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Original article

Prospective intervention study with a microarray-based, multiplexed, automated molecular diagnosis instrument (Verigene system) for the rapid diagnosis of bloodstream infections, and its impact on the clinical outcomes

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ABSTRACT

The Verigene Gram-positive blood culture test (BC-GP) and the Verigene Gram-negative blood culture test (BC-GN) identify representative Gram-positive bacteria, Gram-negative bacteria and their antimicrobial resistance by detecting resistance genes within 3 h. Significant benefits are anticipated due to their rapidity and accuracy, however, their clinical utility is unproven in clinical studies. We performed a clinical trial between July 2014 and December 2014 for hospitalized bacteremia patients. During the intervention period (N = 88), Verigene BC-GP and BC-GN was used along with conventional microbiological diagnostic methods, while comparing the clinical data and outcomes with those during the control period (N = 147) (UMIN registration ID: UMIN000014399). The median duration between the initiation of blood culture incubation and the reporting time of the Verigene system results was 21.7 h (IQR 18.2–26.8) and the results were found in 88% of the cases by the next day after blood cultures were obtained without discordance. The hospital-onset infection rate was higher in the control period (24% vs. 44%, p = 0.002), however, no differences were seen in co-morbidities and severity between the control and intervention periods. During the intervention period, the time of appropriate antimicrobial agents' initiation was significantly earlier than that in the control period (p = 0.001) and most cases (90%; 79/88) were treated with antimicrobial agents with in-vitro susceptibility for causative bacteria the day after the blood culture was obtained. The costs for antimicrobial agents were lower in the intervention period (3618 yen vs. 8505 yen, p = 0.001). The 30-day mortality was lower in the intervention period (3% vs. 13%, p = 0.019).

1. Introduction

Inappropriate antimicrobial therapy is associated with a worse prognosis [1] and the increasing prevalence of multidrug-resistance organisms (MDROs) has led to significant threats to clinical practice. In most developed countries, nearly half of the *Staphylococcus aureus* strains that are clinically isolated are methicillin-resistant [2], and extended-spectrum beta-lactamase-producing *Enterobacteriaceae* have been commonly detected worldwide [3–6]. While these MDROs were classically considered to be causative pathogens of nosocomial or healthcare-associated infections, community-acquired MDROs are now well recognized [7] and cause bacteremia even in previously healthy patients [8,9].

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Bacterial identification and antimicrobial susceptibility testing (AST) are crucial tools for the treatment of bacterial infections, especially for bacteremia. However, bacterial culture requires several days to determine the bacterial phenotypes, and the reports are delayed over weekends or holidays due to the lack of skilled human resources. Thus, information regarding bacterial identification or AST is generally unavailable for clinical decisions regarding treatment for at least two days, which has previously been reported to be the breakpoint for the prognosis of *S. aureus* bacteremia [10].

The Verigene system (Nanosphere Inc., Northbrook, IL, USA) is a rapid diagnostic instrument that employs a microarray-based, multiplexed, automated molecular method. The Verigene Gram-positive blood culture test (BC-GP) and Verigene Gram-negative blood culture test (BC-GN) can identify representative Gram-positive (GP) and Gram-negative (GN) bacteria, along with their antimicrobial resistance, by detecting resistance genes (*mecA*, *vanA*, *vanB*; BC-GP, $bla_{\text{CTX-M}}$, bla_{IMP}, bla_{KPC}, bla_{NDM}, bla_{OXA}, bla_{VIM}; BC-GN). Both of the tests are performed directly on the blood from positive blood culture bottles incubated without pretreatment, and report results are available within 3 h (2.5 h with the BC-GP, 2 h with the GC-GN).

Both the BC-GP and BC-GN have been shown to cover 82–95% of bacteria isolated from the blood of patients with bacteremia [11–19]. The concordance rate of the Verigene system (BC-GP, BC-GN) with conventional microbiological methods has been reported to be 92–98% for BC-GP [11–13,16,20–24] and 90–98% for BC-GN [14,25–27]. Most of the disagreements occurred among cases with multiple-organism bacteremia, and both of the tests were approved by the Food and Drug Administration (FDA) in 2013 as rapid diagnostic tests for bacteremia.

Due to the their rapidity and high performance for bacterial identification and the prediction of antimicrobial resistance, the potential for significant benefits has been anticipated by increasing the rate of early administration of effective antimicrobial agents and reducing the rate of administration of unnecessary antimicrobial agents [11,15,19]. However, these potential benefits have not yet been widely proven in clinical trials, and were only partially performed for *Streptococcus* and *Enterococcus* species [28,29].

In the present study, we performed a clinical trial to investigate the clinical utility of the Verigene tests (BC-GP and BC-GN) for the treatment of patients with bacteremia caused by Gram-positive and -negative bacteria.

1.1. Patients and methods

This clinical trial was performed at Tsukuba Medical Center Hospital (TMCH, 413 beds), which is located next to the University of Tsukuba Hospital and plays a role as a tertiary emergency medical center in the Tsukuba district of Japan. The intervention was prospectively performed between July 1, 2014 and December 31, 2014 after an evaluation of the reliability of the Verigene BC-GP and BC-GN for bacterial identification and antimicrobial resistance prediction (Supplementary file 1), and the clinical data were compared with those of a control period (October 1, 2012–September 30, 2013). During the intervention period, written informed consent was obtained from all patients or from their surrogate decision-maker, as appropriate. This study was performed for patients suffering from bacteremia during the acute phase of infections, and a procedure for inclusion in emergency situations was applied accordance with the ethical guidelines for clinical studies indicated by the Ministry of Health, Labour and Welfare of Japan.

(http://www.mhlw.go.jp/stf/seisakunitsuite/bunya/hokabunya/kenkyujigyou/i-kenkyu/).

If patients or their surrogate decision-makers were unable to provide informed consent at the time when bacteremia was diagnosed, the examination using the Verigene system was tentatively performed with the results provided to each physician, and written informed consent was ultimately obtained from patients who survived or from their surrogate decision-maker. This study was performed with approval from the ethics committee of TMCH (UMIN registration ID: UMIN000014399).

The details of definitions used in current study are summarized in Supplementary file 2.

1.2. Patients

All hospitalized patients with positive blood cultures containing GP or GN detected by the BacT/ALERT 3D system (Sysmex bioMérieux Co., Ltd., Tokyo, Japan) were considered and reviewed for selection both in the intervention and the control periods. The accuracy of BC-GP and BC-GN for multiple organisms were not assured; therefore, we excluded patients with bacteremia caused by more than two species of bacteria, as suspected from the Gram staining examinations. Other exclusion criteria were 1) If only one set of blood cultures was positive. Either one set or two sets of blood cultures were incubated, and *Staphylococcus* spp. or Gram-positive bacilli were suspected by the Gram staining examination. 2) If blood cultures were positive and Gram-positive bacilli were suspected in patients with nosocomial bacteremia. 3) If the Gram staining examination of a positive blood culture indicated the same bacteria as was present in a previous positive blood culture obtained within the past week. 4) If differentiation between GP and GN was difficult by the Gram staining examination. 5) If more than 24 h had passed after the positive blood culture alarm by the BacT/ALERT 3D system. 6) If palliative care was being performed without aggressive treatment for infections. 7) If patients had already died before or on the day when the results of the Gram staining examinations were performed 8) If contamination was suspected based on the clinical findings.

1.3. Diagnostic system for bacteremia and the role of the ID physicians

At TMCH, each blood culture bottle was promptly transferred to an in-house laboratory after blood samples were obtained either in the emergency departments or inpatient wards, and was inserted into the BacT/ALERT 3D system by laboratory staff members 24 h/day. Gram staining examinations were performed on the blood in positive blood culture bottles by laboratory staff members in charge of microbiology from Monday to Sunday, and the results were provided to each hospital physician. Further evaluations were performed mainly with the MicroScan WalkAway-96 (Beckman Coulter, Inc.; Tokyo, Japan) at the microbiology center (Miroku Medical Laboratory Inc.). The ID physician intervened for all of the patients with positive blood cultures on weekdays, and each case was discussed every Wednesday with the faculty of the Department of Infectious Disease, University of Tsukuba Hospital.

During the intervention period, Verigene system examinations were performed from Monday to Sunday with proper ethical processes, and the results with the judgments of the ID physicians were reported to the physicians in charge of each patient. The Verigene system examination was performed once for one of the eligible positive blood culture bottles per case, unless a re-examination was required, and the judgment regarding the selection of antimicrobial agents was facilitated by an antibiogram of the bacteria isolated from blood or cerebrospinal fluid in southern Ibaraki prefecture in 2012 (Supplementary file 3). The final

decisions regarding treatment were made by the physicians in charge of each patient.

1.4. Clinical assessment and outcome measurements

As baseline characteristics, we compared the age, gender, place of the onset of infection, co-morbidities, severity of infections, laboratory findings (WBC and C-reactive protein) at the time when the blood culture was obtained, the sources of infections, causative pathogens, the duration between the initiation of incubation and the day the conventional microbiological identification was reported and the rate of carbapenem use.

The primary outcome analyzed was the duration before the initiation of appropriate antimicrobial therapy after obtaining blood cultures, and the secondary outcomes were the differences in the costs of additional antimicrobial agents, 14-day mortality and 30-day mortality. Appropriate antimicrobial agents were defined as antimicrobial agents based on the *in vitro* susceptibility testing for the causative pathogen of the bacteremia. The costs of additional antimicrobial agents was calculated based on the prescription cost for antimicrobial agents without confirmed *in vitro* susceptibility of the causative pathogens of the bacteremia, and the prescription cost for antimicrobial agents such as vancomycin for methicillin-sensitive *Staphylococcus* spp. or anti-pseudomonal agents, such as carbapenems, for third-generation cephalosporin-sensitive *Escherichia coli*. Additional prescription costs were calculated for the antimicrobial agents prescribed between the day when the blood culture was obtained and the day when the results of conventional microbiological methods were reported.

1.5. Microbiological analysis for bacterial identification and antimicrobial susceptibility testing

All of the strains isolated from blood were preserved at −80 °C during the control and intervention periods. For the strains with discrepancies in the bacterial identification between the Verigene system and conventional microbiological methods or difficulty in the identification by conventional microbiological methods, a genotypic identification was performed using a partial DNA sequence analysis of the 16S rRNA gene with an ABI PRISM BigDye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems, Inc., Foster City, CA, USA). The genotypic results were compared with those of each type strain of bacteria with the Genbank database, and the highest similarity was thus applied to make the final identification. A re-evaluation of the AST with a Dry Plate (Eiken Chemical Co., Ltd., Tokyo, Japan) or E-test (SYSMEX bioMérieux Co., Ltd., Tokyo, Japan) was performed if strains were non-susceptible to the antimicrobial agents used for the treatment of bacteremia analyzed by the MicroScan WalkAway-96 or if the susceptibility of antimicrobial agents administered was not evaluated. These additional evaluations were basically performed after each patient's treatment, and the results were not available during either the control or intervention period.

1.6. Statistical analysis

Categorical variables were analyzed using the $\chi 2$ test or Fisher's exact test as appropriate. Continuous variables were compared using Student's *t*-test or Welch's t-test based on the deviation. The SPSS version 20 software package (IBM, Armonk, NY, USA) was used for all analyses, and statistical significance was defined as a 2-tailed level of significance of $p < 0.05$.

2. Results

During the control and intervention periods, 767 sets of blood culture bottles (469 in the control period, 298 in the intervention period) produced true positive signals and 239 cases (147 in the control period, 92 in the intervention period) were considered to be potential comparable cases (Fig. 1). In the intervention period, a rapid diagnosis with Verigene system could not be performed in four cases, so 88 cases (96%) were included in the final analysis.

The performance of the Verigene system during the intervention period is shown in Table 1. The median duration between the initiation of the blood culture incubation and the reporting time of Verigene system result was 21.7 h (IQR 18.2–26.8), and the results were similar for the Gram-positive (20.8 h, IQR 18.4–24.6) and Gram-negative (22.4 h, IQR 18.2–27.2) bacteria. In three cases, the results of the Verigene system were reported to the hospital physician the same day as the blood cultures were taken from patients. There were no cases of discordance of the bacterial identification and antimicrobial resistance between the conventional microbiological methods and the Verigene system during the intervention period.

The basic characteristic of the cases in the control and intervention periods are summarized in Table 2. Compared with the control period, females were slightly predominant (49% vs. 37%, $p = 0.085$) and hospital-onset infections were significantly less common (24% vs. 44%, $p = 0.002$) during the intervention period. The differences in co-morbidities and the severity of bacteremia were not significant between the two periods. Joint and vertebral infections were prevalent during the intervention period, while there were more cases with infective endocarditis with or without other sites of infections during the control period ($p = 0.009$). Carbapenems were used for 12 patients (8%) with bacteremia during the control period and 3 patients (3%) with bacteremia during the intervention period.

The etiology of bacteremia between the control and the intervention periods are summarized in Table 3. There was no statistical difference between the two periods regarding specific bacteria, including drug-resistant bacteria such as MRSA, non-fermentative Gram-negative bacilli and third-generation cephalosporin-resistant *Enterobacteriaceae*. There were five cases of methicillin-resistant, coagulase-negative staphylococci (MR-CNS) bacteremia in the control period, whereas there was no case of MR-CNS in the intervention period.

The time-dependent rates of cases treated with antimicrobial agents with susceptibility for each causative pathogen are described in Fig. 2. In the intervention period, the administration of an appropriate antimicrobial agent was significantly earlier than during the control period ($p = 0.001$), and most of cases (90%; 79/88) were treated with antimicrobial agents to which the bacteria were susceptible by the next day (day 1) after the blood culture was obtained.

In the intervention period, most cases with the initial inappropriate antimicrobial agent prescription ($n = 15$) were changed to an appropriate agent by day 1 (9/15) or day 2 (10/15) based on the results of the Verigene system examination. For three cases, changes in the prescribed antimicrobial agents were made according to the results of conventional antimicrobial susceptibility testing and clinical response. Appropriate antimicrobial agents were not administered to two cases in the intervention period due to clinical improvement; one case was treated with ampicillin/sulbactam for *E. coli* (ampicillin/sulbactam; MIC = 16/4 μg/mL)-associated cholangitis and another case was treated with cefozopran for *Achromobacter xylosoxydans* (cefozopran; MIC ≥ 32 μg/mL) bacteremia, possibly due to a peripheral catheter infection. None of the 15 cases died during hospitalization. Three patients died within

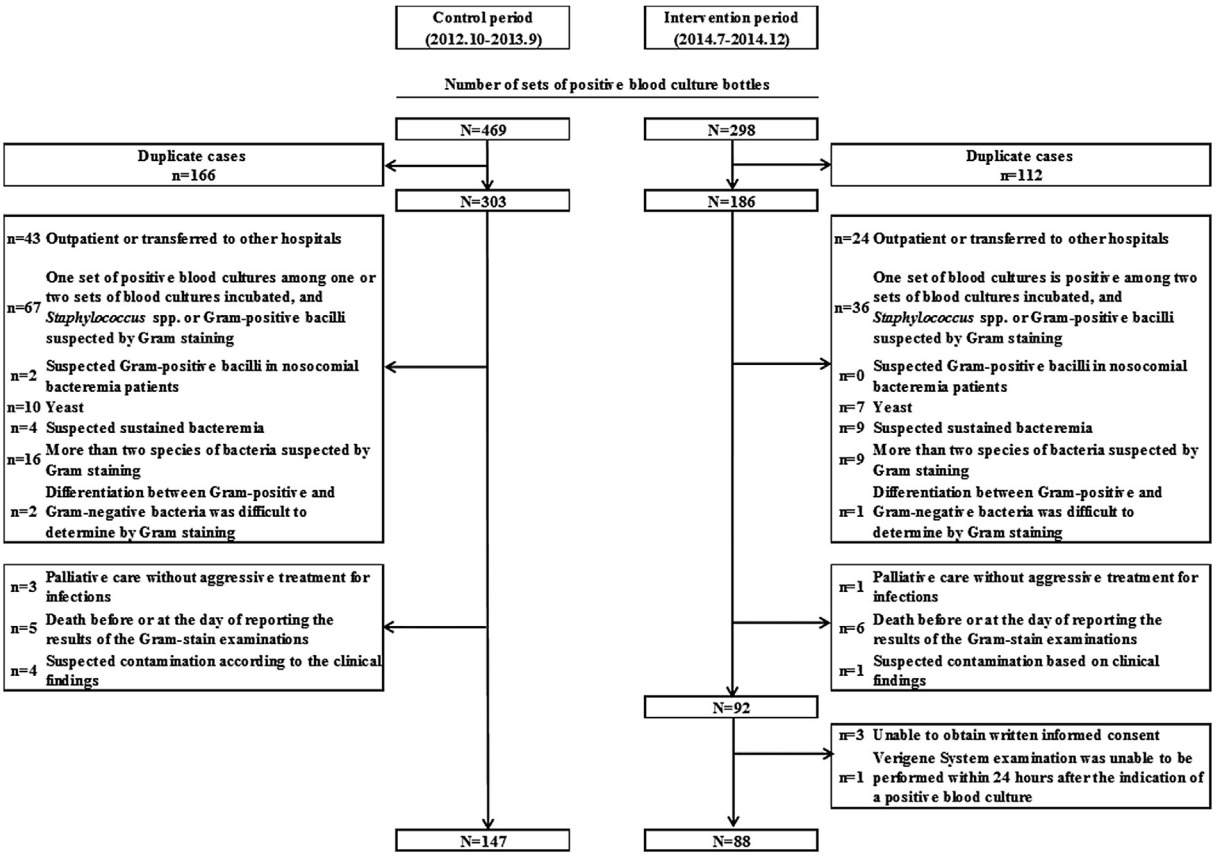

Fig. 1. A flowchart of the case selection process.

Table 1
The results of an analysis of the performance of the Verigene system for evaluating bacteremia during the intervention period.

	Total	GP	GN
	N = 88	N = 20	N = 68
Incubation period for a positive signal obtained from blood culture bottle (hr)	13.6 (12.6–16.8)	14.0 (12.7–16.6)	13.4 (12.6–16.8)
Duration between the initiation of incubation and reporting of the Gram stain examination (hr)	19.2 (15.6–23.5)	17.6 (15.7–20.2)	19.9 (15.3–23.9)
Duration between the initiation of incubation and reporting of the Verigene system result (hr)	21.7 (18.2–26.8)	20.8 (18.4–24.6)	22.4 (18.2–27.2)
Duration between the initiation of incubation and reporting of conventional microbiological identification (hr)	80.8 (71.8–99.4)	74.9 (70.7–115.9)	82.5 (72.9–96.1)
Number of bacterial identifications achieved by the Verigene system[a]	78 (88.6)[b]	20/20 (100)	58/68 (85.3)[b]
(excluding *Klebsiella variicola*)[c]	78/86 (90.7)	N/A	58/66 (87.9)
Concordance rate of the bacterial identification[d]	86 (100)	20/20 (100)	68 (100)
Prediction of antimicrobial resistance			
Methicillin resistance/*mecA* analysis performed[e]	10/10 (100)	10/10 (100)	N/A
Vancomycin resistance/*vanA* or *vanB* analysis performed[f]	1/1 (100)	1/1 (100)	N/A
Cefotaxime/ceftriaxone nonsusceptibility/bla_{CTX-M} analysis performed[g]	45/45 (100)	N/A	45/45 (100)
Meropenem nonsusceptibility/carbapenase gene analysis performed[h]	68/68 (100)	N/A	68/68 (100)

All categorical data are presented as numbers (proportion, %). The continuous data are presented as medians (interquartile range).
GP Gram-positive bacteria, *GN* Gram-negative bacteria, *N/A* not applicable.

[a] Genotypic identification was performed using a partial DNA sequence analysis of the 16S rRNA gene for the strains with discrepancies in the bacterial identification between the Verigene system and conventional microbiological methods or difficulty in the identification by conventional microbiological methods.

[b] *Achromobacter xylosoxydans* (1), *Bacteroides thetaiotaomicron* (1), *Campylobacter jejuni* (1), *Capnocytophaga canimorsus* (1), *Klebsiella variicola* (2), *Moraxella osloensis* (1), *Salmonella* spp. (2), *Shewanella haliotis* (1).

[c] Two strains of *Klebsiella variicola* were not detected during intervention periods with the GN panel, and were accurately detected by improved GN panels, which were performed later.

[d] In one strain of *Streptococcus constellatus*, the Verigene system initially analyzed it as "not detected," and it was accurately judged by re-examination, which was performed soon after the first examination.

[e] There were three strains of *mecA*-positive staphylococci.

[f] *vanA* or *vanB* genes were not detected.

[g] The analysis was performed for strains of *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca* and *Proteus* spp. There were five stains of CTX-M-positive *Enterobacteriaceae*.

[h] bla_{IMP}, bla_{KPC}, bla_{VIM}, bla_{NDM}, bla_{OXA} were measured, and there was one strain of IMP-positive *Pseudomonas aeruginosa*.

Table 2
The characteristics of cases with bacteremia during the control and intervention periods.

	Control period (n = 147)	Intervention period (n = 88)	*P* value
Age (y)	72.7 (17.4)	70.2 (22.3)	0.357
Female	55 (37.4)	43 (48.9)	0.085
Hospital-onset infection	64 (43.5)	21 (23.9)	0.002
Hospital-onset infection or community-onset healthcare-associated infection	84 (57.1)	43 (48.9)	0.227
Charlson's comorbidity index scale	2.0 (1.9)	1.6 (1.8)	0.101
Diabetes mellitus	34 (23.1)	26 (29.5)	0.275
Malignancy	27 (18.4)	12 (13.6)	0.345
Immunosuppressive drugs	5 (3.4)	4 (3.4)	0.999
Clinical severity scale			0.897
Severe sepsis	38 (25.9)	22 (25.0)	
Septic shock	14 (9.5)	7 (8.0)	
WBC (/μL)	11,557 (6791)	12,326 (6970)	0.406
C-reactive protein (mg/dL)	10.2 (8.6)	9.2 (7.0)	0.351
Source of infection			
Urinary tract	52 (35.4)	39 (44.3)	0.213
Pulmonary	8 (5.4)	1 (1.1)	0.159
Intestine or biliary tract	11 (7.5)	11 (12.5)	0.248
Skin and soft tissue	7 (4.8)	5 (5.7)	0.767
Heart (infective endocarditis)	9 (6.1)	2 (2.3)	0.217
Joint and vertebrae	4 (2.7)	10 (11.4)	0.010
Bacteremia or catheter-related	49 (33.3)	19 (21.6)	0.074
Others[a]	7 (4.8)	1 (1.1)	0.264
Duration between the initiation of incubation and day when the results of conventional microbiological identification were reported (days)	3.95 (1.24)	3.84 (1.29)	0.511
Number of patients treated with carbapenems for bacteremia	12 (8.2)	3 (3.4)	0.178

All categorical data are presented as the numbers (proportion, %). Continuous data are presented as the means (standard deviation).
[a] Multiple sites of infections (5), subdural abscess (1), meningitis (1), mycotic aneurysm (1).

7 days without administration of antimicrobial agents with *in vitro* susceptibility testing for causative bacteria during the control period.

The data regarding the additional costs and mortality are shown in Table 4. Compared with the control period, the additional costs for antimicrobial agents were significantly reduced in the intervention period (3618 yen vs. 8505 yen, p = 0.001). Both the cost for antimicrobial agents against non-susceptible causative bacteria of bacteremia (2031 yen vs. 4644 yen, p = 0.013) and the cost for unnecessary anti-MRSA agents or antipseudomonal agents (1586 yen vs. 3915 yen, p = 0.042) were reduced in the intervention period. The cost reduction was prominent among GP cases (2488 yen vs. 11585 yen, p = 0.002), while the difference was not observed in the community-onset GN cases (3797 yen vs. 4272 yen, p = 0.797). The 30-day mortality was significantly lower during the intervention period (3% vs. 13%, p = 0.019).

3. Discussion

During this clinical trial, the Verigene system BC-GP and BC-GN could reliably predict the causative bacteria and their antimicrobial resistance in most cases, and the results were available in 88% of

Table 3
Etiology of bacteremia between the control period and intervention period.

	Control period (n = 147)	Intervention period (n = 88)	*P* value
Gram-positive bacteria			
Staphylococcus aureus	19 (12.9)	9 (10.2)	0.678
(methicillin-resistant *S. aureus*)	10/19 (52.6)	3/9 (33.3)	0.435
Coagulase negative staphylococci	6 (4.1)	1 (1.1)	0.261
(methicillin-resistant CNS)	5/6 (83.3)	0/1 (0)	0.286
Enterococci	6 (4.1)	1 (1.1)	0.261
Streptococci	15 (10.2)	9 (10.2)	0.999
Other Gram-positive bacteria	1 (0.7)	0	0.999
Gram-negative bacteria			
Escherichia coli	43 (29.3)	31 (35.2)	0.385
Klebsiella spp.	15 (10.2)	12 (13.6)	0.527
Proteus spp.	4 (2.7)	2 (2.3)	0.999
(Third-generation cephalosporin-resistant *E. coli*, *Klebsiella* spp. or *Proteus* spp.)	5 (8.1)	4 (8.9)	0.999
SPACE, non-fermentative Gram-negative bacilli	32 (21.8)	17 (19.3)	0.741
Other Gram-negative bacteria	2 (1.4)	6 (6.8)	0.055
Polymicrobial[a]	4 (2.7)	0	0.3

All categorical data are presented as number (proportion, %).
CNS coagulase-negative *staphylococci*, SPACE *Serratia marcescens*, *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Citrobacter* spp., *Enterobacter* spp., N/A not applicable.
[a] The record of Gram stain examinations for blood obtained from positive blood cultures showed one organism, however, multiple species of bacteria were finally cultivated. All of the four cases had polymicrobial infections of Gram-negative bacteria and survived at discharge.

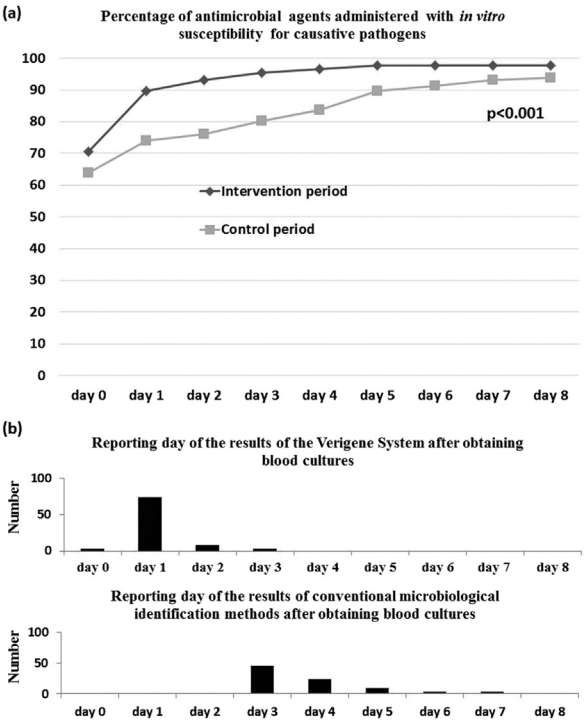

Fig. 2. (a) The rates of treatment with antimicrobial agents with susceptibility for each causative pathogen of the bacteremia. (b) Reporting day of the results of the Verigene system and conventional microbiological identification methods after obtaining blood cultures.

cases by the next day after the blood culture was obtained. The intervention contributed to earlier initiation of treatment with an appropriate antimicrobial agent, and led to favorable outcomes without an increase in the carbapenem use. A decrease in the prescribed antimicrobial agents against non-susceptible causative bacteria and refrainment of unnecessary anti-MRSA agents or antipseudomonal agents resulted in cost reduction in the intervention period, especially among cases with Gram-positive bacteremia.

Several important points should be considered when interpreting the present results. First, we used local antibiogram data, along with the results of the Verigene system examinations, when making the choice of antimicrobial agents during the intervention period. While the Verigene system can identify most of the causative bacteria of bacteremia and their major antimicrobial resistance for beta-lactam agents and vancomycin, we could not obtain information regarding the resistance to other antimicrobial agents, such as fluoroquinolones. In addition, the presence of a deficiency or loss of porin expression, active efflux pump systems, overproduction of AmpC beta-lactamase [30], production of ESBLs other than CTX-M ESBL [3] or mutation of penicillin-binding protein [31] were not detectable by the Verigene system. Thus, the availability of the local antibiogram had an important role in the decision-making regarding the choice of antimicrobial agent in this study.

In this clinical trial, ID physicians were involved in deciding on the treatments for all cases of bacteremia. The presence of ID physicians is considered to be useful for the treatment of bacteremia [32], and the choice of antimicrobial agent was the leading question from hospitals physicians to ID physicians according to a recent prospective multi-countries study [33]. There is a current need to select the antimicrobial agent based on the clinical manifestations and rapid testing results, including the urinary antigen test and/or Gram stain examinations for bacteremic patients in the acute phase, and the present study indicated the significant assistance provided by the Verigene system due to rapid availability of bacterial identification and prediction of antimicrobial resistance.

The most important benefit of the rapid diagnostic methods, including the Verigene system examinations, is their rapidity in providing results. For example, there were limited clinical benefits recognized in a recent clinical trial that utilized a multiplexed molecular method for bacteremia, which required 51 h to report the results of the molecular method after the blood culture obtained [34]. In addition, the results might not be useful if the physician does not understand the results or change antimicrobial agents as appropriate. Therefore, cooperation among the laboratory, ID physicians and the clinicians in charge of patients with bacteremia is essential for maximizing the effectiveness of the Verigene system examinations.

As limitations, this study was performed in Japan, where infections with vancomycin intermediate *S. aureus* and vancomycin-resistant enterococci were rarely encountered [35,36], but MRSA and CTX-M-producing Enterobacteriaceae were commonly isolated [35,37]. Thus, the current results might not be applicable in other counties, especially in countries with a low prevalence rate of MDRO infections [38]. In addition, matrix-assisted laser desorption/ionization time (MALDI-TOF) has been introduced into clinical practice as a rapid identification method for bacteria, and a recent study indicated that there was a reduction of the time to identification by 28 h (84 h vs. 56 h) among patients with bacteremia [39]:

Table 4
The secondary outcomes of cases with bacteremia during the control and intervention periods.

	Control period (n = 147)	Intervention period (n = 88)	*P* value
Additional costs of antimicrobial agents (yen)			
Gram-positive and Gram-negative bacteria	8505 (14,274)	3618 (7623)	0.001
(Cost for antimicrobial agents against non-susceptible causative bacteria of bacteremia)	4644 (9975)	2031 (6038)	0.013
(Cost for unnecessary anti-MRSA agents or antipseudomonal agents)	3915 (11,920)	1586 (5239)	0.042
Gram-positive bacteria	11585 (17,503)	2488 (4666)	0.002
(Gram-positive cocci in clusters)	14,611 (20,449)	4498 (5891)	0.136
(Gram-positive cocci in chains)	7989 (13,333)	477 (1510)	0.019
Gram-negative bacteria (GN)	7057 (12,309)	3951 (8294)	0.052
(Hospital-onset GN infection)	10,326 (13,454)	4376 (9516)	0.092
(Community-onset GN infection)	4272 (10,591)	3797 (7908)	0.797
(Community-onset healthcare-associated GN infection)	4450 (3869)	3010 (6051)	0.461
14-day Mortality (%)	8 (5.4)	1 (1.1)	0.159
30-day Mortality (%)	19 (12.9)	3 (3.4)	0.019

All categorical data are presented as numbers (proportion, %). The continuous data are presented as the means (standard deviation).

the effectiveness of the Verigene system has not been clarified under such circumstances. Third, while co-morbidity and severity were similar between both periods, hospital-onset infection was more common in the control period and there was a difference in the ratio of infection sites between the two periods, which may have affected the difference in the prognosis. In addition, we did not perform the Verigene system examination for patients with bacteremia caused by multiple organisms or with suspected contamination. Furthermore, we could not evaluate the necessity of the Verigene system for each specific organism or specific place of onset in the present trial. The accumulation of *in vitro* and clinical data will be necessary to evaluate the utility of the Verigene system for these situations.

In conclusion, the results of the Verigene Gram-positive blood culture test and the Verigene Gram-negative blood culture test were available for most of the cases, and the rapid diagnosis of the causative pathogens improved the rate of administration of appropriate antimicrobial agents without increasing the use of carbapenems. Benefits were also recognized for the antimicrobial prescription costs and patient prognosis.

Conflicts of interest

The Verigene BC-GP and BC-GN used in the current study were provided by Hitachi High-Technologies Corporation, and one of the two processors for the Verigene system was graciously loaned to us by Hitachi High-Technologies Corporation during the study period. TMCH, MML and the Nagasaki University Graduate School of Biomedical Sciences received fees for experiments and research expenses for the quality evaluation of the BC-GN panel, and some of these data are shown in Supplementary file 1. The cost for the validation of bacterial identification and drug susceptibility testing was supported by TMCH.

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A portion of the data from this study was presented at the 89th General Meeting of the Japanese Association for Infectious Diseases (Kyoto, Japan, 2015).

Mr. Tetsuo Miura (Hitachi High-Technologies Corporation) reviewed our protocol before submission to the ethics committee of TMCH to evaluate the requirements for supply support (the estimated number of Verigene BC-GP, BC-GN and processors for the Verigene system) and provided technical support for the Verigene system examination, including system maintenance, during the study period. They were not allowed to check the clinical database or patient outcomes during the study period.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jiac.2015.08.019.

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