A Comparative Study to Determine the Recovery Rate of Microorganisms of Bloodstream Infections: Two Versus Three Blood Culture Specimens

Teerarat Shanthachol MD*,
Sumanee Nilgate Msc**, Chusana Suankratay MD, PhD*

** Division of Infectious Diseases, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*
*** National Streptococcal Reference Center in Thailand, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*

Objective: *There has been a development of automated and continuous-monitoring blood culture systems that are more sensitive than conventional systems for the detection of microorganisms. Whether two or three blood culture specimens obtained during a 24-hour period using these automated systems achieving a higher recovery rate of microorganism remains to be determined. The present study was aimed to compare the recovery rates of microorganism of blood-stream infections (BSIs) using two and three blood culture specimens.*
Material and Method: *A prospective investigator-blinded study was carried out in patients who needed to have blood cultures in medicine wards and intensive care units as well as an emergency room of King Chulalongkorn Memorial Hospital, Bangkok, Thailand, between October 1, 2010 and March 31, 2011. Three blood culture specimens were obtained from each patient during a 24-hour period. Each specimen was inoculated into an aerobic bottle of blood culture broth (TREK Diagnostics, Cleveland, OH, US), and then incubated at 37°C for seven days.*
Results: *Of 568 patients, there were 116 (20.4%) unimicrobial episodes with three blood cultures obtained during a 24-hour period. There were 70 (12.3%) and 46 (8.1%) episodes of true pathogen and contaminant, respectively. The recovery rates of true pathogen were 75.7% (53 isolates), 87.1% (61 isolates), and 100% (70 isolates) with the first, second, and third blood culture specimens, respectively ($p < 0.05$ between the recovery rate with the first two and the third blood culture specimens). There were 25 (35.7%), 38 (58.6%) isolates, and four (5.7%) of Gram-positive, Gram-negative bacteria, and fungi, respectively. Among 25 Gram-positive bacteria, Staphylococcus aureus was the most common isolate (10, 14.3%), followed by Streptococcus pneumoniae (5, 7.1%) and Enterococcus faecalis, Enterococcus faecium, coagulase-negative Staphylococcus (3, 10% each). Among 38 Gram-negative bacteria, Escherichia coli was the most common isolate (13, 18.6%), followed by Pseudomonas aeruginosa (8, 11.4%), and Klebsiella pneumoniae (6, 8.6%). The sensitivity and specificity of the recovery rate of microorganisms using two blood culture specimens were 85.7% and 92.3%, respectively. The sensitivity and specificity of the recovery rate of microorganisms using three blood culture specimens were 100% and 90.8%, respectively.*
Conclusion: *To the best of the authors' knowledge, the present study is the first prospective study to compare the recovery rate of microorganisms of BSIs between the two and three blood culture specimens using the VersaTREK blood culture system. Three blood culture specimens are required to achieve the recovery rate of more than 99%.*

Keywords: *Blood cultures, Cultures, automated blood cultures, Microorganisms, Recovery rate, Bloodsteam infections*

J Med Assoc Thai 2012; 95 (8): 1053-8
Full text. e-Journal: *http://jmat.mat.or.th*

A blood culture is defined as a specimen of blood obtained from a single venipuncture or intravenous access device[1]. There has been a development of blood culturing techniques to improve the recovery rate of microorganisms of bloodstream infections (BSIs)[2]. The previous studies were carried out to determine the optimal blood volume[1-4], the number of specimens[5,6], and the incubation period[5] for the detection of bloodstream microorganisms, using the conventional manual blood culture systems. Since then, there has been a development of automated, continuous-monitoring culturing systems that are more sensitive for the detection of microorganisms. To the authors' knowledge, there have been no studies to determine the recovery rate of microorganisms using the automated VersaTREK blood culture system

Correspondence to:
Suankratay C, Division of Infectious Diseases, Department of Medicine, Chulalongkorn University Hospital, Bangkok 10330, Thailand.
Phone & Fax: 0-2256-4578
E-mail: chusana.s@chula.ac.th

despite its wide availability in Thailand for more than a decade. In addition, the sensitivity for detection of bloodstream microorganisms varies among each automated, continuous-monitoring blood culture systems. The present study was thus aimed to compare the recovery rates of microorganisms of BSIs using two vs. three blood culture specimens at King Chulalongkorn Memorial Hospital (KCMH), Bangkok, Thailand.

Material and Method

Study design

A prospective investigator-blinded study was carried out in all patients who needed to have blood cultures in medicine wards and intensive care units as well as an emergency room of KCMH, Thailand, between October 1, 2010 and March 31, 2011. Written informed consent was obtained from all patients, and the institutional review board approved the protocol.

Patients

The present study enrolled the patients older than 15 years who had clinical features compatible with bacteremia/fungemia requiring of blood cultures taken from peripheral veins. Three blood culture specimens were obtained from each patient during a 24-hour period. Skin preparation before taking blood cultures using 2% chlorhexidine in 70% alcohol. The volume of blood that was obtained for each blood culture specimens was 5 mL. Each specimen was directly inoculated into an aerobic bottle of blood culture broth (TREK Diagnostics, Cleveland, OH, US), and then incubated at 37°C for seven days. The isolated microorganism and their antibiotic susceptibility pattern were determined using the standard methods recommended by the Clinical Laboratory Standards Institute (CLSI)[7]. The medical records of all patients who had positive blood cultures were reviewed by one of the investigators (TS) to determine the clinical significance of each isolate from blood cultures. All blood culture isolates were categorized into two groups including a true pathogen and a contaminant. A contaminant was classified if (a) a common skin flora including coagulase-negative *Staphylococcus* (CoNS), *Corynebacterium* species, *Micrococcus* species, *Bacillus* species, or *Propionibacterium* species was isolated from one of the three blood culture specimens without an isolation of the same organism from another potentially infected site (for example, intravenous catheter tip)[8] or (b) a common flora of the skin was isolated in a patient with incompatible clinical features, no contributing risks, and improvement without specific treatment for that microorganism.

Statistical analysis

In order to calculate the sample size to determine the difference in the recovery rate of microorganisms using two versus three blood culture specimens, the authors assumed that it would be 6.9% at King Chulalongkorn Memorial Hospital from the authors' previous study[9], and the authors' small pilot study in 2009 which showed that the recovery rates of microorganisms using two and three blood culture specimens were 70% and 100%, respectively (unpublished data). The authors' sample size was calculated using the 2-related population, non-parametric test for a difference. Hence, 124 positive blood cultures provided 90% power to detect the difference of the recovery rate of microorganisms using two versus three blood culture specimens. All data were analyzed using the SPSS 16.0 software program. The categorical variables were compared using McNemar's test, and $p < 0.05$ was considered statistically significant.

Results

Five hundred sixty eight patients needed to have blood cultures during the study period. There were 116 (20.4%) unimicrobial episodes with three blood cultures obtained during a 24-hour period. There were 70 (12.3%) and 46 (8.1%) episodes of true pathogen and contaminant, respectively.

The recovery rates of true pathogen were 75.7%, 87.1%, and 100% with the first, second, and third blood culture specimens, respectively ($p < 0.05$ between the recovery rate with the first two and the third blood culture specimens) (Table 1). The recovery rates of contaminant were 69.6%, 82.6%, and 100% with the first, second, and third blood culture

Table 1. The recovery rates of true pathogen and contaminant with the first 1, 2, and 3 blood culture specimens

Numbers of specimens	Number of recovery (percent)	
	True pathogen (n = 70)	Contaminant (n = 46)
First 1 specimen	53 (75.7)	32 (69.6)
First 2 specimens	61 (87.1)	38 (82.6)
First 3 specimens	70 (100)	46 (100)

specimens, respectively. The sensitivity and specificity of the recovery rate of microorganisms using two blood culture specimens were 85.7% and 92.3%, respectively. The sensitivity and specificity of the recovery rate of microorganisms using three blood culture specimens were 100% and 90.8%, respectively. Among Gram-positive bacteria, the sensitivity and specificity of the recovery rate using two blood culture specimens were 96% and 92.8%, respectively. The sensitivity and specificity of the recovery rate of Gram-positive bacteria using three blood culture specimens were 100% and 91.3%, respectively. Among Gram-negative bacteria, the sensitivity and specificity of the recovery rate using two blood culture specimens were 78.1% and 100%, respectively. The sensitivity and specificity of the recovery rate of Gram-negative bacteria using three blood culture specimens were 100% and 100%, respectively.

There were 53 (75.7%), 61 (87.1%), and 70 (100%) episodes of true pathogen detected with the first, second, and third blood culture specimens, respectively. Among 70 true pathogens, there were 25 (35.7%), 41 (58.6%), and four (5.7%) isolates of Gram-positive bacteria, Gram-negative bacteria, and fungi, respectively. Among 25 Gram-positive bacteria, *Staphylococcus aureus* was the most common isolate (10 isolates, 40.0%), followed by *Streptococcus pneumoniae* (5, 20.0%), and *Enterococcus faecalis*, *Enterococcus faecium*, CoNS (3, 12.0% each). Among 41 Gram-negative bacteria, *Escherichia coli* was the most common isolate (13 isolates, 31.7%), followed by *Pseudomonas aeruginosa* (8, 19.5%), and *Klebsiella pneumoniae* (6, 14.6%). Among 46 contaminants, all were Gram-positive bacteria including CoNS (23, 50%), *Bacillus* species (8, 17.4%), *Corynebacterium* species (7, 15.2%), and *S. aureus* (7, 15.2%) (Table 2).

Table 2. The species of bacteria and fungi isolated from all three blood culture specimens

Microorganisms	Number of isolates (percent)	
	True pathogen	Contaminant
Gram-positive bacteria	25 (35.7)	46 (100)
Staphylococcus aureus	10 (14.3)	7 (15.2)
Streptococcus pneumoniae	5 (7.1)	0
Coagulase-negative *Staphylococcus*	3 (4.3)	23 (50.0)
Enterococcus faecalis	3 (4.3)	0
Enterococcus faecium	3 (4.3)	0
Lactobacillus species	1 (1.4)	0
Bacillus species	0	8 (17.4)
Corynebacterium species	0	7 (15.2)
Streptococcus salivarius	0	1 (2.2)
Gram-negative bacteria	41 (58.6)	0
Escherichia coli	13 (18.6)	0
Pseudomonas aeruginosa	8 (11.4)	0
Klebsiella pneumoniae	6 (8.6)	0
Burkholderia cepacia	1 (1.4)	0
Acinetobacter baumannii	5 (7.1)	0
Salmonella Choleraesuis	3 (4.3)	0
Proteus mirabilis	1 (1.4)	0
Stenotrophomonas maltophilia	1 (1.4)	0
Enterobacter cloacae	3 (4.3)	0
Fungi		
Candida albicans	4 (5.7)	0
Total	70	46

Among 10 patients with positive culture in the third specimen only, there were four (40%) females and six males. Nine (90%) patients were older than 50 years. Among these 10 isolates, there was one (10%) Gram-positive (*E. faecium*) and nine (90%) Gram-negative bacteria (4 *P. aeruginosa*, 3 *E. coli*, 1 *Enterobacter cloacae*, and 1 *Salmonella Choleraesuis*). The final diagnosis was primary bacteremia (4 patients, 40%), urinary tract infection (2, 20%), catheter-related BSI (2, 20%), pneumonia (1, 10%), and spontaneous bacterial peritonitis (1, 10%). Among these 10 patients, antibiotics were changed in three patients from a carbapenem to an antipseudomonal cephalosporin with and without an aminoglycoside according to the susceptibility pattern.

Discussion

Two or three blood culture specimens obtained during a 24-hour period are generally considered appropriate for the detection of microorganisms of BSIs in adult patients using the conventional manual blood culture systems[2,5,6,8,10,11]. Weinstein and colleagues studied 282 adult patients and found that the recovery rates of microorganisms were 91.5% and 99.3% with the first and second blood culture specimens, respectively[6]. Bartlett et al studied 80 adults and found that recovery rates of microorganisms were 80%, 89%, and 99% with the first, second, and third blood culture specimens, respectively[10].

However, in the present study, three blood culture specimens were required to achieve the recovery rate of more than 99% in adults using the automated, continuous-monitoring blood culture system (TREK Diagnostics, Cleveland, OH, US). These results are consistent with those two previous studies using different automated, continuous-monitoring systems. A study by Cockerill et al using the automated BACTEC 9240 culture system (Becton Dickinson Diagnostic Instrument Systems, Bohemia, NY, USA) showed that the recovery rates of microorganisms were 65.1%, 80.4%, 95.7%, and 100% with the first, second, third, and fourth blood culture specimens, respectively[12]. Another study by Lee and colleagues was carried out in two university hospitals in the United States, using the automated BACTEC 9240 or BACT/ALERT (bioMerieux Clinical Diagnostics, Durham, NC, US) blood culture system[13]. Of 629 unimicrobial episodes with more than two blood culture specimens obtained during a 24-hour period, the recovery rates were 73.1%, 89.7%, 98.2%, and 99.8% with the first, second, third, and fourth blood culture specimens, respectively. The potential explanation for these findings is discussed by Cockerill et al[12]. They speculate that the automated, continuous-monitoring blood culture systems may be more sensitive than the conventional systems to detect the low-level of microorganisms of BSIs. Hence, a greater number of blood culture specimens may be required to detect such low level of microorganisms.

In the present study, the recovery rates of Gram-positive bacteria were not significantly different between using two and three blood culture specimens (96% and 100%, respectively). In contrast, the recovery rate of Gram-negative bacteria using three blood culture specimens was higher than that using two blood culture specimens (87.1% and 100%, respectively). These observations are consistent with a study by Lee et al, which showed that *S. aureus* BSIs was recovered using the first two blood culture specimens in about 90% of episodes, whereas *P. aeruginosa* was detected in 60% of BSIs using the first two blood culture specimens[13]. The authors' potential explanation could be the relatively higher sensitivity of the modern automated, continuous-monitoring blood culture systems to detect Gram-positive bacteria BSIs, compared to Gram-negative bacteria BSIs.

In the present study, the most frequently isolated microorganism was *E. coli* (18.6%), followed by *S. aureus* (14.3%), *P. aeruginosa* (11.4%), and *K. pneumoniae* (8.6%). A study by Cockerill et al showed that S. aureus was the most common isolate, followed by *E. coli* and CoNS. Another study by Lee et al also found that the most commonly isolated microorganism was *S. aureus*, followed by CoNS and *Enterococcus* species.

Even though the present study was carried out in a prospective fashion, it still has some limitations. The present study was aimed to determine the optimal number of blood culture specimens, and hence there was no standardization of appropriate practice of all procedures for blood cultures by health personnel especially the volume of each blood sample, which also is much important for the recovery of bacteria of BSIs. Aseptic technique is also important to decrease the contamination rate. However, no healthcare personnel were informed regarding the ongoing study, and hence there should be no influence of the results of the present study. In addition, the present study was carried out only at the Department of Medicine in one institute. Hence, it could not be generalized to other departments or hospitals unless

a well-designed multicenter study was conducted in the future to confirm the present results.

In conclusion, the recovery rates of micro-organisms of BSIs between the second and third blood culture specimens using the VersaTREK blood culture system were 87.1% and 100%, respectively. Three blood culture specimens are required to achieve the recovery rate of more than 99%.

Acknowledgement

The authors wish to thank all the healthcare personnel of King Chulalongkorn Memorial Hospital for assistance with the present study.

Potential conflicts of interest

None.

References

1. Dunne WM, Nolte FS, Wilson ML. Blood cultures III. In: Hindler JA, editor. Cumulative techniques and procedures in clinical microbiology (Cumitech). Washington, DC: American Society for Microbiology; 1997:1-21.
2. Hall KK, Lyman JA. Updated review of blood culture contamination. Clin Microbiol Rev 2006; 19: 788-802.
3. Tenney JH, Reller LB, Mirrett S, Wang WL, Weinstein MP. Controlled evaluation of the volume of blood cultured in detection of bacteremia and fungemia. J Clin Microbiol 1982; 15: 558-61.
4. Ilstrup DM, Washington JA II. The importance of volume of blood cultured in the detection of bacteremia and fungemia. Diagn Microbiol Infect Dis 1983; 1: 107-10.
5. Washington JA II. Blood cultures: principles and techniques. Mayo Clin Proc 1975; 50: 91-8.
6. Weinstein MP, Reller LB, Murphy JR, Lichtenstein KA. The clinical significance of positive blood cultures: a comprehensive analysis of 500 episodes of bacteremia and fungemia in adults. I. Laboratory and epidemiologic observations. Rev Infect Dis 1983; 5: 35-53.
7. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility testing; nineteenth informational supplement. CLSI document M100-S19. Pennsylvania: CLSI; 2008.
8. MacGregor RR, Beaty HN. Evaluation of positive blood cultures. Guidelines for early differentiation of contaminated from valid positive cultures. Arch Intern Med 1972; 130: 84-7.
9. Suwanpimolkul G, Pongkumpai M, Suankratay C. A randomized trial of 2% chlorhexidine tincture compared with 10% aqueous povidone-iodine for venipuncture site disinfection: Effects on blood culture contamination rates. J Infect 2008; 56: 354-9.
10. Bartlett RC. Contemporary blood culture practices. In: Sonnenwirth AC, editor. Bacteremia: laboratory and clinical aspects. Springfield, IL: Charles C Thomas Publisher; 1973: 15-35.
11. Nicola FG. Assessing how many blood cultures are needed for detecting bloodstream infections. J Clin Microbiol 2008; 46: 1155-6.
12. Cockerill FR 3rd, Wilson JW, Vetter EA, Goodman KM, Torgerson CA, Harmsen WS, et al. Optimal testing parameters for blood cultures. Clin Infect Dis 2004; 38: 1724-30.
13. Lee A, Mirrett S, Reller LB, Weinstein MP. Detection of bloodstream infections in adults: how many blood cultures are needed? J Clin Microbiol 2007; 45: 3546-8.

การศึกษาเปรียบเทียบอัตราการเพาะเชื้อจุลชีพของการติดเชื้อในกระแสเลือด: สองหรือสามตัวอย่างเลือดเพาะเชื้อ

ธีรารัตน์ ฉันทชล, สุมาณี นิลเกตุ, ชุษณา สวนกระต่าย

วัตถุประสงค์: *ได้มีการพัฒนาระบบการเพาะเชื้อในเลือดเป็นระบบอัตโนมัติและติดตามการขึ้นของเชื้อตลอดเวลา ซึ่งพบว่ามีความไวมากกว่าระบบการเพาะเชื้อแบบดั้งเดิมในการตรวจพบเชื้อจุลชีพ การเพาะเชื้อในเลือดในช่วงเวลา 24 ชั่วโมง โดยใช้ระบบการเพาะเชื้อในเลือดแบบอัตโนมัติจะให้อัตราการขึ้นจุลชีพมากกว่าระหว่าง 2 และ 3 ตัวอย่างเลือดยังต้องการการศึกษา ดังนั้นการศึกษานี้จึงเป็นการศึกษาเปรียบเทียบอัตราการเพาะเชื้อจุลชีพจากการเก็บตัวอย่างเลือดระหว่าง 2 และ 3 ตัวอย่างเลือดเพาะเชื้อ*

วัสดุและวิธีการ: *เป็นการศึกษาไปข้างหน้าในผู้ป่วยที่มีความจำเป็นที่จะเก็บตัวอย่างเลือด เพื่อนำมาเพาะเชื้อจุลชีพในกลุ่มผู้ป่วยในของแผนกอายุรกรรมและในผู้ป่วยแผนกฉุกเฉิน โรงพยาบาลจุฬาลงกรณ์ ระหว่าง 1 ตุลาคม พ.ศ. 2553 ถึง 31 มีนาคม พ.ศ. 2554 โดยเก็บตัวอย่างเลือดเพาะเชื้อ 3 ตัวอย่างในระยะเวลา 24 ชั่วโมง แต่ละตัวอย่างเลือดใส่ในเพาะเชื้อในเลือดชนิดแอโรบิก (Trek Diagnostic Systems, Cleveland, OH, US) โดยเพาะเลี้ยงเชื้อจุลชีพที่ 37°C เป็นเวลา 7 วัน*

ผลการศึกษา: *จาก 568 ผู้ป่วย พบว่ามี 116 (20.4%) ตัวอย่างเลือดเพาะเชื้อขึ้นเชื้อจุลชีพ 1 ชนิด โดยพบว่า 70 (12.3%) เป็นเชื้อที่ก่อให้เกิดโรคจริง และ 46 (8.1%) ที่เป็นเชื้อปนเปื้อน อัตราการเพาะเชื้อจุลชีพของตัวอย่างเลือด 1, 2, และ 3 ตัวอย่างเลือดเพาะเชื้อ เท่ากับ 75.7% (53 จุลชีพ) 87.1% (61 จุลชีพ) และ 100% (70 จุลชีพ) ตามลำดับ ($p < 0.05$ ระหว่างอัตราการเพาะเชื้อจุลชีพจากการเก็บตัวอย่างเลือด 2 และ 3 ตัวอย่าง ตามลำดับ) มีการขึ้นแบคทีเรียแกรมบวก แกรมลบ และรา เท่ากับ 25 (35.7%) 38 (58.6%) และ 4 (5.7%) จุลชีพ ตามลำดับ จากจำนวน 25 แบคทีเรียแกรมบวก พบ Staphylococcus aureus มากที่สุด (10 ครั้ง 14.3%) ตามมาด้วย Streptococcus pneumoniae (5 ครั้ง 7.1%) และ Enterococcus faecalis, Enterococcus faecium, coagulase-negative staphylococcus (3 ครั้ง 10% ในแต่ละจุลชีพ) จากจำนวน 38 ชนิดของแบคทีเรียแกรมลบ พบ Escherichia coli มากที่สุด (13 ครั้ง 18.6%) ตามมาด้วย Pseudomonas aeruginosa (8 ครั้ง 11.4%) และ Klebseilla pneumoniae (6 ครั้ง 8.6%) ความไวและความจำเพาะของการเพาะเชื้อจุลชีพจากการเก็บตัวอย่างเลือด 2 ตัวอย่าง เท่ากับ 87.1% และ 92.5% ความไวและความจำเพาะของการเพาะเชื้อจุลชีพจากการเก็บตัวอย่างเลือด 3 ตัวอย่าง เท่ากับ 100% และ 90.8% ตามลำดับ*

สรุป: *อัตราการพบเชื้อก่อโรคจากการเพาะเชื้อจุลชีพจากการเก็บตัวอย่างเลือดเพาะเชื้อ 2 และ 3 ตัวอย่าง โดยใช้ระบบการเพาะเชื้อในเลือดแบบอัตโนมัติ VersaTREK โดยพบว่าการเก็บ 3 ตัวอย่างเลือดเพาะเชื้อมีอัตราการเพาะเชื้อจุลชีพมากกว่า 99%*