glass followed by Gram staining and microscopic examination. The broth that indicated Gram-positive cocci bacteria was then processed further for direct AST, which was done by taking out 0.1 mL of broth from the positive blood culture bottle using a 1 mL syringe. Then, 4 drops of the broth were rubbed evenly in all directions on the Mueller-Hinton agar plate. Furthermore, five antibiotic discs – piperacillin/tazobactam 110 µg, gentamicin 10 µg, ceftazidime 30 µg, erythromycin 15 µg, and penicillin 10 IU – were placed on it and incubated for 16–18 hours at 37 °C (Figure 1). The inhibition zones were measured by a caliper and then recorded and reported as sensitive, intermediate, or resistant according to the Clinical Laboratory Standard Institute guidelines [12]. *S. aureus* ATCC 29213 was used for quality control of AST.

For conventional AST, the broth was sub-cultured from the flagged-positive blood culture on both chocolate and MacConkey agar media and then incubated over-night at 37 °C. Next, 0.5 McFarlan standard of bacterial suspension was prepared, which was then further processed for identification and AST using VITEK-2 (VITEK® 2 GP; VITEK® 2 AST-GP67, Marcy L'Étoile, France) and interpreted according to the Clinical Laboratory Standard Institute guidelines [13].

The accuracy of direct AST was measured by measuring minor errors, major errors, and very major errors. The errors were determined by comparing the results of the direct AST to the conventional method as the reference [14]. Very major errors were determined whenever the result of direct AST was sensitive, but the result of the conventional method was resistant. When the result of direct AST was resistant, but the conventional method tested as sensitive, it was determined as a major error. Minor errors were determined whenever the test result of direct AST was categorized as either sensitive or resistant, but the conventional method indicated intermediate response for each replicate. In addition, minor errors could occur when the direct AST method concluded the sample as intermediate, but the conventional method concluded the sample as either sensitive or resistant. Minor errors were calculated by dividing the number of minor errors found by the total number of isolates tested for their antibiotic susceptibility and then expressed as percentage.[14]

Statistical analysis

The data were analyzed using descriptive statistics. The accuracy of direct AST was determined by calculating the error rates. The reliability of this susceptibility test was determined by calculating

Figure 1. Direct antibiotic susceptibility test. A, the broth from a positive blood culture is taken; B, four drops of the broth are streaked on Muller Hinton (MH) agar plat; C, antibiotic discs are placed on MH agar plate.

Table 1. Characteristics of the study subjects.

Variable	n	%
Gender		
Male	51	62.9
Female	30	37.1
Age		
< 60 years old	49	60.5
> 60 years old	32	39.5
Ward		
Intensive	18	22.2
Non-intensive	63	77.8
Use of medical device		
Intravenous (IV) line	81	100
IV line and central venous catheter	7	8.6
IV line and dialysis catheter	22	27.1

weighted Kappa index. Principally, this index was calculated by the following formula: observed agreement minus agreement on the basis of chance divided by potential actual agreement, as described in a previous publication [15]. Z-tests were used to test different proportions in one population. Differences in proportion were considered statistically significant if the p value was < 0.05.

Results

This study included 81 patients, consisting of 51 males (62.9%) and 30 females (37.1%.) Most of subjects were < 60 years old (60.5%) and received care in the non-intensive ward (77.8%). All subjects were placed on an intravenous line. There were 7 (8.6%) placed on both intravenous line and central venous catheter, whereas 22 (27.1%) were placed on both intravenous catheter and dialysis catheter. The use of central venous catheters and dialysis catheters were reported by Sahli *et al.* and Parameswaran *et al*. as the biggest risk factors for bloodstream infection due to Gram positive bacteria [5,16]. A summary of the characteristics of the patients is presented in Table 1.

The critical values of methicillin resistant coagulase negative *Staphylococcus* (MRCoNS), methicillin resistant *Staphylococcus aureus* (MRSA), and methicillin resistant *Staphylococcus epidermidis* (MRSE) were 29.6%, 17.2%, and 9.8%, respectively (Table 2). Yamada *et al.* reported that the increased risk of bacteremia due to MRCoNS was associated with the condition of patients with hematologic malignancies, chemotherapy-induced neutropenia, skin or mucosal disorders or infection, and prolonged use of central venous catheters [8].

Table 2. Clinical isolates of Gram-positive cocci bacteria causing bloodstream infection identified by conventional blood culture technique.

	n	%
Clinical isolates found in blood culture		
Staphylococcus aureus	34	41.9
Staphylococcus hominis	11	13.6
Staphylococcus haemolyticus	11	13.6
Staphylococcus epidermidis	10	12.3
Enterococcus faecalis	10	12.3
Enterococcus faecium	3	3.7
Staphylococcus saphropyticus	2	2.4
Critical values of blood culture result		
MRCoNS	24	29.6
MRSA	14	17.2
MRSE	8	9.8

MRSA: methicillin resistant *Staphylococcus aureus*; MRSE: methicillin resistant *Staphylococcus epidermidis*; MRCoNS: methicillin resistant coagulase negative *Staphylococcus*.

The resistance of Gram-positive cocci bacteria to piperacillin/tazobactam, gentamicin, ceftazidime, erythromycin, and penicillin measured by direct AST were 64.2%, 51.9%, 74%, 53%, and 82.7% respectively. Similar rates of resistance of these bacteria to piperacillin/tazobactam, gentamicin, ceftazidime, erythromycin, and penicillin were also detected when tested by conventional AST (Table 3).

There were no minor, major, nor very major errors detected when three types of antibiotics (piperacillin/tazobactam, ceftazidime, and penicillin) were tested by direct AST (Table 4). Minor errors of 4.93% (p = 0.063) and major errors of 2.50% (p = 0.396) were found when direct AST was used to test gentamicin. The minor errors were not statistically different from the acceptable minor error of < 10%. The major errors were not statistically different from the acceptable major error of < 3%. Minor errors of 1.23% and major errors of 2.85% (p = 0.469) were found when

Table 3. Results of antibiotic susceptibility tests (AST) using direct AST and conventional AST.

Method	Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Direct AST	Piperacillin/Tazobactam	29 (35.8)	0	52 (64.2)
	Gentamicin	38 (46.9)	1 (1,2)	42 (51.9)
	Ceftazidime	21 (26)	0	60 (74)
	Erythromycin	34 (42)	4 (5)	43 (53)
	Penicillin	14 (17.3)	0	67 (82.7)
Conventional AST	Piperacillin/Tazobactam	29 (35.8)	0	52 (64.2)
	Gentamicin	43 (53)	3 (3.7)	35 (43.3)
	Ceftazidime	21 (26)	0	60 (74)
	Erythromycin	34 (42)	5 (6.1)	42 (51.9)
	Penicillin	14 (17.3)	0	67 (82.7)

Table 4. Accuracy of the direct antibiotic susceptibility tests (AST).

Method	Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Direct AST	Piperacillin/Tazobactam	29 (35.8)	0	52 (64.2)
	Gentamicin	38 (46.9)	1 (1,2)	42 (51.9)
	Ceftazidime	21 (26)	0	60 (74)
	Erythromycin	34 (42)	4 (5)	43 (53)
	Penicillin	14 (17.3)	0	67 (82.7)
Conventional AST	Piperacillin/Tazobactam	29 (35.8)	0	52 (64.2)
	Gentamicin	43 (53)	3 (3.7)	35 (43.3)
	Ceftazidime	21 (26)	0	60 (74)
	Erythromycin	34 (42)	5 (6.1)	42 (51.9)
	Penicillin	14 (17.3)	0	67 (82.7)

AST was used to test erythromycin. The occurrence of these two errors was not statistically higher than the acceptable error limit.

Reliability evaluation of the direct AST was done by comparing it with the conventional AST in 81 clinical isolates of Gram-positive cocci bacteria (Table 5). Complete agreements were found in three types of antibiotics, namely piperacillin/tazobactam, ceftazidime, and penicillin. Agreement percentages of these two methods of AST in testing gentamycin and erythromycin were 93.83% and 97.53%, respectively. The weighted Kappa index of 1 was found in the case of piperacillin/tazobactam, ceftazidime, and penicillin. The values of this index were 0.92 and 0.96 for gentamicin and erythromycin, respectively.

Discussion

It is uncertain whether the cause of bacteremia is Gram-negative bacteria or Gram-positive bacteria. Some studies have reported that Gram-positive cocci were more predominant [17–23]. However, other studies concluded that Gram-negative bacteria are more frequently associated with bacteremia [24–28]. One study reported that Gram-positive cocci were more frequently found in pediatric patients, whereas Gram-negative bacteria were predominant in the adult and elderly populations [29].

The AST that is done directly from the blood culture with bacterial growth is considered to be more beneficial since the results of this test are available earlier. However, this test should be evaluated in terms of both accuracy and reliability. Coorevits *et al.* conducted a study by comparing AST conducted directly from various clinical specimens to conventional AST; both AST were conducted using the disc diffusion method [9]. Based on AST test results of 97 clinical specimens of Gram-negative bacteria, they reported that the accuracy of the results of direct AST were 93.4% in agreement, with 1.6% minor errors, 4.6% major errors, and 0.4% very major errors. The two AST methods showed perfect agreement (100% agreement) when conducted on 26 clinical specimens of *Staphylococci* [9]. Another study was conducted to compare AST directly from blood cultures which indicated bacterial growth (flagged growth) to the conventional AST method. The direct ASTs were conducted by Kirby Bauer disc diffusion method whereas the conventional method was done by micro broth dilution method (VITEK-2 Compact; BioMerieux, Marcy L'Étoile, France) which required prior sub-culture on solid agar media. The results on Enterobacteriaceae showed 98.95% agreement, while the very major, major, and minor errors were 0.21%, 0.42%, and 0.425%, respectively. There was 94.44% agreement when testing *Staphylococci* isolates, along with 1.39% very major errors, 1.39% major errors, and 2.78% minor errors. Agreement of these two AST methods conducted on clinical isolates of non-fermenter Gram-negative rod and *Enterococcus* spp. were 98.21% and 97.83%, respectively. No very major errors were found in these two kinds of bacteria. Major errors of 1.19% and minor errors of 0.6% were found in the non-fermenter Gram-negative rods. In addition, there were major and minor errors of 1.45% and 0.72%, respectively in the *Enterococcus* spp. [30]. Chandrasekaran *et al.* evaluated direct AST by comparing it with three different systems of blood culture as reference standards. Twenty isolates of Enterobacteriaceae, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* were inoculated respectively into blood culture bottles of Bactec™ Plus Aerobic/F,

Table 5. Reliability of the direct antibiotic susceptibility test (AST) compared to the conventional method.

Antibiotics	n	Agreement (%)	Weighted Kappa index
Piperacillin/Tazobactam	81	100	1
Gentamicin	81	93.83	0.92
Ceftazidime	81	100	1
Erythromycin	81	97.53	0.96
Penicillin	81	100	1

VersaTREK Redox 1, and BacT/Alert FA Plus (Becton Dickinson and Company, Sparks, USA) and subsequently incubated into systems of automated blood culture. The categorical agreements of the direct AST with the blood culture system of Bactec™ Plus Aerobic/F, VersaTREK Redox 1, and BacT/Alert FA Plus were 87.8%, 88.4%, and 92.2%, respectively. No very major errors were found. Major errors of 3%, 2.3%, and 1.7% were found when direct AST was compared to the blood culture system of Bactec™ Plus Aerobic/F, VersaTREK Redox 1, and BacT/Alert FA Plus. They also found that the best time duration for incubation was 18 hours. Bacterial density ranged from 7.6×10^7 to 5.0×10^8 CFU/mL which resulted in categorical agreement of the direct AST ranging from 94.7% to 96.2% [10]. A similar study was conducted in order to evaluate the accuracy of EUCAST rapid disc diffusion AST, as well as to study its duration of incubation (4, 6, and 8 hours). The reference standard was conventional blood culture which used Bactec™ Plus aerobic and instrument of blood culture Bactec™ FX (Becton Dickinson and Company, Sparks, USA). Clinical isolates used in this study were *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *E. faecalis*, *E. faecium*, and *S. pneumoniae*. The results of the study showed good categorical agreement, and the error rate was acceptable. Study of each clinical isolate showed total very major and major errors < 3% and minor error < 10% in 4, 6, 8 hours incubation periods. In general, a lesser error was found in 8 hours of incubation time [31].

In the present study, the 5 types of antibiotics that are commonly used as the first line of antibiotic treatment for Gram-positive cocci bacteria were selected. It is possible to extend the study to other types of antibiotics including cefoxitin so that the possibility of resistance to methicillin can be detected earlier.

The accuracy of direct AST in this study was similar to the findings in a previous study and acceptable for all of the five types of antibiotics studied since both the very major and major errors were < 3%, and minor errors were < 10% for each antibiotic tested [32]. Reliability of this AST could be seen in the value of categorical agreement in which the value of > 90% was acceptable. The agreements of direct AST with the reference standard of conventional AST for each of the five types of antibiotics were > 90%. Perfect agreement (categorical agreement: 100%; weighted-Kappa index: 1) among these two methods of AST was observed when testing the antibiotics piperacillin/tazobactam, ceftazidime, and penicillin. Categorical agreement when testing the antibiotics gentamicin and erythromycin were 93.83% and 97.53%, respectively. In addition, the weighted-Kappa index for testing of these two antibiotics were 0.92 and 0.96, respectively. These two had a weighted-Kappa index which indicated a value of almost perfect agreement [15]. Determining weighted-Kappa index is considered as an important parameter of reliability because this agreement index excludes agreement that might have occurred on the basis of chance and takes into account the potential actual agreement.

Conclusions

Direct AST was conducted from the broth of blood culture which indicated bacterial growth and was compared with conventional AST (broth micro dilution) as a reference standard in order to evaluate its accuracy and reliability. The results showed that the direct AST method was accurate and reliable for analyzing clinical isolates of Gram-positive cocci bacteria, and therefore it could be recommended for routine testing in laboratory services.

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Author Contributions

OS, RNF, and IP: conception and research planning; RNF submission of ethical clearance to the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada, data acquisition/collection, data analysis and preparing the manuscript. OS and IP: supervising the research, data analysis, feedback on the manuscript. All the authors have critically reviewed the manuscript.

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