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Original article

How should microbiology laboratories interpret cultures of the sonicate of closed needleless connectors?

María Jesús Pérez-Granda[a,b,c,d,*], José María Barrio[a,b], Raquel Cruces[e], Beatriz Alonso[c,e], Pablo Martín-Rabadán[b,c], Inmaculada Collado[a], María Guembe[c,e]

[a] Department of Anesthesiology, Hospital General Universitario Gregorio Marañón, Madrid, Spain
[b] CIBER Enfermedades Respiratorias-CIBERES (CB06/06/0058), Madrid, Spain
[c] Department of Clinical Microbiology and Infectious Diseases, Hospital General Universitario Gregorio Marañón, Madrid, Spain
[d] Department of Nursing. School of Nursing, Physiotherapy and Podiatry, Universidad Complutense de Madrid, Madrid, Spain
[e] Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain

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ABSTRACT

Introduction: Our objective was to determine whether there is a cut-off in the needleless connectors' (NCs) cultures that when combined with skin cultures it was as efficient as conventional superficial cultures to rule-out catheter colonization (CC) and catheter-related bloodstream infection (CRBSI).
Methods: During 10 months, we collected samples and then we analyzed the validity values of skin + NCs cultures for CC and CRBSI considering the best cut-off showing at least >90% of specificity to have a high negative predictive value using a ROC curve.
Results: We collected a total of 167 catheters. The optimal cut-off of NCs culture was 1000 cfu/NC. The validity values for CC and CRBSI combining skin cultures and NCs cultures using the selected cut-off were, respectively: S, 42.9%/16.7%; SP, 83.6%/75.8%; PPV, 27.3%/2.5%; and NPV, 91.0%/96.0%.
Conclusions: The combination of skin cultures and quantitative NCs cultures could be used for ruling-out CC and CRBSI.

¿Cómo deben los laboratorios de microbiología interpretar los cultivos del «sonicado» de los conectores cerrados sin aguja?

Palabras clave:
Colonización
Cultivos superficiales
Conectores sin aguja
Sonicación
Punto de corte

RESUMEN

Introducción: Nuestro objetivo fue determinar si existe un punto de corte en los cultivos de conectores sin aguja (NC) que, cuando se combina con cultivos de piel, sea tan eficiente como los cultivos superficiales convencionales para descartar colonización de catéter (CC) y bacteriemia relacionada con el catéter (BRC).
Métodos: Durante 10 meses se coleccionaron muestras, y después se analizaron los valores de validez de los cultivos de piel + NC para CC y BRC considerando el mejor punto de corte aquel que mostrara al menos > 90% de especificidad para tener un alto valor predictivo negativo usando una curva ROC.
Resultados: Se estudiaron un total de 167 catéteres. El punto de corte óptimo del cultivo de NC fue de 1.000 ufc/NC. Los valores de validez para CC y BRC combinando cultivos de piel y cultivos de NC utilizando el punto de corte seleccionado fueron, respectivamente: S: 42,9/16,7%; ES: 83,6/75,8%; VPP: 27,3/2,5% y VPN: 91,0/96,0%.
Conclusiones: La combinación de cultivos de piel y cultivos cuantitativos de NC podría usarse para descartar CC y BRC.

* Corresponding author.
E-mail address: massus@hotmail.es (M.J. Pérez-Granda).

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Introduction

The use of conservative diagnostic methods to detect catheter colonization (CC) and catheter-related bloodstream infection (C-RBSI) are of high importance in the clinical management of Major Heart Surgery (MHS) patients.[1] Conventional superficial cultures (from the skin and hubs) demonstrated to have a high negative predictive value (90.4–96.7), for both colonization and C-RBSI, allowing to maintain catheters and to find another source of infection.[2–5] However, hub cultures require rubbing inside the catheter lumen, which may be a risk for bacteraemia because the biofilm can be dislodged.[6,7] Therefore, we demonstrated in previous studies that combining skin cultures with cultures of withdrawn needleless connectors (NCs) was an alternative and safer method to rule out CC and C-RBSI, showing no inferiority to conventional superficial culture.[8–10]

The aim of the present study was to assess whether there is a cut-off in the NCs culture that when combined with skin cultures was as efficient as conventional superficial cultures to rule-out CC and C-RBSI in patients admitted to MHS-intensive care unit (MHS-ICU).

Methods

Hospital setting and patients

Our institution is a general referral hospital with 1550 beds and approximately 50,000 admissions/year. More than 500 MHS procedures are performed annually in the Department of Cardiovascular Surgery, which is a large referral unit.

Study design

We performed an ecological prospective study and included the patients on the MHS-ICU when a CVC remained in place ≥7 days after insertion.

At catheter withdrawal, simultaneous superficial samples (from the skin surrounding the catheter insertion site and from the inside of the hubs) and NCs (CLAVE^{TM} systems, ICU Medical, Inc., San Clemente, CA, USA) were obtained.

The skin samples were obtained by lifting the dressing and rubbing the area around the insertion site (in a 3-cm radius) with dry cotton swab. The inner hub samples were obtained using alginate swabs that were introduced into the hub and rubbed repeatedly against its inner surface (1 swab per hub).

Superficial cultures were processed following standard semi-quantitative microbiological techniques.

Catheter tips were cultured using the roll-plate (Maki) technique and sonication onto a blood agar plate.

All groups of NCs belonging to a single catheter lumen (hub) were sonicated (1 min) together into 20 ml of BHI and 100 μl were cultured onto blood agar plates and incubated for 24–48 h at 37 °C. The number of colony forming units (cfu) was counted for each set of cultures and the no. of cfu/NC was calculated (cfu/plate × 200/no. NCs). We considered the lumen colonized when ≥1 culture was positive. The number of cultured NCs varied depending on the number of lumens in each catheter (1–5 lumens).

The gold standard to confirm catheter colonization was positivity of the catheter tip culture either by the semiquantitative Maki technique or by the sonication method.

We analyzed the validity values of skin and NCs' cultures for CC and C-RBSI considering the best cut-off showing >90% of specificity to have a high negative predictive value using a ROC curve.

The microorganisms recovered were fully identified using standard microbiological methods.

We also used a pre-established protocol to record patient characteristics, underlying diseases, comorbidity conditions, severity-of-illness scores, such as acute physiology and chronic health evaluation II (APACHE II) scores, the maximum severity reached before catheter withdrawal, and microbiological data on blood cultures.

Ethics

The Ethics Committee of our institution (Hospital Gregorio Marañon) approved the study (MICRO, HGUGM. 2015-083) and written informed consent was obtained from the study participants.

Definitions

Catheter tip colonization

Isolation of either ≥15 cfu/plate with the semiquantitative Maki technique or ≥100 cfu/segment with the sonication method.

Skin and hub colonization

Isolation of ≥15 cfu/plate in semiquantitative culture.

Table 1
Main patients' characteristics.

Variables	Total $N = 105$	Colonized $N = 19$	Non-colonized $N = 86$	p
Median age (IQR), years	68 (60.5–77.0)	73 (53.0–79.0)	67.5(61.0–77.0)	0.94
Male sex, N (%)	66 (62.8%)	14 (73.7%)	52 (60.5%)	0.31
Underlying conditions, N (%)				
Myocardial infarction	13 (12.5)	3 (15.8)	10 (11.6)	0.45
Congestive heart failure	91 (86.7)	18 (94.7)	73 (84.9)	0.45
Central nervous system disease	12 (11.4)	5 (26.3)	7 (8.1)	0.04
Chronic obstructive pulmonary disease	26 (24.8)	7 (36.8)	19 (22.1)	0.24
Renal dysfunction	16 (15.2)	2 (14.5)	14 (16.3)	0.73
Diabetes mellitus	28 (26.7)	6 (31.6)	22 (25.6)	0.58
Peptic ulcer disease	14 (13.3)	4 (21.1)	10 (11.6)	0.28
Peripheral vascular disease	12 (11.4)	2 (10.5)	10 (11.6)	1.00
Median Euroscore (IQR)	8.0 (5.5–10.0)	6.0 (5.0–10.0)	8.0 (6.0–10.0)	0.39
Median Apache II score (IQR)	8.0 (6.5–10.0)	9.0 (7.0–11.0)	8.0 (6.0–10.0)	0.20
Type of surgery, N (%)				
Valve replacement	49 (46.7)	7 (36.8)	42 (48.8)	0.45
CABG	20 (19.0)	5 (26.3)	15 (17.4)	0.35
Mixed (valve and CABG)	7 (6.7)	2 (10.5)	5 (5.8)	0.60

IQR, interquartile range; CABG, coronary artery bypass grafting.

Table 2
Main catheters' characteristics.

Variables	Total $N=167$	Colonized $N=21$	Non-colonized $N=146$	p-Value
Type of catheter, N (%)				0.79
Non-tunneled central venous catheter	122 (73.0)	15 (71.4)	107 (73.3)	
Guidewire	45 (26.9)	6 (28.6)	39 (26.7)	
Location, N (%)				0.86
Jugular	155 (92.8)	20 (95.2)	135 (92.5)	
Subclavian	11 (6.6)	1 (4.8)	10 (6.8)	
Femoral	1 (0.6)	0 (0.0)	1 (0.7)	
Total no. of parenteral nutrition/propofol, N (%)	33 (19.8)	5 (23.8)	28 (19.3)	0.57
Reasons for catheter withdrawal, N (%)				0.001
End of use	133 (79.6)	12 (57.1)	121 (82.9)	
Suspicion of infection	17 (10.2)	7 (33.3)	10 (6.8)	
Others	17 (10.2)	2 (9.5)	15 (10.3)	
Median catheter days (IQR)	10 (7.0–16.0)	9.0 (7.0–13.0)	10.5 (7.0–16.2)	0.39
Total no. of catheter days	2360	263	2097	NA

IQR, interquartile range.

Table 3
Microorganisms isolated in colonized catheters.

Tip	Skin + NCs[a]	Skin + Hubs	C-RBSI
Staphylococcus epidermidis	–	–	No
Staphylococcus epidermidis	*Staphylococcus epidermidis* *Corynebacterium tuberculoestearicum*	*Staphylococcus epidermidis*	No
Streptococcus simulans	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*	No
Enterobacter aerogenes	*Enterobacter aerogenes*	*Enterobacter aerogenes*	No
Enterococcus faecium	–	–	Yes
Staphylococcus epidermidis	–	–	No
Staphylococcus hominis	*Staphylococcus capitis* *Staphylococcus epidermidis*	–	No
Burkholderia cepacia	–	–	Yes
Staphylococcus epidermidis	*Staphylococcus haemolyticus*	–	No
Staphylococcus epidermidis	–	–	Yes
Staphylococcus saprophyticus	–	–	No
Serratia marcescens	–	*Serratia marcescens*	Yes
Proteus mirabilis	*Staphylococcus epidermidis* *Enterococcus faecalis*	*Staphylococcus epidermidis* *Enterococcus faecalis*	No
Staphylococcus epidermidis	*Staphylococcus epidermidis* *Staphylococcus hominis*	*Staphylococcus epidermidis*	No
Staphylococcus aureus	–	–	Yes
Staphylococcus epidermidis	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*	
Enterococcus faecalis Klebsiella pneumoniae	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	Yes
Staphylococcus epidermidis	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*	No
Staphylococcus epidermidis	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*	No
Staphylococcus epidermidisBurkholderia cepacia	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*	No
Staphylococcus epidermidis	Staphylococcus capitis	Staphylococcus capitis	No

NC, needleless connector; C-RBSI, catheter-related bloodstream infection.
[a] Using the cut-off in NCs' cultures of 1000 cfu/NC.

We considered an episode of C-RBSI to be confirmed when the same microorganism was isolated both from peripheral blood cultures and from the catheter tip.

To calculate the validity values for NCs for predicting catheter colonization, we considered positive NCs those with the same microorganism(s) as isolated from the catheter tip.

Statistical analysis

Values are expressed as the mean (SD) or median (IQR) for continuous variables and as percentages, with the 95% confidence interval (95% CI), when applicable, for categorical variables. Categorical variables were evaluated using the chi-square test or the two-tailed Fisher exact test. Statistical significance was set at $p<0.05$ (two-tailed). We calculated the validity values of the superficial culture (skin + hubs) and skin + NCs culture by comparing them with the gold standard of CC. The sensitivity, specificity, and positive and negative predictive values, with 95% CI, were calculated using EPIDAT 3.1. Finally, to assess a point of count of the NCs we conducted a ROC curve to determine the optimal cut-off to classify colonized NCs.

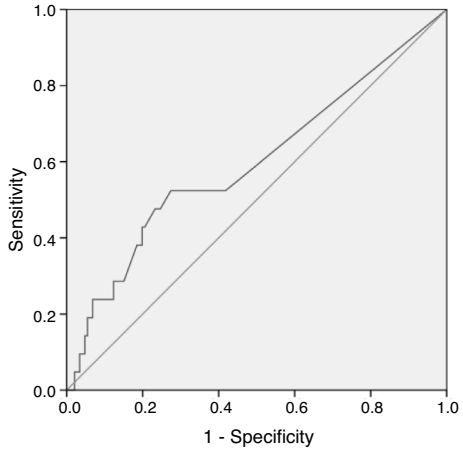
Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp, Armonk, NY, USA).

Results

During 10 months, 309 patients with 609 catheters were admitted in ICU-MHS. We finally included in the study 105 (34.0%) patients who had 167 (27.4%) central catheters inserted for ≥7days.

A total of 2166 cultures were made (167 tips, 251 skins, 874 hubs and 874 groups of NCs).

The mean (SD) age was 68 (60.5–77.0) years. The main underlying conditions of the patients were congestive heart failure (86.7%) and diabetes mellitus (26.7%). The median (QIR) APACHE II at inclusion, and EuroScore were 8.0 (6.5–10.0) and 8.0 (5.5–10.0), respectively.



S% (95% CI)	SP% (95% CI)	PPV% (95% CI)	NPV% (95% CI)	LR+ (95% CI)	LR- (95% CI)
19.0 (0.0-38.2)	93.8 (89.6-98.0)	30.8 (1.8-60.0)	89.0 (83.7-94.2)	3.09 (1.04-9.15)	0.86 (0.70-1.07)

Fig. 1. Receiver operating characteristic (ROC) curve. Specificity, sensitivity, likelihood ratio for positive test (LR+), and likelihood ratio for negative test (LR−) of NCs with a cut-off of >1000 cfu in quantitative culture of the sonicate.

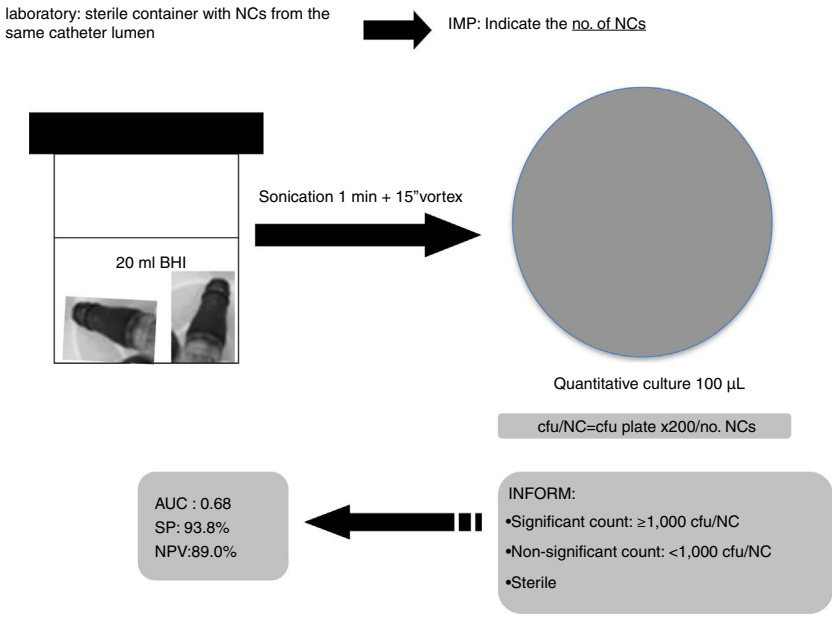


Fig. 2. Laboratory procedure for NCs' processing and interpretation of cultures.

No significant differences in the underlying conditions and situation of the populations between colonized and non-colonized catheters were obtained (Table 1).

The main reason for catheter withdrawal was end of use (79.6%), followed by suspicion of infection (10.2%), and other reasons (10.2%). Other patient and catheter data are detailed in Table 2.

The overall catheter tip colonization rate was 12.6% (21/167) and 6 episodes of C-RBSI (2.5 episodes/1000 catheter days) were confirmed. Table 3 shows the microorganisms isolated from the colonized central venous catheters.

Cut-off points of 100 cfu, 500 cfu and 1000 cfu were determined. As show in Fig. 1, a threshold value counts shows a sensitivity of 19% and specificity of 94%; if the cut-off of >1000 cfu was selected; ROC curve 0.6 (0.45–0.74; IC). Although the auc is not very good, it is the best to have a high VPN.

Table 4 shows the validity values for skin + NCs' cultures using the selected cut-off and conventional superficial cultures (skin + hubs) for prediction of CC and C-RBSI. Skin + NCs' cultures had 42.9% sensitivity and a 91.0% negative predictive value for CC compared with 42.9% and 91.5% for conventional superficial cultures. The validity values for skin + NCs' cultures using the selected cut-off and conventional superficial cultures for C-RBSI were, respectively: sensitivity, 16.7%/33.3%; specificity, 75.8%/80.1%; positive predictive value, 2.5%/5.9%; and negative predictive, 96.0%/97.0% (Table 4).

Discussion

Our study demonstrates that the combination of skin and NCs' cultures considering a new cut-off had the same validity values as conventional skin and hub cultures to rule out CC and C-RBSI.

Table 4
Validity values of conventional superficial cultures and skin + NCs' cultures for prediction of catheter colonization and C-RBSI.

	S% (95% CI)	SP% (95% CI)	PPV% (95% CI)	NPV% (95% CI)	Validity index (95% CI)	Prevalence (95% CI)	LR+ (95% CI)	LR− (95% CI)
Catheter colonization								
Skin + Hubs	42.9 (19.3–66.4)	88.4 (82.8–93.9)	34.6 (14.4–54.8)	91.5 (86.5–96.4)	82.6 (76.6–88.7)	12.6 (7.2–17.9)	3.68 (1.89–7.16)	0.65 (0.44–0.94)
Skin + NCs[a]	42.9 (19.3–66.4)	83.6 (77.2–89.9)	27.3 (10.6–43.9)	91.0 (85.8–96.2)	78.4 (71.9–84.9)	12.6 (7.2–17.9)	2.61 (1.41–4.82)	0.68 (0.47–1.00)
C-RBSI								
Skin + Hubs	33.3 (0.0–72.4)	80.1 (73.6–86.6)	5.9 (0.0–15.3)	97.0 (93.7–100)	80.1 (73.6–86.6)	3.6 (0.5–6.7)	1.68 (0.52–5.42)	0.83 (0.47–1.47)
Skin + NCs[a]	16.7 (0.0–54.8)	75.8 (68.8–82.7)	2.5 (0.0–8.6)	96.0 (92.3–99.8)	73.6 (66.7–80.6)	3.6 (0.5–6.7)	0.69 (0.11–4.20)	1.10 (0.76–1.59)

S, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR−, negative likelihood ratio; CI, confidence interval; NA, not applicable; NCs, needleless connectors; C-RBSI, catheter-related bloodstream infection.
[a] Using the cut-off in NCs' cultures of 1000 cfu/NC.

Patients admitted to MHS-ICU are at high risk for CC and, consequently for C-RBSI. When there is suspicion of C-RBSI it is important to achieve a rapid diagnosis for a proper management of the patient.[11,12] The actual guidelines for the diagnosis of catheter-related infections recommend performing conservative diagnostic methods when there is suspicion of C-RBSI, such as superficial cultures from skin and hubs.[13] These cultures have demonstrated their efficacy to rule out C-RBSI in several populations.[1–5] However, hub cultures require rubbing a swab inside the catheter lumen that can dislodge the biofilm into the bloodstream.[6,7] In order to reduce this risk, our study group have recently reported an alternative and safer diagnostic procedure with similar validity values to rule out CC and C-RBSI based on culturing the sonicate of withdrawn NCs. This was assessed in central venous systems from MHS-ICU patients.[8–10] However, in these studies NCs' were processed considering a positive culture of the sonicate when any number of cfu was counted (qualitative culture), which implied a high number of false positive results. In the present study we solved this issue by considering a cut-off (1000 cfu/NC) in the NCs' culture of the sonicate which demonstrated that, when combined with skin cultures, the validity values for CC and C-RBSI were similar to that obtained with conventional superficial cultures from skin and hubs.

Based on our data, we suggest to Microbiology laboratories a new proposal for the processing and interpretation of NCs' cultures by sonication for a proper management in the diagnosis of catheter colonization and C-RBSI (Fig. 2).

Conclusion

Despite the combination of skin cultures and quantitative NCs' cultures did not show good sensitivity, they could be used as a conservative diagnostic procedure for ruling-out catheter colonization and C-RBSI when no catheter withdrawal is possible.

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Conflict of interests

None of the authors have conflicts of interest to declare.

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References

1. Bouza E, Munoz P, Burillo A, Lopez-Rodriguez J, Fernandez-Perez C, Perez MJ, et al. The challenge of anticipating catheter tip colonization in major heart surgery patients in the intensive care unit: are surface cultures useful? Crit Care Med. 2005;33:1953–60. PMID: 16148465.
2. Bouza E, Alvarado N, Alcala L, Perez MJ, Rincon C, Munoz P. A randomized and prospective study of 3 procedures for the diagnosis of catheter-related bloodstream infection without catheter withdrawal. Clin Infect Dis. 2007;44:820–6. PMID: 17304454.

3. Bouza E, Rojas L, Guembe M, Marin M, Anaya F, Luno J, et al. Predictive value of superficial cultures to anticipate tunneled hemodialysis catheter-related bloodstream infection. Diagn Microbiol Infect Dis. 2014;78:316–9. PMID: 24428979.
4. Cercenado E, Ena J, Rodriguez-Creixems M, Romero I, Bouza E. A conservative procedure for the diagnosis of catheter-related infections. Arch Intern Med. 1990;150:1417–20. PMID: 0262196.
5. Guembe M, Martin-Rabadan P, Echenagusia A, Camunez F, Rodriguez-Rosales G, Simo G, et al. Value of superficial cultures for prediction of catheter-related bloodstream infection in long-term catheters: a prospective study. J Clin Microbiol. 2013;51:3025–30. PMID: 23850957.
6. Raad II. The pathogenesis and prevention of central venous catheter-related infections. Middle East J Anesthesiol. 1994;12:381–403. PMID: 8938007.
7. Sitges-Serra A, Pi-Suner T, Garces JM, Segura M. Pathogenesis and prevention of catheter-related septicemia. Am J Infect Control. 1995;23:310–6. PMID: 6438585.
8. Pérez-Granda MJ, Cruces R, Barrio JM, Bouza E. Assessment of central venous catheters colonization using surveillance culture of withdrawn connectors and insertion site skin. Crit Care. 2016 [Published online 02.02.16].
9. Perez-Granda MJ, Guembe M, Cruces R, Bouza E. Vascular catheter colonization: surveillance based on culture of needleless connectors. Crit Care. 2016;20:166. PMID: 27234944.
10. Guembe M, Perez-Granda MJ, Cruces R, Martin-Rabadan P, Bouza E. Cultures of needleless connectors are useful for ruling out central venous catheter colonization. J Clin Microbiol. 2015;53:2068–71. PMID: 25878353.
11. Kumar A. Early antimicrobial therapy in severe sepsis and septic shock. Curr Infect Dis Rep. 2010;12:336–44. PMID: 21308515.
12. Kumar A, Zarychanski R, Light B, Parrillo J, Maki D, Simon D, et al. Early combination antibiotic therapy yields improved survival compared with monotherapy in septic shock: a propensity-matched analysis. Crit Care Med. 2010;38:1773–85. PMID: 20639750.
13. Mermel LA, Allon M, Bouza E, Craven DE, Flynn P, O'Grady NP, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2009;49:1–45. PMID: 19489710.