Development and Clinical Validation of a Droplet Digital PCR Method for Detection of Acinetobacter baumannii and Klebsiella pneumonia in Patients with Suspected Bloodstream Infections

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Abstract

The relatively long turnaround time and low sensitivity of traditional blood culture may delay the effective antibiotic therapy in patients with bloodstream infection (BSI). To reduce the morbidity and mortality of BSI, a rapid and sensitive pathogen detection method is urgently required. Acinetobacter baumannii and Klebsiella pneumonia are two major microorganisms responsible for BSI. Here we reported a novel droplet digital PCR (ddPCR) method that can detect A. baumannii and K. pneumonia in whole blood samples within 4 h, with a specificity of 100% for each strain and limit of detection at 0.93 copies/microliter for A. baumannii and 0.27 copies/microliter for K. pneumonia. Clinical validation in 170 patients with suspected BSIs showed that, compared with blood culture that reported 4 (2.4%) A. baumannii cases and 7 (4.1%) K. pneumonia cases, ddPCR detected 23 (13.5%) A. baumannii cases, 26 (15.3%) K. pneumonia cases, and 4 (2.4%) dual infection cases, including the 11 positive patients reported by blood culture. In addition, the positive patients reported by ddPCR alone (n = 42) had significantly lower serum concentrations of procalcitonin and lactate, SOFA and APACHE II scores, and 28-day mortality than those reported by both blood culture and ddPCR (n = 11), suggesting that patients with less severe manifestations can potentially benefit from the guidance of ddPCR results. In conclusion, our study suggests that ddPCR represents a sensitive and rapid method to identify causal pathogens in blood samples and to guide the treatment decisions in the early stage of BSI.

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Abstract

The relatively long turnaround time and low sensitivity of traditional blood culture may delay the effective antibiotic therapy in patients with bloodstream infection (BSI). To reduce the morbidity and mortality of BSI, a rapid and sensitive pathogen detection method is urgently required. *Acinetobacter baumannii* and

*Klebsiella pneumonia*are two major microorganisms responsible for BSI. Here we reported a novel droplet digital PCR (ddPCR) method that can detect *A. baumannii* and *K. pneumonia* in whole blood samples within 4 h, with a specificity of 100% for each strain and limit of detection at 0.93 copies/microliter for *A. baumannii* and 0.27 copies/microliter for *K. pneumonia.* Clinical validation in 170 patients with suspected BSIs showed that, compared with blood culture that reported 4 (2.4%) *A. baumannii* cases and 7 (4.1%) *K. pneumonia* cases, ddPCR detected 23 (13.5%) *A. baumannii* cases, 26 (15.3%) *K. pneumonia* cases, and 4 (2.4%) dual infection cases, including the 11 positive patients reported by blood culture. In addition, the positive patients reported by ddPCR alone (n = 42) had significantly lower serum concentrations of procalcitonin and lactate, SOFA and APACHE II scores, and 28-day mortality than those reported by both blood culture and ddPCR (n = 11), suggesting that patients with less severe manifestations can potentially benefit from the guidance of ddPCR results. In conclusion, our study suggests that ddPCR represents a sensitive and rapid method to identify causal pathogens in blood samples and to guide the treatment decisions in the early stage of BSI.

Keywords: *Acinetobacter baumannii, Klebsiella pneumonia,*droplet digital PCR, Bloodstream infection, pathogen

Introduction

Bloodstream infection (BSI) represents a major cause of death worldwide, contributing to increased healthcare costs, length of hospital stay, and in-hospital morbidity (McNamara, et al., 2018). Timely and accurate pathogen identification is critical to guide antimicrobial treatment for patients in the early stage of BSI. Blood culture remains the gold standard for identifying the pathogens in BSI (Blevins and Bronze, 2010). However, it is limited by the low sensitivity and the long turnaround time (Riedel and Carroll, 2016, Tabak, et al., 2018). In septic patients within the first 6 h of documented hypotension, every 1-h delay in appropriate antibiotic therapy leads to an average increase of mortality rate by 7.6% (Kumar, et al., 2006). For hospitalized patients with bacterial infections, inappropriate initial antimicrobial treatment almost doubles the risk of 30-day mortality (Fraser, et al., 2006). Thus, it is necessary to develop a rapid and accurate method to identify the causal pathogens in BSI.

Recently, the culture-independent, real-time PCR-based or microarray-based methods, such as SeptiFast (Roche, Switzerland), Magicplex (Seegene, Korea), and TaqMan array card assay (Academy of Military Medical Science, China), have shown promising performance in rapidly identifying the pathogens and initiating early targeted antibiotic therapy in BSI. However, the low sensitivities ranging from 29% to 79.4% may limit the clinical application of these methods (Warhurst, et al., 2015, Buehler, et al., 2016, Riedel and Carroll, 2016, Zhang, et al., 2018, Zboromyrska, et al., 2019). Droplet digital polymerase chain reaction (ddPCR) is a novel molecular method to detect and quantify nucleic acids. In ddPCR, the template is partitioned into thousands of nanoliter-sized droplets and amplified. After amplification, the numbers of positive and negative reactions are counted, and the copy number of the template is calculated using Poisson statistics (Huggett, et al., 2015, Kuypers and Jerome, 2017). As an emerging versatile tool with high sensitivity, accuracy, and precision, ddPCR has been increasingly applied in multiple clinical scenarios, including oncology (Gevensleben, et al., 2013, Taly, et al., 2013, Jennings, et al., 2014, Postel, et al., 2018, Galimberti, et al., 2019), non-invasive prenatal testing (Barrett, et al., 2012, Tan, et al., 2019), and infectious diseases (Kelley, et al., 2013, Pholwat, et al., 2013, Sedlak, et al., 2014, Sedlak, et al., 2014, Whale, et al., 2016, Wouters, et al., 2019).

Acinetobacter baumannii and *Klebsiella pneumoniae* are two major Gram-negative bacteria involved in BSI, with high capabilities to develop antibiotic resistance. BSIs due to multidrug-resistant *A. baumannii* and *K. pneumoniae* significantly contribute to the mortality in the intensive care unit (ICU), with a mortality rate over 50% (Balkhair, et al., 2019, Brink, 2019). In this study, we developed and validated a ddPCR-based method to detect *A.baumannii* and*K. pneumonia* in blood samples of patients with suspected BSI. Our results provide ddPCR as a promising method to accurately and rapidly diagnose BSIs caused by *A. baumannii* and *K. pneumoniae.*

Materials and methods

Blood culture and control bacterial strains

Upon clinical suspicion of BSI, whole blood samples were simultaneously obtained for blood culture and molecular diagnosis. Two sets of blood cultures were collected for each patient according to routine clinical practice, and each set consisted of an aerobic bottle and an anaerobic bottle. The blood cultures were incubated at 37 in a BacT/ALERT® 3D System (BioMérieux, France). When the system showed a positive signal, Gram staining was performed, followed by subculture on a Columbia blood agar plate at 37 with 5% CO_2. Following overnight incubation, the pathogens were further identified by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) (VITEK(r) MS system, BioMerieux, France). The positive control bacteria were *A. baumannii* ATCC 19606 with 5 clinical isolates and *K. pneumonia* CMCC 46117 with 33 clinical isolates. A total of 131 other clinical isolates that are commonly found in BSI were used as negative controls (**Table 1**).

DNA extraction

Total DNA was isolated from 1 μL of purified control bacterial strains using a TIANamp bacteria DNA kit (TIANGEN Biotech, Beijing, China) following the manufacturer's instructions. Plasma was obtained by centrifuging the blood sample at 1,600 × g for 20 min. Plasma DNA extraction was isolated from 2 mL plasma using a magnetic serum/plasma DNA kit (TIANGEN Biotech, Beijing, China) and the Auto-Pure20B nucleic acid purification system (Hangzhou Allsheng Instruments, Zhejiang, China). DNA was eluted in 50 μL of elution buffer, and stored at -80°C until use.

Primers and probes

Primers and TaqMan MGB probes (**Table 2**) were designed using Primer Express (Thermo Fisher Scientific, Waltham, MA, USA) and synthesized by General Biosystems (Chuzhou, China). A ROX or Cy5 reporter was incorporated at the 5' end of the probes, and a nonfluorescent quencher at the 3' end. The sensitivity and specificity were evaluated using the sequence alignments in GenBank and the Basic Local Alignment Search Tool on NCBI, respectively.

Droplet digital PCR

ddPCR was carried out using a ddPCR platform (Pilot Gene Technology, Hangzhou, China). A duplex ddPCR assay was performed to detect *A. baumannii* and *K. pneumonia* simultaneously in one chip. ddPCR analysis was performed using a Pilot Gene Droplet Digital PCR System following the manufacturer's protocol. Briefly, the ddPCR master mix for each testing panel had a final volume of 15 μL and contained 1 × ddPCR premix, 1 μM forward and reverse primers, 300 nM each probe, 5 μL of isolated plasma DNA, and DNase-free water. The mixture was loaded on a ready-to-use disposable plastic chip. About 20,000 droplets were generated using a droplet generator (DG32; Pilot Gene Technology), followed by amplification on a TC1 thermal cycler (Pilot Gene Technology). The thermal cycling parameters were 95 °C for 5 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s. After PCR amplification, droplets were analysed using an iScanner 5 chip scanner. Data analysis for droplet counts and amplitudes was performed with 30 min of hands-on time using GenePMS software version v2.0.01.20011.

Evaluation of specificity, sensitivity, linearity, and precision

Analytical specificity was evaluated by testing genomic DNA isolated from the positive and negative control bacterial strains. Analytical sensitivity was determined using LoB and LoD assays with samples in 22 replicates. Probit analysis was performed to measure LoD for each bacterium. The mean value of the copy number and the standard deviation (SD) were calculated. LoB was calculated as $\text{mean}_{\text{blank}} + 1.645(\text{SD}_{\text{blank}})$. LoD was calculated as $\text{mean}_{\text{blank}} + 3(\text{SD}_{\text{blank}})$(Armbruster and Pry, 2008). The linearity was determined by a 2-fold serial dilution of the DNA template, and each dilution was measured in 8 replicates. Precision was evaluated by testing five different concentrations of genomic DNA in 8 replicates.

Patients

A total of 170 patients were recruited from Zhejiang Provincial People's Hospital from March 2019 to October 2020. The inclusion criteria were age > 18 years and suspicion of BSI. Suspected BSI was defined if the patient had a sudden high fever (T [?] 38.5 °C) accompanied by hemodynamic instability that could not be explained by a site-specific infection at another body site. In addition, the recruited patient also presented life-threatening organ dysfunction with an increase of 2 points or more in the sepsis-related organ failure assessment score (Warhurst, et al., 2015, Timsit, et al., 2020). The demographic and clinical characteristics of each patient were collected within the first 24 h of suspicion of BSI and summarized in**Table 3** . Telephone follow-up interviews were conducted with surviving patients. An unfavorable outcome was defined as 28-day all-cause mortality after ICU admission. This study was approved by the Institutional Review Board and the Ethics Committee of Zhejiang Provincial People's Hospital (No. 2019KY002). All patients provided informed written consent.

Statistical analysis

SAS 9.13 (SAS Institute, NC, USA) was used for database management and statistical analyses. Continuous variables were expressed as the mean and standard deviation (SD) or median and interquartile range (IQR) where appropriate. The t test was used to analyze normally distributed continuous variables, whereas the Mann–Whitney U test was used to analyze nonnormally distributed continuous variables. Categorical variables were reported as frequencies and percentages, and analyzed using the chi-square test. P values less than 0.05 were considered statistically significant.

Results

Analytical specificity

To detect *A. baumannii* and *K. pneumonia* in blood samples, we designed a specific primer-probe set for each strain. The specificity test showed that all 5 *A. baumannii* isolates and 33 *K. pneumonia* isolates from ATCC were detected by corresponding primer-probe set, whereas the 131 negative control isolates showed no response to the primer-probe sets (**Table 4**). These results suggest that the primer-probe sets are specific to *A. baumannii*and *K. pneumonia* , respectively.

Analytical sensitivity

Analytical sensitivity was measured using the limit of blank (LoB) and limit of detection (LoD) assays. As shown in **Table 4** , LoB of*A. baumannii* and *K. pneumonia* were 0.55 copies/μL and 0.15 copies/μL, respectively; LoD of *A. baumannii* and *K. pneumonia* were 0.93 copies/μL and 0.27 copies/μL, respectively. LoD served as the lowest concentration that gave positive results in the ddPCR assay.

Analytical repeatability, reproducibility, and linearity

Within-run precision (repeatability) and within-laboratory precision (reproducibility) were measured using different concentrations of sheared *A. baumannii* and *K. pneumonia* DNA samples. The within-run coefficients of variations (CVs) of *A. baumannii* and*K. pneumonia* were 2.7%–5.3% and 2.3%–9.5%, respectively. The within-laboratory CVs of *A. baumannii* and *K. pneumonia*were 5.3%–7.5% and 7.4%–9.7%, respectively (Tables 5 and 6).

Linearity was determined by a 2-fold serial dilution of the DNA template. Figure 1 shows the regression lines that represent the linear relationships between DNA copy numbers and concentrations of *A. baumannii* ($R^2 = 0.9925$) and *K. pneumonia* ($R^2 = 0.9915$), respectively. Taken together, these results indicate that ddPCR has excellent repeatability, reproducibility, and linearity in detecting *A. baumannii* and*K. pneumonia* in blood samples.

Turnaround time of diagnosis

The blood samples were obtained and transferred to ddPCR laboratory in about 10 min. Plasma was immediately isolated after centrifugation for 20 min. DNA extraction and PCR amplification were completed within 3 h. Data analysis was done within 30 min using GenePMS software. The average turnaround time

of ddPCR was 4.2 ± 0.51 h, which was remarkably shorter than that of the blood culture test (90.6 ± 10.9 h, $P < 0.01$).

Clinical performance

Of the 170 patients with suspected BSIs tested by ddPCR, 53 showed positive results, including 23 (13.5%, 23/170) *A. baumannii*cases, 26 (15.3%, 26/170) *K. pneumonia* cases, and 4 (2.4%, 4/170) dual infection cases. On the other hand, blood culture detected 4 (2.4%) *A. baumannii* cases, 7 (4.1%) *K. pneumonia*cases, and 22 (12.9%) cases with other bacterial infections, with an overall positivity rate of 19.4% (33/170). Of note, all the 11 cases (100%, 11/11) with *A. baumannii* or *K. pneumonia*infections reported by blood culture showed positive results in ddPCR assay. The positivity rate of *A. baumannii* and *K. pneumonia* in the ddPCR assay was much higher than that in the blood culture (31.2%, 53/170 vs. 6.5%, 11/170) (**Table 7**).

The clinical characteristics of the 53 ddPCR-positive patients were summarized in **Table 3** . No significant differences were observed in age, systolic and diastolic blood pressure, the plasma level of CRP, white blood cell counts, serum creatinine, and use of vasoactive drugs (all P [?] 0.05) between the patients reported by ddPCR alone (n = 42) and the patients reported by both ddPCR and blood culture (n = 11). Importantly, compared with the 11 positive patients reported by both ddPCR and blood culture, the 42 positive patients reported by ddPCR alone had significantly decreased serum concentrations of procalcitonin (3.70 *vs.* 12.9 pg/L, $P = 0.03$) and lactate (2.76*vs.* 4.26 mmol/L, $P = 0.04$), APACHE II scores (21.9*vs.* 27.9, $P = 0.02$), SOFA scores (10.5 *vs.* 13.8,$P = 0.03$), and 28-day mortality rates (70.6% *vs.* 90.1%,$P = 0.04$) (**Table 3**). These data suggest that the less severe BSI patients who have been missed by blood culture may benefit from ddPCR test and have better clinical outcomes.

Discussion

By studying 165,593 blood specimens from 13 USA hospitals, Tabak et al. have shown that the median time to identify BSI pathogens using traditional blood culture was 44.0 h, with a sensitivity of approximately 70% in critically ill patients and even lower for fastidious microorganisms (Tabak, et al., 2018). In this study, to overcome the shortcomings of the blood culture in BSI diagnosis, we developed a culture-independent ddPCR method to rapidly and accurately identify *A. baumannii* and *K. pneumonia* in blood samples of patients with suspected BSI. Our results showed that ddPCR could identify *A. baumannii* and *K. pneumonia* in whole blood samples within 4 h, with a specificity of 100% for each strain and limit of detection at 0.93 copies/μL for *A. baumannii* and 0.27 copies/μL for *K. pneumonia* . Clinical validation in 170 patients with suspected BSIs showed that ddPCR not only detected the positive patients who were identified by blood culture but also detected the patients who were missed by blood culture. Of note, compared with the patients reported by both blood culture and ddPCR assay, the patients reported by ddPCR alone had less severe clinical manifestations and better clinical outcomes, suggesting that these patients benefit from the guidance of ddPCR results in the early stage of BSI. Thus, ddPCR may serve as a rapid and reliable method to identify causal pathogens in BSI and to guide the treatment decisions in the early stage of BSI.

In BSI, the human immune system and antibiotic treatment kill invading pathogens, leading to the release of the nucleic acids from the pathogens into the blood, which become a part of circulating cell-free DNA (cfDNA) (Lo, et al., 1999, Diehl, et al., 2008, Lu, et al., 2018, Papadopoulos, 2020). Thus, the presence of specific pathogenic DNA in cfDNA can reflect the presence of pathogens in the bloodstream. Accumulating evidence have demonstrated the feasibility of next-generation sequencing (NGS) of plasma cfDNA to identify the pathogens in BSI (Blauwkamp, et al., 2019, Farnaes, et al., 2019, Grumaz, et al., 2019, Rossoff, et al., 2019). However, the typical turnaround time of 2 days and the high cost of NGS remain barriers for cfDNA NGS in clinical practice (Long, et al., 2016, Simner, et al., 2018, Chiu and Miller, 2019, Crawford, et al., 2019). In this study, we took advantage of the ultra-high sensitivity of ddPCR and the feasibility of cfDNA in pathogen identification to develop a ddPCR-based method using cfDNA as the template. The turnaround time of ddPCR to diagnosis was 4 h, which was significantly shorter than that of NGS (2–3 d) or blood culture (90.6 ± 12.9 h in this study).

Wouters et al. have developed a ddPCR method to detect bacteria or fungi using metagenomic DNA as the template and broad-range primer-probe sets; however, the overall specificity in clinical validation was only 80% (Wouters, et al., 2019). In this study, we used cfDNA as the template and designed specific primer-probe sets for *A. baumannii* and*K. pneumonia* , respectively. We achieved 100% specificity in well-characterized ATCC isolates of each strain, which was also higher than other reported PCR-based methods, such as SeptiFast (50%, (Warhurst, et al., 2015); 85.5%, (Korber, et al., 2017)), Magicplex (29%) (Zboromyrska, et al., 2019), TAC assay (79.4%) (Zhang, et al., 2018), T2Bacteria (90%) (Maki, 2019, Nguyen, et al., 2019), and cfDNA NGS (93.7%) (Blauwkamp, et al., 2019).

The sensitivity of blood culture is typically lower than expected. The blood culture positivity rate of septic patients was 51% over 22 years in the United States (Martin, et al., 2003). Cheng et al. have reported a 71.7% blood culture positivity rate in severe sepsis patients from ten university hospitals in China (Cheng, et al., 2007). Similarly, 70% of infected patients in a one-day international investigation in ICU have been reported positive by blood culture test (Vincent, et al., 2009). The low sensitivity of blood culture may attribute to the low bacteria abundance in the blood, the antibiotic treatment before sampling, and the culture techniques. Molecular detection methods are less affected by these factors, thus usually have higher positivity rates than blood culture. The positivity rates of different molecular methods are 1.56 – 6.45-fold higher than that of blood culture (Long, et al., 2016, Korber, et al., 2017, Farnaes, et al., 2019, Grumaz, et al., 2019, Nguyen, et al., 2019). In the present study, the positivity rates of *A. baumannii* and *K. pneumonia* were 6.8-fold ((23+4)/4) and 4.3-fold ((26+4)/7) higher than those of blood culture, respectively. Thus, molecular detection methods may disclose the missed diagnosis in blood culture, allowing timely diagnosis and appropriate antibiotic treatment for the patients with BSI.

In this study, the 53 positive patients detected by ddPCR had typical manifestations resulting from BSI, including body temperature greater than 38.5, abnormally elevated serum levels of C-reactive protein and procalcitonin, hemodynamic instability, and severe organ dysfunction. Of note, the positive patients reported by ddPCR alone exhibited less severe manifestations than those reported by both ddPCR and blood culture, suggesting that ddPCR is more sensitive than blood culture for early diagnosis of BSI.

In conclusion, we developed a novel ddPCR method to detect two major pathogens in patients with suspected BSI. Clinical validation revealed that our method outperformed the blood culture in specificity, sensitivity, and turnaround time, serving as a promising method for early and accurate diagnosis of BSI. However, in this pilot study, we only included two major Gram-negative bacteria responsible for BSI. More clinically important pathogens will be further investigated in future studies.

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Competing interests

All authors declare that they have no competing interests.

Author contributions

Yang Zheng Jun Jing and Bangchuan Hu conceived the study design, analyzed and interpreted the data, and drafted the manuscript. Ziqiang Shao and Renhua Sun performed research and contributed analytic tools. Jingquan Liu and Run Zhang participated in study design, acquired the data, and helped revise the manuscript. All authors read and approved the final manuscript.

Ethics statement

The study protocol was approved by the Institutional Review Board and Ethics Committee (No. 2019KY002) of Zhejiang Provincial People's Hospital, and adhered to the guidelines of Institutional Review Board and Ethics Committee.

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Figure Legend

Figure 1. Linear regression analysis. Linearity was determined by a 2-fold serial dilution of the DNA template.

Table 1. Microorganisms used in specificity assay

Organism	No.	Source/strain
Acinetobacter baumannii	5	ATCC 19606; Clinical isolates (4)
Klebsiella pneumonia	33	CMCC 46117; Clinical isolates (32)
Escherichia coli	8	Clinical isolates (8)
Pseudomonas aeruginosa	19	CMCC 10104; Clinical isolates (18)
Enterococcus faecalis	9	ATCC 19433; Clinical isolates (8)
Enterococcus faecium	5	ATCC 19434; Clinical isolates (4)
Serratia marcescens	7	CMCC 41002; Clinical isolates (6)
Salmonella enterica	1	CMCC 41002
Streptococcus pneumoniae	1	CMCC 31001
Enterobacter cloacae	15	CMCC 43501; Clinical isolates (14)
Burkholderia cepacia	13	ATCC25416; Clinical isolates (12)
Enterobacter aerogenes	1	ATCC 13048
Proteus mirabilis	18	BNCC 107943; Clinical isolates (17)
Candida albicans	2	CMCC 98001; Clinical isolates (1)
Candida glabrata	1	Clinical isolates (1)
Stenotrophomonas maltophilia	3	Clinical isolates (3)
Staphylococcus aureus	9	Clinical isolates (9)
Staphylococcus epidermidis	3	Clinical isolates (3)
Staphylococcus hominis	1	Clinical isolates (1)
Staphylococcus haemolyticus	13	Clinical isolates (13)
Staphylococcus capitis	2	Clinical isolates (2)

Table 2. Primers and probes for *A. baumannii* and*K. pneumonia* detection.

Name	Target Gene	Sequence (5'–3')
Ab-F	OXA-51-like β-lactamase ($bla_{OXA\text{-}51\text{-}like}$)	CAC ACT ACG GGT GTT TTA GTT ATC CA
Ab-R		CGA GCA AGA TCA TTA CCA TAG CTT T

Name	Target Gene	Sequence (5'–3')
Ab-Probe		Cy5-CAA GGC CAA ACT C-MGB
Kp-F	*Klebsiella pneumoniae hemolysin (khe)*	GGG CGA GGT TTA CGT CTC AA
Kp-R		GCG TGT GGA TAA GAG GTG CG
Kp-Probe		ROX-CCA CCA CGA GCG GC-MGB

Table 3. Comparison of clinical characteristics of positive patients reported by different detection methods.

Clinical characteristics	Positive patients by BC and/or ddPCR (n=53)	ddPCR-only (+) (n=42)	BC & ddPCR (+) (n=11)	*P*
Age (years)	65.7 ± 13.3	64.7 ± 13.8	69.4 ± 11.2	0.29
Male, n (%)	38 (71.7)	30 (71.4)	8 (72.7)	0.93
Use of vasoactive drugs	35 (66.0)	27 (64.3)	8 (72.7)	0.60
Norepinephrine, n (%)	34 (64.2)	26 (61.9)	8 (72.7)	0.51
Epinephrine, n (%)	6 (11.3)	5 (11.9)	1 (9.10)	0.79
Vasopressin, n (%)	12 (22.6)	8 (19.1)	4 (36.5)	0.22
Mechnical ventilation, n (%)	44 (83.2)	35 (83.3)	9 (81.8)	0.91
Renal replacement therapy, n (%)	14 (26.4)	11 (26.2)	3 (27.3)	0.94
Physical examinations				
Temperature (°C)	38.7 ± 0.37	38.8 ± 0.39	38.6 ± 0.21	0.10
Systolic blood pressure (mmHg)	86.4 ±17.6	88.4 ±15.7	78.6 ± 22.8	0.11
Diastolic blood pressure (mmHg)	44.2 ± 9.57	46.4 ± 8.10	41.5 ± 7.88	0.09
Complete blood counts and blood biochemistry				
Platelet counts, median (IQR) $\times 10^9$/L	61.2 (54.4 -93.4)	83.0 (63.5 - 108.5)	40.3 (15.7 - 103.6)	0.12
White blood cell, median (IQR) $\times 10^9$/L	10.1 (7.72 -13.3)	10.5 (8.19 - 13.3)	8.99 (3.02 - 26.7)	0.77
C reactive protein (mg/L), median (IQR)	152.1 (125.0 - 185.1)	140.6 (110.4-179.1)	204.4 (150.4 -277.8)	0.06

Clinical characteristics	Positive patients by BC and/or ddPCR (n=53)	ddPCR-only (+) (n=42)	BC & ddPCR (+) (n=11)	*P*
Procalcitonin (pg/L), median (IQR)	4.84 (3.04 - 7.72)	3.70 (2.20 - 6.21)	12.9 (4.13 - 40.4)	0.03
Serum creatinine (μmol/L), median (IQR)	125.6 (107.2 -147.1)	119.2 (98.9 - 143.6)	151.5 (103.9 - 220.9)	0.22
Serum lactate (mmol/L), median (IQR)	3.01 (2.50 - 3.62)	2.76 (2.28 -3.43)	4.26 (2.75 -6.89)	0.04
SOFA score	11.2 ± 4.77	10.5 ± 4.54	13.8 ± 4.85	0.03
APACHE II score	23.2 ± 7.46	21.9 ± 6.63	27.9 ± 8.77	0.02
28 - day mortality, n (%)	34 (64.2)	24 (70.6)	10 (90.1)	0.04

BC, blood culture; ddPCR, droplet digital PCR; IQR, interquartile range; SOFA, sequential organ failure assessment score; APACHE II, acute physiology and chronic health evaluation II score; Values are mean ± SD or number of subjects (percentage of the column total). *P* values for characteristics difference were calculated for comparisons by the standard normal *z* -test (means) or Fisher's exact test (proportions).

Table 4 . Analytical specificity and sensitivity of *A. baumannii* and *K. pneumonia* .

Microorganism	Specificity	Specificity	Sensitivity	Sensitivity	Sensitivity	Sensitivity
	A. baumannii	*K. pneumonia*	Mean	SD	LoB	LoD
A. baumannii	5/5 (100)	0/5 (0)	0.09	0.28	0.55	0.93
K. pneumonia	0/33 (0)	33/33 (100)	0.03	0.08	0.16	0.27
Other 131 isolates	0/131 (0)	0/131 (0)				

A. baumannii	*A. baumannii*	*K. pneumonia*	*K. pneumonia*
Concentration (copies/μL)	CV%	Concentration (copies/μL)	CV%
578.4 ± 15.9	2.7	551.5 ± 12.6	2.3
261.2 ± 13.4	5.1	240.4 ± 8.2	3.4
91.9 ± 3.2	3.5	90.5 ± 5.6	6.2
46.2 ± 1.4	2.9	62.0 ± 2.6	4.2
26.1 ± 1.4	5.3	36.1 ± 3.4	9.5

Table 5. Analytical repeatability of A. *baumannii* and*K. pneumonia* detection

CV, coefficients of variations.

Table 6. Analytical reproducibility of *A. baumannii* and*K. pneumonia* detection

A. baumannii	*A. baumannii*	*K. pneumonia*	*K. pneumonia*
Concentration (copies/μL)	CV%	Concentration (copies/μL)	CV%
580.1±30.6	5.3	541.1±40.0	7.4
91.0±5.5	6.0	91.5±7.0	7.6

A. baumannii	*A. baumannii*	*K. pneumonia*	*K. pneumonia*
27.1±2.0	7.5	38.5±3.7	9.7

CV, coefficients of variations.

Table 7. Clinical validation of ddPCR analysis *vs* . blood culture

Species	ddPCR	Blood culture
A. baumannii	23	4
K. pneumonia	26	7
A. baumannii & K. pneumonia	4	0
Other microorganisms	Not detected	22
Negative	117	137