Clinical Diagnostic Performance of Digital PCR for Pathogen Detection in Patients with *Klebsiella pneumoniae* Bloodstream Infections

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Introduction

Rapid diagnosis and early administration of appropriate antimicrobials are crucial to improve the prognosis and decrease the mortality of patients with bacterial bloodstream infections (BSIs). In recent years, digital PCR (dPCR) has emerged as a new molecular method for the diagnosis of BSIs [1,2]. This study aimed to examine the sensitivity and specificity of dPCR and the association between bacterial DNA load in whole blood and the time-to-positivity (TTP) of blood cultures (BCs) in patients with *Klebsiella pneumoniae* (KP) BSIs.

Material and methods

Study design

This study was conducted at Hiroshima University Hospital from June 2023 to June 2024. The inclusion criteria for patient enrollment were as follows: (1) ≥ 18 years of age, (2) patients in whom two sets of BC were obtained simultaneously, along with whole blood samples, and (3) BC positive for KP. The exclusion criteria were as follows: (1) patients who were treated with antimicrobial agents 1 week within collection of BCs, (2) presence of polymicrobial bacteremia, (3) patients whose residual volume of whole blood sample was less than 400 µL, and (4) patients who died on the same day of BC sampling.

Whole blood samples from 50 patients with BC positive for pathogens other than KP (n=25) as well as those with BC negative (n=25) were evaluated using dPCR with *K. pneumoniae* specific primer and probe as negative control. Two sets of BC specimens were drawn from each patient and BCs were performed with BacT/ALERT Virtuo System (bioMérieux). Only the TTP of the first positive BC bottle was used for analysis. Whole blood samples which were simultaneously collected with BCs, were used for dPCR detection. DNA was extracted from 400 µL of whole blood sample using the automatic extraction system magLEAD with magLEAD Dx SV reagent (Precision System Science Co.). The final eluate (50 µL) was stored at –80 ° C until further use. All clinical data were collected from the medical record.

dPCR

The primers and probes designed to target the KP-specific *khe* and *CTX-M* genes were used [3,4]. The dPCR reactions were loaded onto the QIAcuity Nanoplate 26K 24-well and carried out in a QIAcuity ONE 2-Plex system (Qiagen, Germany), with following thermal cycling 95° C for 2 min, 40 cycles at 95° C for 15 s and 57° C for 30 min, and a final cycle at 98° C for 10 min (Figure 1). The number of target DNA molecules in the whole blood samples was calculated as copies/mL.

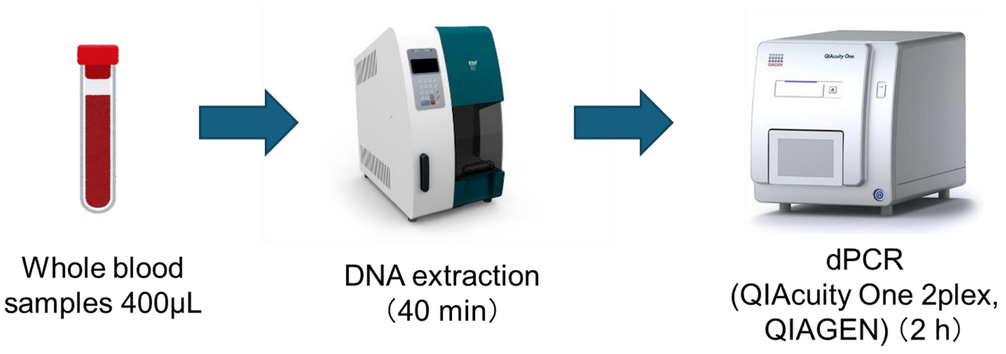

Figure 1. DNA extraction and digital PCR

Results and Discussion

Of the 72 patients with KP BSIs, dPCR detected KP DNA in 63 (87.5%). The results of dPCR for KP had a sensitivity of 87.5% (95%CI 77.9-93.3), specificity of 100% (95%CI 92.8-100) (table 1). Patients with positive dPCR results had significantly shorter TTP than those with negative results (median, 9.6 h vs. 10.4 h, p=0.046) (table 2). The positivity rate for both BC sets was significantly higher in patients with positive dPCR results than in those with negative results (92.1% vs. 55.6%, p=0.011). Among dPCR-positive patients, patients with septic shock (n=11) had higher KP DNA load in whole blood (median, 98,932 copies/mL vs. 983 copies/mL, p < 0.001) and shorter TTP (median, 7.1 h vs. 9.8 h, p=0.004) compared with patients without septic shock (table 3). Among dPCR-positive patients, all patients with ESBL-positive KP BSIs were positive for CTX-M gene. The KP DNA load in whole blood inversely correlated with TTP (p=0.023, figure 2).

Table 1. The results of blood culture and dPCR for *khe* gene

	Blood culture for *K. pneumoniae*		
	Positive	Negative	Total
dPCR for *khe* gene			
Positive	63	0	63
Negative	9	50	59
Total	72	50	122

Table 2. Clinical characteristics and detailed information of the blood culture results of patients who had positive or negative dPCR results for *khe* gene

	Patients with positive dPCR results (n=63)	Patients with negative dPCR results (n=9)	P value
Age (years), median (IQR)	77 (66-81)	65 (49-71)	0.009
Male, n (%)	37 (58.7)	4 (44.4)	0.49
Primary site of bacteremia			
Intra-abdominal infection, n (%)	42 (66.7)	3 (33.3)	0.071
Bile duct infection, n (%)	35 (55.6)	1 (11.1)	0.028
Intestinal perforation, n (%)	1 (1.6)	1 (11.1)	0.24
Urinary tract infection, n (%)	11 (17.5)	5 (55.6)	0.022
Others, n (%)	10 (15.8)	1 (11.1)	1.0
BSI caused by ESBL-producing *K. pneumoniae*, n (%)	9 (14.3)	2 (22.2)	0.62
Septic shock, n (%)	11 (17.5)	0(0)	0.34
Blood culture			
TTP (h), median (IQR)	**9.6 (8.8-10.7)**	**10.4 (9.4-13.1)**	**0.046**
Positive for two sets of blood culture, n (%)	**58 (92.1)**	**5 (55.6)**	**0.011**
No. of positive bottle of two sets of blood culture			
4 bottles, n (%)	44 (69.8)	3 (33.3)	0.057
3 bottles, n (%)	8 (12.7)	1 (11.1)	1.0
2 bottles, n (%)	9 (14.3)	2 (22.2)	0.62
1 bottle, n (%)	2 (3.2)	3 (33.3)	0.012

ESBL, extended-spectrum β-lactamase; IQR, interquartile range; TTP, Time-to-positivity

Table 3. Clinical characteristics and detailed information of the blood culture results of dPCR-positive patients with and without septic shock

	Patients with septic shock (n=11)	Patients without septic shock (n=52)	P value
***K. pneumoniae* DNA load in whole blood (copies/mL), median (IQR)**	**98,932 (10852-1674450)**	**983 (196-3906)**	**<0.001**
Blood culture			
TTP (h), median (IQR)	7.1 (6.3-10.7)	9.8 (9.1-10.7)	0.004
Positive for 4 bottles of blood culture, n (%)	11(100)	33 (63.5)	0.025

IQR, interquartile range; TTP, Time-to-positivity

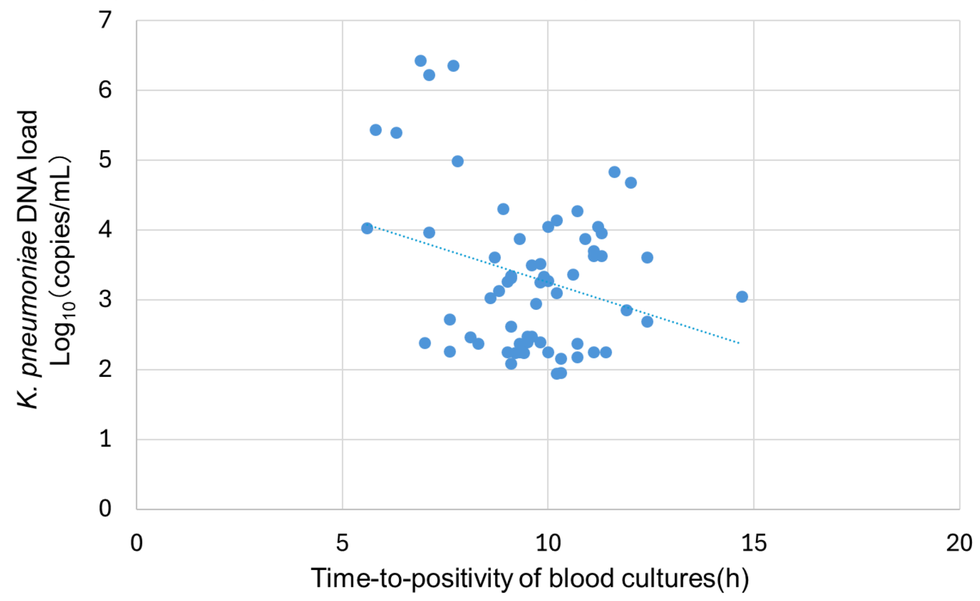

Figure 2. Correlation between *khe* DNA load in whole blood and time-to-positivity of blood cultures.

Conclusion

This study showed that 87.5% of whole blood samples from patients with *K. pneumoniae* BSI diagnosed via BC were dPCR-positive. Patients with septic shock had significantly higher levels of *K. pneumoniae* DNA in the whole blood than those without septic shock. Further studies are needed to evaluate the accuracy of dPCR for the diagnosis of patients with BSI caused by other organisms and the association of pathogen DNA load with disease severity and mortality.

1. Clin Microbiol Infect. 2022;28:195-201.
2. Kitagawa et al. BMC Infect Dis. 2025;25:22. (QR code)
3. Microbiol Spectr. 2023;11:e0041523.
4. Water Environ Res. 2024;96:e11145.