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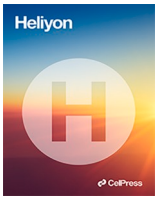

Research article

A retrospective study of the detection of sepsis pathogens comparing blood culture and culture-independent digital PCR

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ABSTRACT

Fast and precise identification of microorganisms in the early diagnosis of sepsis is crucial for enhancing patient outcomes. Digital PCR (dPCR) is a highly sensitive approach for absolute quantification that can be utilized as a culture-independent molecular technique for diagnosing sepsis pathogens. We performed a retrospective investigation on 69 ICU patients suspected of sepsis. Our findings showed that a multiplex dPCR diagnostic kit outperformed blood culture in detecting the 15 most frequent bacteria that cause sepsis. Ninety-two bacterial strains were identified using dPCR at concentrations varying from 34 copies/mL to 105,800 copies/mL. The detection rate of dPCR was much greater than that of BC, with 27.53% (19/69) versus 73.91% (51/69). The sensitivity of dPCR was 63.2%. Our research indicated that dPCR outperforms blood culture in the early detection of sepsis-causing microorganisms. The diagnostic kit can detect a greater variety of pathogens with quantitative data, including polymicrobial infections, and has a quicker processing time. DPCR is a valuable technique that could aid in the proper management of sepsis.

1. Introduction

Sepsis is an illness of severe organ dysfunction resulting from the body's abnormal response to infection [1]. Sepsis is characterized by significant morbidity and mortality. In 2017, there were around 48.9 million documented cases of sepsis globally, resulting in 11.0 million fatalities [2]. China has 5.68 million sepsis patients annually [3]. The administration of antimicrobials that are effective against the specific organism causing sepsis is crucial to treatment. Administering antibiotics to septic patients within the initial hour of confirmed hypotension was associated with an 80% survival probability. Each hour of delay in antibiotic administration within the first 6 h raised the risk of mortality by 7.6% [4]. Liu found that the adjusted odds ratio for hospital mortality increased by 1.09 for every hour of delay in administering antibiotics after admission [5]. Fast and precise identification of microorganisms in blood samples is crucial to guaranteeing septic patients receive rapid, appropriate, and precise treatment for sufficient antibiotic coverage, enabling adjustment or discontinuation of empirical antimicrobial therapy.

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Traditional blood culture (BC) is still considered the most reliable method for identifying sepsis-causing bacteria. Nevertheless, it possesses inherent constraints that hinder a timely diagnosis of sepsis, such as extended processing times and restricted sensitivity [6]. Acquiring BC during antibiotic treatment has been correlated with a notable decrease in identifying pathogens [7]. Aerobic and anaerobic microbial cultures need 20–30 mL of blood, which may be difficult to collect from old or neonatal patients. Pathogen recovery, identification, and antimicrobial susceptibility testing (AST) can take several days to detect antibiotic resistance, thereby delaying treatment and leading to increased rates of adverse events, mortality, and medical costs.

Various methods are utilized to identify sepsis pathogens [8], including matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS), multiplex fluorescent quantitative PCR, and fluorescent in situ hybridization (FISH). However, these techniques can only identify pathogens in blood culture-positive samples. Methods for directly detecting whole blood include metagenomics-based assays using NGS sequencing, multiplex real-time PCR [9], and PCR coupled with T2 magnetic resonance [10]. Some tests can expedite the process for hours but may lack the sensitivity to detect pathogens at low levels [9]. Certain techniques necessitate costly equipment and a lengthy turnaround time. These approaches are currently inadequate for fully satisfying the requirements for quick and precise detection of sepsis pathogens.

Digital PCR (dPCR) has demonstrated significant promise for detecting pathogens in patients with suspected sepsis because of its exceptional sensitivity, accuracy, and precision [11,12]. DPCR is a test that utilizes the concepts of limited dilution PCR and Poisson statistics. During the dPCR experiment, the sample is distributed into tens of thousands of partitions. Fluorescent signals from each partition are quantified at the end of the PCR process to determine the total number of target molecules in the sample. The dPCR approach quantifies nucleic acid molecules without using a standard curve, leading to less error and enhanced accuracy. DPCR may identify a small number of viral genomes and is being more commonly used in identifying infectious diseases [13–15]. DPCR can identify minimal quantities of a pathogen's DNA from blood samples in 3–6 h, aiding in subsequent therapy. DPCR may reduce the hindrance caused by the high quantity of human genomic DNA. Utilizing a multiplexed dPCR assay can enhance cost efficiency. Nevertheless, there is a shortage of studies focused on confirming the effectiveness of multiplex dPCR in the prompt identification of sepsis pathogens in ICU settings.

In this retrospective study, we identified the top 15 clinically significant pathogens in patients with sepsis-like symptoms using a multiplex dPCR panel. Along with nine other bacterial species, the panel also contains "ESKAPE" pathogens (*E. faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *E. coli*). From April 1, 2020, to March 31, 2021, we assessed the dPCR approach for the quick and precise identification of the pathogenic bacteria in 69 ICU patients who were suspected of having sepsis. In order to assess the consistency of dPCR and BC procedures as well as whether dPCR can give doctors additional information for more accurate antibiotic treatment, we also conducted a head-to-head comparison of the two techniques.

2. Materials and methods

2.1. Patients

The study was authorized by the General Hospital Ethics Committee of Ningxia Medical University. Between April 1, 2020, and March 31, 2021, 69 clinical blood samples were obtained from the General Hospital of Ningxia Medical University's acute care unit. This study recruited patients suspected of sepsis, aged 18–90 years, consecutively from April 1, 2020, to March 31, 2021. Demographic and clinical data, as well as blood culture results, were collected with the informed consent of the patients or their legal representatives. The criteria for inclusion were a clinical suspicion of sepsis, characterized by a rapid high fever ($T > 38.5\ ^{\circ}C$) and severe organ dysfunction with a sequential organ failure assessment (SOFA) score increase of two points or more. Acute physiology and chronic health evaluation II (APACHE II) and SOFA scoring systems were used daily to evaluate organ failure and disease severity during the hospital stay. The exclusion criteria included any terminal-stage condition, severe malignancy, or pre-existing sepsis at the time of hospitalization. Patients' mortality was monitored for up to 28 days.

2.2. Blood culture and detection of pathogens

Whole blood samples were taken concurrently for molecular diagnostics and blood culture when sepsis was clinically suspected. According to standard clinical procedure, one or more sets of blood cultures—each consisting of an anaerobic culture and an aerobic culture—were acquired for every patient [16]. The BacT/ALERT 3D System from BioMérieux, France, was used to incubate the blood cultures at 37 °C. Upon receiving a positive signal from the system, Gram staining was conducted, and then a Columbia blood agar plate was used for a subculture at 37 °C with 5% CO_2. The infections were further detected by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS; VITEK MS systembioMérieux, France) after an overnight incubation, as previously outlined [17].

2.3. Extraction of DNA from whole blood and DPCR

Each participant provided 1 mL of peripheral venous blood using an EDTA-anticoagulant tube. 1 mL of whole blood was used to extract DNA with a magnetic DNA kit (TIANGEN Biotech, Beijing, China), following the manufacturer's instructions. 50 μL of DNA was collected and then kept at −80 °C for dPCR analysis. Fifteen bacterial pathogens were tested utilizing the sepsis pathogenic microorganism detection kit in conjunction with the RainSure DropX-2000 Droplet Digital PCR System, both from Rainsure Scientific in Suzhou, China. The 15 target pathogens are listed in Supplementary Table 1 DPCR analysis was conducted according to the

Table 1
Pathogens detected by blood culture method.

Sample ID	Bacterial strains	SOFA score	APACHE II score	28-day mortality	Antibiotics used
1	*Escherichia coli* *Bacteroides fragilis* *Eggerthella lenta*	2	19	Dead	Meropenem Piperacillin Sulbactam sodium Tigecycline
2	*Enterococcus faecium*	10	23	Dead	Meropenem Levofloxacin Etimicin sulfate Ceftazidime Avibactam
6	*Escherichia coli* *Klebsiella pneumoniae*	16	30	Dead	Piperacillin Tazobactam Ciprofloxacin Meropenem Vancomycin
7	*Acinetobacter baumannii*	21	37	Dead	Meropenem Imipenem Cilastatin Ganciclovir Voriconazole Azithromycin Sulbactam sodium Tigecycline
8	*Escherichia coli* *Bacteroides thetaiotaomicron*	3	9	Dead	Efoperazone and Sulbactam Imipenem
9	*Escherichia coli* *Candida tropicalis*	10	12	Alive	Meropenem Tigecycline
10	*Escherichia coli*	16	8	Alive	Cefoperazone and Sulbactam Imipenem Cilastatin
30	*Klebsiella pneumoniae*	10	25	Alive	Meropenem Cefoperazone and Sulbactam Etimicin Ciprofloxacin Tigecycline Carbofenozin Teicoplanin
32	*Enterobacter cloacae*	16	24	Dead	Teicoplanin and Imipenem Meropenem and Tigecycline Piperacillin and Sulbactam Ceftazidime Avibactam Tigecycline and Sulbactam sodium
43	*Klebsiella pneumoniae*	7	4	Alive	Piperacillin Tazobactam
65	*Enterococcus faecalis*	8	17	Alive	Piperacillin and Sulbactam Imipenem Teicoplanin
70	*Enterococcus faecium*	6	13	Dead	Imipenem Cefoperazone and Sulbactam Tigecycline
72	*Escherichia coli*	7	14	Alive	Cefoperazone and Sulbactam Imipenem Teicoplanin Laxocephalosporin Ceftazidime Fluconazole Voriconazole Carbofenozin Tigecycline
87	*Bacteroides fragilis*	6	13	Dead	Cefuroxime sodium Piperacillin and Sulbactam Teicoplanin Imipenem
102	*Escherichia coli*	11	22	Alive	Piperacillin Tazobactam
108	*Klebsiella pneumoniae*	11	27	Dead	Piperacillin Tazobactam Tigecycline Imipenem
130	*Viridans Streptococci*	9	18	Alive	Meropenem Tigecycline
134	*Escherichia coli*	5	10	Dead	Piperacillin Tazobactam Teicoplanin Tigecycline

(*continued on next page*)

Table 1 (*continued*)

Sample ID	Bacterial strains	SOFA score	APACHE II score	28-day mortality	Antibiotics used
143	*Escherichia coli*	7	20	Alive	Cefoperazone and Sulbactam Levofloxacin Ceftezole sodium Laxocephalosporin Piperacillin Sulbactam sodium Meropenem Amikacin Fluconazole

manufacturer's instructions. Each dPCR mix for the four testing panels had a final volume of 20 μL, consisting of 10 μL digital PCR buffer, 2 μL panel primer probe mix, and 8 μL extracted whole blood DNA. 75 μL of droplet generation oil and 20 μL of the dPCR mixture were loaded into the oil wells and sample wells of the cartridge sequentially. The equipment autonomously conducted droplet production and executed the PCR reaction following the heat cycling methodology. Step 1: Heat to 95 °C for 10 min to activate DNA polymerase. Step 2: Conduct denaturation at 94 °C for 30 s and annealing at 60 °C for 1 min for 40 cycles. Step 3: Heat to 98 °C for 10 min to deactivate the enzyme. Step 4: Cool to 20 °C for 2 min. The cartridge was moved and inserted into a DScanner4-1000 (Rainsure Scientific, Suzhou, China) for droplet analysis using multi-channel fluorescence detection. The four fluorescence channels were examined to identify the microbes in each panel. The data analysis was conducted with GeneCount Analysis System software version v1.63.0222 by RainSure Scientific in Suzhou, China. The manufacturer's criteria for positive and negative outcomes are detailed in Supplementary Table 2.

An internal control for supervising the DNA extraction and PCR reaction system was the human RPP30 gene. The positive control, consisting of 15 genomic DNA samples from bacteria and human genomic DNA, was utilized to establish a suitable threshold for positive clusters in the samples. The negative control, comprising genomic DNA from *Citrobacter freundii*, *Staphylococcus Pasteuri*, *Acinetobacter calcium acetate*, *Pseudomonas fluorescens*, *Staphylococcus mimicking*, and *Enterobacter aerogenes*, was employed to identify exterior or reagent bacterial contamination and cross-sample contamination.

2.4. DPCR's limit of detection (LOD)

Genomic DNA of the 15 bacteria in the test panel was isolated from 15 quantified strains. The stock solutions were diluted to a concentration of 15625 copies/μL. Subsequently, a fivefold series of dilutions ranging from 15625 to 1 copies/μL were prepared. Thereafter, these diluted solutions were utilized to assess the LoD for each bacterium in single-plex analyses. The bacterial strains are listed in Supplementary Table 3. The LOD for the strain was determined to be the lowest detectable concentration in 19 out of 20 replicates, with a 95% confidence interval. Data analysis was conducted with GeneCount Analysis System software version v1.63.0222 from RainSure Scientific in Suzhou, China.

2.5. Data analysis

To determine the target concentration, the dPCR data were processed with GeneCount Analysis System software, V1.63.0222 (Rainsure Scientific, Suzhou, China). Categorical data were displayed as numbers in percentages, while continuous data were shown as the mean ± SEM. The ANOVA test was used to compare groups based on continuous variables. The category variables were evaluated by Chi-square tests. Statistical significance was defined as a p-value below 0.05. IBM SPSS software version 27.0 was used to conduct the statistical tests (IBM, Armonk, NY, SA).

3. Results

3.1. Baseline clinical and demographic data for the participants

In this study, 69 patients with a suspected case of sepsis were successively recruited. Clinical information, such as demographics, comorbidities, organ dysfunction, surgical intervention, and clinical outcomes, was taken from the patient's medical records. Using a 28-day survival follow-up, SOFA and APACHE II were used to evaluate each patient. as displayed in Supplementary Table 4. When analyzing categorical variables, the Chi-square test was utilized; conversely, the ANOVA test was applied to continuous variables. The patients' median age was sixty-seven years old. Procalcitonin and C-reactive protein in plasma had mean values of 3.1 ± 1.0 ng/mL and 170.6 ± 96.4 mg/L, respectively. The SOFA and APACHE II scores had mean values of 8.9 ± 5.1 and 16.9 ± 7.5, respectively. There is a 43.5% 28-day mortality rate among the 69 patients.

The four groups did not show any notable variations in hospital length of stay, vasoactive and hormonal drug treatment, underlying disease, local infection, or other clinical factors listed in Supplementary Table 4.

3.2. Bacteria identified by blood culture

Peripheral blood samples from 69 sepsis patients were examined in this investigation; 19 (19/69, 27.5%) of them were culture-

Table 2
DPCR positive results detected from 69 patients' samples.

Group	Bacterial strains	Sample ID	Concentration in blood (copies/mL)	BC results
Group1	*Acinetobacter baumannii*	9	163	*Candida tropicalis Escherichia*
BC and dPCR positive and	*Escherichia coli*		79	*coli*
consistent	*Acinetobacter baumannii*	6	77	*Escherichia coli*
	Escherichia coli		77	*Klebsiella pneumoniae*
	Klebsiella pneumoniae		83	
	Staphylococcus haemolyticus		83	
	Enterobacter cloacae	32	124	*Enterobacter cloacae*
	Escherichia coli		581	
	Enterobacter cloacae	43	116	*Klebsiella pneumoniae*
	Escherichia coli		454	
	Klebsiella pneumoniae		610	
	Enterococcus faecium	2	136	*Enterococcus faecium*
	Escherichia coli		978	
	Stenotrophomonas maltophilia		120	
	Enterococcus faecalis	72	423	*Escherichia coli*
	Escherichia coli		54625	
	Stenotrophomonas maltophilia		366	
	Enterococcus faecalis	8	431	*Bacteroides thetaiotaomicron*
	Escherichia coli		49125	*Escherichia coli*
	Pseudomonas Aeruginosa		139	
	Staphylococcus epidermidis		246	
	Stenotrophomonas maltophilia		203	
	Escherichia coli	10	36	*Escherichia coli*
	Escherichia coli	102	112	*Escherichia coli*
	Escherichia coli	134	888	*Escherichia coli*
	Escherichia coli	143	56	*Escherichia coli*
	Escherichia coli	1	63	*Bacteroides fragilis*
	Staphylococcus epidermidis		38	*Eggerthella lenta Escherichia coli*
Group 2	*Acinetobacter baumannii*	65	184	*Enterococcus faecalis*
BC & dPCR positive but different	*Staphylococcus aureus*		34	
	Enterobacter cloacae	87	150	*Bacteroides fragilis*
	Escherichia coli		753	
	Staphylococcus aureus		226	
	Enterobacter cloacae	30	95	*Klebsiella pneumoniae*
	Escherichia coli		759	
	Staphylococcus aureus		230	
	Stenotrophomonas maltophilia		133	
	Enterococcus faecalis	108	540	*Klebsiella pneumoniae*
	Escherichia coli		48250	
	Stenotrophomonas maltophilia		263	
	Escherichia coli	70	57	*Enterococcus faecium*
	Escherichia coli	130	186	*Viridans Streptococci*
	Escherichia coli	7	223	*Escherichia coli*
	Klebsiella pneumoniae		112	
	Staphylococcus aureus		94	
Group3 dPCR positive only	*Acinetobacter Baumannii*	125	106	Negative
	Staphylococcus hominis		1058000	
	Bacteroides fragilis	90	44	Negative
	Enterobacter cloacae	81	84	Negative
	Enterobacter cloacae	39	54	Negative
	Escherichia coli		399	
	Enterobacter cloacae	44	86	Negative
	Escherichia coli		1663	
	Enterobacter cloacae	62	76	Negative
	Escherichia coli		885	
	Enterobacter cloacae	116	59	Negative
	Escherichia coli		200	
	Staphylococcus aureus		153	
	Enterobacter cloacae	40	62	Negative
	Escherichia coli		1950	
	Stenotrophomonas maltophilia		64	

(*continued on next page*)

Table 2 (*continued*)

Group	Bacterial strains	Sample ID	Concentration in blood (copies/mL)	BC results
	Enterobacter cloacae	109	78	Negative
	Escherichia coli		1079	
	Staphylococcus aureus		71	
	Stenotrophomonas maltophilia		1425	
	Enterococcus faecalis	66	653	Negative
	Pseudomonas aeruginosa		238	
	Escherichia coli	19	139	Negative
	Escherichia coli	20	859	Negative
	Escherichia coli	21	1124	Negative
	Escherichia coli	23	1013	Negative
	Escherichia coli	36	538	Negative
	Escherichia coli	37	130	Negative
	Escherichia coli	41	1325	Negative
	Escherichia coli	42	1663	Negative
	Escherichia coli	64	133	Negative
	Escherichia coli	67	1043	Negative
	Escherichia coli	85	399	Negative
	Escherichia coli	99	110	Negative
	Escherichia coli	101	81	Negative
	Escherichia coli	128	20125	Negative
	Escherichia coli	136	93	Negative
	Escherichia coli	131	88	Negative
	Pseudomonas aeruginosa		88	
	Escherichia coli	35	378	Negative
	Staphylococcus aureus		62	
	Escherichia coli	74	1375	Negative
	Stenotrophomonas maltophilia		99	
	Klebsiella pneumoniae	14	60	Negative
	Staphylococcus aureus	119	153	Negative
	Staphylococcus aureus	120	576	Negative
	Staphylococcus hominis	92	38	Negative

positive. By using traditional culture, 11 species and 26 strains of pathogens were found, comprising 3 g-positive bacteria, 7 g-negative bacteria, and 1 fungus. The species excluded from the dPCR panel were *Viridans streptococci*, *Bacteroides thetaiotaomicron*, *Eggerthella lenta*, and *Candida tropicalis*. The most often found bacterium, *E. coli*, was found in 9 samples (9/19, 47.4%). Additionally, there were four polymicrobial infections (Table 1): 1 *E. coli* and *K. pneumonia*, 1 *E. coli* and *B. thetaiotaomicron*, 1 *E. coli* and *C. tropicalis*, 1 *E. coli*, *B. fragilis*, and *E. lenta*.

3.2.1. DPCR processing time

It took approximately 4–5 h from blood collection to getting the test results. 1 h for sample lysis and pathogen DNA extraction, 15

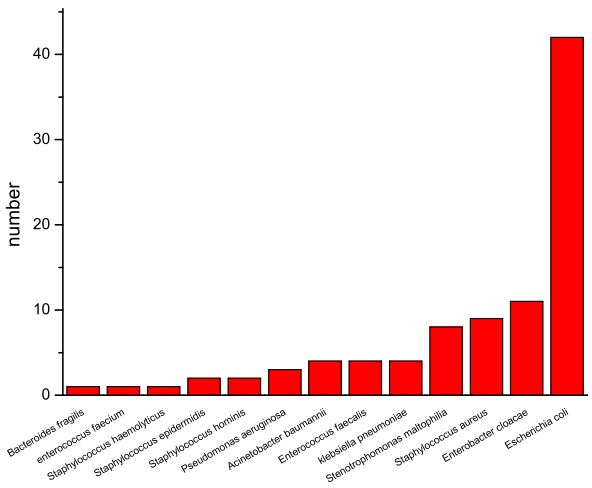

Fig. 1. Distribution of 13 bacteria species detected by dPCR.

min for dPCR reaction buffer mixing and sample loading, 2 h for droplet generation and dPCR amplification, and 45 min for scanning and analysis of dPCR results.

3.3. Pathogens detected by dPCR

A total of 51 (73.9%, 51/69) sepsis patients' blood samples were detected positive by dPCR (Table 2). There were 13 bacterial species (92 strains) identified. The top three bacteria Among the 92 strains found were *Staphylococcus aureus* (n = 9), *Enterobacter cloacae* (n = 11), and *Escherichia coli* (n = 42) (Fig. 1). A two-dimensional schematic of the dPCR results is shown in Supplementary Fig. 1. There were 24 cases of polymicrobial infections, including 12 double microbial infections, 8 triple microbial infections, 3 fourfold microbial infections, and 1 fivefold microbial infection. In patient 8, the main infections were *Escherichia coli* (49125 copies/mL in blood), *Enterococcus faecalis* (431 copies/mL in blood), *Staphylococcus epidermidis* (246 copies/mL in blood), *Stenotrophomonas maltophilia* (203 copies/mL in blood), and *Pseudomonas aeruginosa* (139 copies/mL in blood). The strain with the lowest concentration detected was *Staphylococcus aureus* (34 copies/mL in blood), and the strain with the highest concentration was *Staphylococcus hominis* (105800 copies/mL in blood). 50 (54.3%) strains were detected with copy numbers less than 200 copies/mL. *E. coli* (n = 13), *Staphylococcus hominis* (n = 1), and *Stenotrophomonas maltophilia* (n = 1) were among the 15 strains with copy numbers >1000 copies/mL. The top 3 high-concentration *E. coli* infection patients were also polymicrobial infection patients.

3.4. Concordance analysis of dPCR and BC

The formula for calculating sensitivity was 100% x (TP/TP + FN). Four scenarios were classified as true positives (TP): the dPCR findings and the BC results were entirely consistent; the dPCR results contained extra bacteria; the dPCR and the BC results shared results and both independently detected pathogens; the dPCR results were included in the BC results. False-negative (FN) denoted the presence of pathogens discovered by the BC and dPCR separately, without any overlap. Alternatively, the BC results were positive, but the dPCR results were negative. The formula for calculating specificity is 100% x (TN/TN + FP). False-positive (FP) meant that the BC results were negative but the dPCR findings were positive. True negative (TN) denotes that both the dPCR and the BC results were negative.

Compared to BC (19/69, 27.5%), the dPCR (51/69, 73.9%) yielded a larger percentage of positive results. The findings of the dPCR analysis were as follows: sensitivity was 63.2%, specificity was 36%, positive predictive value was 27.3%, and negative predictive value was 72%.

One strain of *S. viridis* that was identified solely by BC was outside the dPCR detection range, while 12 samples out of the 19 positive BC were concordantly positive by BC and dPCR, and 7 samples independently detected pathogens without overlapping. Thirteen more pathogens, including five *S. maltophilia*, four *S. aureus*, two *S. epidermidis*, one *P. aeruginosa*, and one *S. haemolyticus*, were identified solely by dPCR and not by BC among these twelve samples that tested positive for both dPCR and BC. Four polymicrobial infections were found in 19 (21%) positive BC samples. Of the 51 positive dPCR samples, 24 polymicrobial illnesses were found (47%).

3.5. DPCR's LOD

We used five-fold serial dilutions of quantitative bacterial genomic DNA stocks of 15 bacteria at various concentrations ranging from 15625 to 1 copies/μL to investigate the LOD of the test. The lowest concentration that could be detected in 19 out of 20 replicates of the assay was defined as the strain's LOD (95% confidence interval). Bacterial genomic DNA ranging from 1.45 copies/reaction for *E. faecalis* to 80.33 copies/reaction for *S. pneumonia* was detected by the dPCR in this experiment (Table 3 and Supplementary Fig. 2). In each test, the NTC showed no signs of contamination.

Table 3
LOD detected using dPCR assay described in this invention.

	Target Strain	Strain Stock solution concentration (CFU/μL)	LOD (8 μL) copies/reaction	LOD* (copies/mL)
PanelA	*Bacteroides fragilis*	2.30E+06	28.84	180.26
	Staphylococcus epidermidis	2.30E+06	28.84	180.26
	Enterococcus faecalis	1.50E+06	1.45	9.04
	Streptococcus pneumoniae	1.00E+07	80.33	502.08
PanelB	*Acinetobacter baumannii*	2.10E+06	2.17	13.56
	Enterobacter cloacae	2.10E+06	4.14	25.91
	Enterococcus faecium	1.30E+06	1.95	12.20
	Staphylococcus aureus	2.40E+06	18.71	116.95
Panel C	*Pseudomonas aeruginosa*	2.80E+06	14.10	88.12
	Staphylococcus haemolyticus	1.20E+06	15.13	94.57
	Klebsiella pneumoniae	1.90E+06	1.75	10.91
PanelD	*Escherichia coli*	2.30E+06	3.22	20.13
	Staphylococcus cephalosporus	2.60E+06	12.97	81.08
	Stenotrophomonas maltophilia	1.50E+06	12.60	78.76
	Staphylococcus hominis	1.60E+06	15.25	95.30

4. Discussion

It is generally accepted that early treatment of sepsis patients will bring benefits to patients, both in hospital costs and outcomes [4,5,18]. But early recognition of pathogens in sepsis patients remains a challenge. BC has the shortcomings of prolonged processing time and low positive findings. Clinicians typically administer empirical antimicrobial treatment prior to pathogen identification, potentially resulting in bacterial resistance and treatment inefficacy. DPCR is an advanced PCR technique that provides increased sensitivity, repeatability, and absolute quantification to address these difficulties. Several research have investigated its use in infectious diseases [18], targeting 16S rRNA for bacterial identification [19–22]. This study utilized the multiplex dPCR technique to quickly identify pathogens in whole blood samples without the need for culture, with an expected completion time of 4–5 h. In order to identify the 15 bacterial pathogens at the species level, the species-specific genes were selected as target genes.

We conducted a direct comparison between the dPCR and BC approaches. The positive rate for dPCR was 73.9%, greatly above the 27.5% rate for BC. Of the 19 positive blood cultures, the concordance rate was 63.2%. Clinical validation demonstrated that the dPCR approach outperformed BC in terms of positivity rate and turnaround time, showing potential for quick and early sepsis detection. There was one strain of *A. baumannii*, two strains of *K. pneumoniae*, one strain of *B. fragilis*, one strain of *E. faecium*, and one strain of *E. faecalis* that were positive by BC but negative by dPCR. The primers/probes used were sufficiently sensitive, as they successfully detected three *A. baumannii*, four *K. pneumoniae*, two *B. fragilis*, one *E. faecium*, and four *E. faecalis* strains in the dPCR-positive samples. The disparity could be attributed to the contamination of BC, a frequent issue in BC assays. There is a common belief that a specific proportion of BC will be contaminated regardless of the precautions taken [23]. Washer discovered that 13% of positive BCs were due to contamination. The overall contamination rate was 0.8% when blood for culture was collected peripherally by phlebotomists performing venipuncture [24]. Rupp stated that 23% of positive BCs were due to contamination, with an overall contamination incidence of 1.8% for a specific research period [25]. To confirm contamination in the blood culture laboratory, we should incorporate testing of environmental samples in upcoming clinical studies using dPCR and BC methods simultaneously to test environmental samples in the laboratory to verify the presence of contamination.

For the 15 bacterial detection panels, only *S. pneumonia* and *S. capitis* weren't detected in 69 samples for the dPCR method, which are two Gram-positive bacteria. It might be because of the small size of the cohort or because the epidemic trend of pathogenic bacteria is different in different regions of China. *S. pneumonia was* also not detected in Wu's report [26]. DPCR shows a higher rate of polymicrobial infection (47%) due to the lower detection limit of dPCR (shown in Table 3). Research indicates that 50% of BSI episodes are linked to bacterial concentrations ranging from 0.01 to 1.0 CFU/mL [27] to 1×10^3 and 1×10^4 CFU/mL [28]. Our data showed that the detection limit for different bacteria is different, ranging from 9 to 500 copies/mL. The ratio of copy numbers from PCR to CFU numbers from counting live bacteria is not always the same. This is because different nucleic acid extraction kits can break down bacterial cell walls in very different ways, which can lead to huge differences in the amount of nucleic acid extracted. Irwin reported [29] that 3–27 copies/CFU of 16S rDNA were detected for *E. coli*, 3–11 copies/CFU for *E. faecalis*, 0.3–43 copies/CFU for *S. aureus, and* 7–9 copies/CFU for *S. pneumonia* using different extract methods.

Our results were consistent with those of several studies using the dPCR method [19,22,30–33]. Hu [30] conducted a comparison of the detection of microorganisms and AMR genes using metagenomic next-generation sequencing (mNGS) and dPCR, as well as BC, in samples from septic patients suspected of having BSIs. dPCR has a quicker processing time and higher sensitivity compared with mNGS and BC. Ziegler [19,33] reported that dPCR can be applied to measure bacterial DNA in critically ill patients' blood with 16S ribosomal DNA or species-specific genes.

Prior research has demonstrated that molecular tests can detect positive cases in 10–40% of initially negative BCs, leading to potential improvements in patient outcomes through targeted antibiotic therapy [34]. Clinicians should assess if a positive dPCR result with a low bacterial concentration indicates a genuine bloodstream infection. Additional parameters such as blood biochemistry analysis, demographic and clinical aspects, and procalcitonin tests should be taken into account. Possible explanations for BC-negative and dPCR-positive results could be nonviable or nonproliferating bacteria, intracellular organisms in circulating phagocytic cells, antibiotic-induced suppression of bacterial growth, or contamination [35]. Our research found that 26.08% (18/69) of BC-negative and dPCR-positive cases could be false positives due to the low concentration, less than 100 copies/mL in the blood.

The dPCR in this work exhibited low sensitivity and specificity, possibly attributed to the small sample amount of 1 mL utilized. To enhance sensitivity and specificity, the initial approach is to augment the template loading volume, which may lead to improved detection rates in dPCR. Factors influencing dPCR sensitivity include the volume of template added and the volume of template examined. The Rainsure dPCR system can analyze 15 μL, leaving a non-analyzed volume of 35% that is difficult to minimize. We intended to raise the blood volume for DNA extraction from 1 mL to 2 mL and increase the DNA template amount used in 20 μL of reaction solution from 8 μL to 12 μL to enhance the kit's sensitivity. The second approach involves a molecular assay like mNGS, which should be utilized to assess conflicting outcomes resulting from using BC as a reference with limited sensitivity.

There are several limitations of dPCR compared to qPCR. First, DPCR is less versatile than qPCR in terms of equipment and reagents. It is difficult to apply one dPCR kit to different dPCR devices because of the different methods of reaction buffer partition. Second, the economic cost of dPCR is still higher than BC and qPCR. As dPCR technology advances and becomes more commonly used for diagnosing suspected sepsis, the cost of the test will decrease. Third, because dPCR has an additional buffer partitioning step compared to qPCR, it requires a higher level of skill in operation. However, with the commercialized all-in-one machines, the operation will become more and more simple, and this will no longer be a problem.

Our study has several limitations: i) Due to the inconsistent results of dPCR and BC, there are not enough blood samples to be verified with the NGS method. ii) For the samples with positive dPCR results but a concentration lower than 100 copies/mL, repeat testing was not conducted due to the limited DNA amount. iii) Since this was a retrospective analysis, active antimicrobial stewardship

interventions were not involved.

The results of digital PCR can help physicians give more precise antibiotic treatments rather than just treating patients based on empirical antimicrobial therapy. Of the 19 patients who tested positive for both digital PCR and blood cultures, 1–9 antibiotics were given. It may not have been necessary to give the patients so many antibiotics if the results of digital PCR had been available as a reference. For example, in patient #72, blood cultures detected only *Escherichia coli* infection, and it took nine antibiotics in the actual antibiotic treatment in 4 days in the ICU to make the condition better (cefoperazone and sulbactam, Imipenem, Teicoplanin, Laxocephalosporin, Ceftazidime, Fluconazole, Voriconazole, Carbofenozin, and Tigecycline). DPCR detected *Escherichia coli*, *Enterococcus faecalis*, and *Stenotrophomonas maltophili* infections. Blood cultures of patient #7 detected only Acinetobacter baumannii infection, and the patient's status did not improve after treatment with meropenem and imipenem cilastatin on the first day of ICU, after which the physician gave ganciclovir, voriconazole, azithromycin, sulbactam sodium, and tigecycline for the next 3 days. DPCR results of this patient were Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae infection. If the dPCR result is available to the physician, it can help the physician treat patients with precise antibiotics.

5. Conclusion

Sepsis is a time-dependent illness, and early pathogen detection might help clinicians treat patients with the right antibiotics and possibly improve their prognosis. This study illustrated that dPCR can provide rapid and quantificational information on 15 pathogens within 5 h, thereby significantly reducing turnaround time and improving diagnostic sensitivity. However, there is still a need for the dPCR kit to be refined, namely for higher sensitivity and specificity. Optimization of nucleic acid auto-extraction procedures for shorter turnaround times, increasing the sample volume, decreasing the elution volume, and selection of kits with higher extraction efficiency are highly desirable. In ICU settings, dPCR is a helpful tool that can be used as a supplemental test to the traditional BC approach. It can aid with appropriate medication and may even improve patient outcomes while treating sepsis. To evaluate the effectiveness, additional value, and viability of dPCR in ICU practice, large multicenter trials will be necessary.

Ethics approval

The study was approved by the General Hospital Ethics Committee of Ningxia Medical University. (Documentation No 2020-690).

Data availability statement

Data will be made available on request.

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CRediT authorship contribution statement

Zhijun Zhao: Writing – original draft, Methodology, Formal analysis. **Yixuan Wang:** Writing – original draft, Methodology, Investigation. **Yuting Kang:** Investigation, Data curation. **Geng Wu:** Data curation. **Jing He:** Writing – review & editing, Writing – original draft, Funding acquisition. **Zhanying Wang:** Methodology. **Ju Yang:** Formal analysis, Data curation. **Yaqi Wang:** Writing – review & editing, Supervision, Resources. **Xiaojun Yang:** Investigation. **Wei Jia:** Writing – review & editing, Supervision, Investigation, Funding acquisition.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used [deepl/https://www.deepl.com/write] and [quillbot] (https://quillbot.com/] in order to check the plagiarism and use proper verbiage and terminology throughout the manuscript. After using these tools/services, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27523.

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