Case Report: Recovery of Pathogenic *Leptospira* spp. from Routine Aerobic Blood Culture Bottles

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Abstract. *Leptospira* spp. are fastidious and slow-growing bacteria, making recovery difficult and diagnostic sensitivity in the clinical setting low. However, collection of *Leptospira* isolates is valuable for epidemiological and laboratory research. Severe leptospirosis cases may present as septic shock, and the differential diagnosis often includes bacterial septicemia, leading clinicians to collect blood cultures. Here, we report the successful isolation of pathogenic *Leptospira* spp. from blood culture bottles (targeting aerobic bacteria incubated at 37°C) from a 64-year-old man admitted with septic shock. The patient presented with 4 days of fever, severe hypotension, transient atrial fibrillation, jaundice, and oliguric renal failure. After admission, intravenous ceftriaxone plus azithromycin was given with fluid resuscitation, norepinephrine infusion, invasive mechanical ventilation, and renal replacement therapy. He was discharged from the hospital 16 days after admission. Using the blood sample obtained on admission, the diagnosis of leptospirosis was confirmed by multiplex real-time PCR (targeting bacterial 16S rRNA and *LipL32* gene). We collected 200 µL from the blood culture bottle to inoculate a 5-mL Ellinghausen, McCullough, Johnson, and Harris media supplemented with 5% fetal bovine serum. After 2 weeks of incubation at 30°C, *Leptospira* strains were identified and confirmed by real-time PCR. Genotyping was undertaken using the multi-locus sequence typing (MLST) scheme#1. The isolate matched with ST50 isolates in the PUBMLST database. This case provides evidence that in tropical countries, severe leptospirosis should be considered in patients who present with symptoms of sepsis. Pathogenic *Leptospira* may be successfully isolated from aerobic blood cultures in routine clinical settings.

INTRODUCTION

Leptospirosis is a bacterial zoonosis of global importance caused by spirochetes of the genus *Leptospira*. Leptospirosis has recently been recognized as a common cause of acute undifferentiated fever in tropical countries, including Thailand.^{1,2} The transmission occurs mostly after indirect contact with bacteria shed in the urine of reservoir animals, mainly rodents. Clinical manifestations of human leptospirosis vary from flu-like febrile illnesses to rapidly fatal infection. Many severe leptospirosis cases present as septic shock, and the differential diagnosis often includes bacterial septicemia, leading clinicians to perform blood cultures.² *Leptospira* isolation is of limited use for diagnosis and treatment of human leptospirosis because they are fastidious and slow-growing bacteria, resulting in low sensitivity. However, collection of *Leptospira* isolates remains crucial to better understand the epidemiology of this complex zoonosis and to provide researchers with different isolates. We report a case of severe leptospirosis confirmed by real-time PCR using an acute blood sample and by the successful isolation of pathogenic *Leptospira* from the routine aerobic blood culture bottles.

CASE REPORT

A previously healthy 64-year-old Thai man, who had been living in Bangkok, Thailand, for the preceding 20 years, was admitted to the intensive care unit at our hospital in September

2018 for septic shock. He developed fever for 4 days and watery diarrhea on the second day. He reported that he felt dizzy with weakness of lower limbs and had knocked his head in the bathroom the day before admission, resulting in a superficial 1-cm laceration on his forehead. Vital signs in the emergency department were notable for a temperature of 36.5°C, a pulse of 133 beats/minute, a respiratory rate of 24 breaths/minute, a blood pressure of 62/52 mmHg, and an O₂ sat of 99% at room air. He was awake, alert, and oriented to person and place. His skin and sclerae were icteric, but no conjunctival injection or hemorrhage was observed. There was a healing, 1-cm laceration at the base of the left big toe. Scattered rales were heard throughout the lungs. There were no neurological deficits, except for generalized muscle power grade IV/V for both upper and lower extremities. There was no calf or thigh-muscle tenderness.

Initial laboratory studies were remarkable for a total white blood cell count of 13,200 cells/µL (93% neutrophils and 8% bands), a hemoglobin of 9.2 g/dL, and a total platelet count of 27×10^9 cells/L. Plasma creatinine was 2.99 mg/dL (peak 3.21 mg/dL), and venous bicarbonate was 16 mEq/L. Aspartate transaminase was 95 U/L, alanine transaminase 25 U/L, total bilirubin 3.76 mg/dL (peak 10.69), direct bilirubin 3.46 mg/dL, and creatinine phosphokinase 1,131 U/L. Urinalysis revealed significant proteinuria, 5–10/white blood cells per high power field (HPF), and 50–100 red blood cells/HPF. HIV antibodies were negative.

After admission, intravenous ceftriaxone and azithromycin were initiated with fluid resuscitation, and norepinephrine infusion was given to maintain his blood pressure to 100/58 mmHg. On the following day, a presumptive diagnosis of severe leptospirosis was made based on history and examination. His respiratory status declined and required endotracheal

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intubation within 6 hours of admission. Chest X-rays progressed from no definite infiltration to diffuse bilateral patchy opacities in that time frame. Hemodialysis was initiated to manage oliguric acute renal failure and volume overload. Jaundice, respiratory, and renal parameters gradually improved. There was no major bleeding complication. He was discharged in good clinical condition after 2 weeks of hospitalization. Routine bacterial cultures of blood, urine, sputum, and stool were unremarkable.

Multiplex real-time PCR assay using hydrolysis (TaqMan) probes targeting the genes encoding 16S rRNA and LipL32 of pathogenic *Leptospira* spp. was used to screen the patient's potassium ethylenediaminetetraacetic acid (EDTA) blood collected on admission. The results were positive for both primers (Figure 1). The quantification cycle (Cq) values were around 20, suggesting a high bacterial load in the patient sample. Two hundred microliters was collected from one of his aerobic blood culture bottles (BacT/Alert® SA for adults, BioMérieux, Marcy-l'Etoile, France) to inoculate a 5-mL Ellinghausen, McCullough, Johnson, and Harris (EMJH) media supplemented with 5% fetal bovine serum.³ In addition, pellets from another 200 µL blood centrifugation were inoculated in a second tube. Both tubes were incubated at 30°C. Spirochetes were identified under dark-field microscopy 2 weeks after inoculation. The isolates were confirmed as pathogenic *Leptospira* spp. by multiplex quantitative real-time PCR (Figure 2). Genotyping of this pathogenic isolate was undertaken using the multi-locus sequence typing

(MLST) scheme#1 developed for seven major pathogenic *Leptospira* spp.⁴ The seven MLST loci were amplified, and the amplicons were undertaken for Sanger sequencing. The quality of sequence chromatographs and concordant between forward and reverse reads were checked using free-ware (M. W. Davis, A plasmid Editor). All allele numbers and sequence types (STs) were assigned through the PubMLST webpage using BIGSdb.⁵ The isolate matched with *Leptospira interrogans* ST50 in the database.

DISCUSSION

Real-time PCR has been increasingly used for the early diagnosis of leptospirosis and other diseases in the clinical setting. However, *Leptospira* isolates provide biological material for serological and/or molecular identification for epidemiological study and laboratory research. For example, living *Leptospira* cells are used for the identification of serovars. It has been well documented that *Leptospira* spp. survive in the commercial blood culture system.^{6,7} A prospective study from New Caledonia has recently shown that isolation of *Leptospira* spp. from blood culture bottles (BacT/Alert SA for adults) was successful in about one-third (13 of 39) of patients with positive *Leptospira* real-time PCR. In this report, blood culture bottles were incubated at 37°C between 0.7 and 2.5 days before sampling for subculture into EMJH media.⁸ Moreover, a recent study has demonstrated that a fastidious strain of leptospires even grows in a new media

FIGURE 1. PCR amplification curve and result of patient's EDTA blood and positive control.

FIGURE 2. PCR amplification curve and result of a leptospire isolate that was obtained from a patient's hemoculture sample grown in Ellinghausen, McCullough, Johnson, and Harris (EMJH) medium.

formulation (HAN media) at 37°C.⁹ Evidence from these previous studies suggests that optimal conditions with certain nutrients can support the survival or growth of leptospires at body temperature. We successfully isolated the pathogenic *Leptospira* strains from aerobic blood culture bottles after 5 days without growth. Using MLST, we determined that the isolates belonged to *L. interrogans* ST50, which was not identified during the human leptospirosis outbreak in Thailand from 1998 to 2003.¹⁰ This ST50 was reported previously from a human case in Indonesia⁴ and commonly found in rodents in Malaysia.^{11,12} The ST5 isolates from both reports were *L. interrogans* serovar Bataviae.^{4,12} This case report provides evidence that in tropical countries, severe leptospirosis should be considered among the common causes of sepsis or septic shock and that isolation of pathogenic *Leptospira* spp. is possible in the routine clinical setting. Further studies are required to determine the rate or yield of successful isolation in rural hospitals where severe leptospirosis is common.

CONCLUSION

We report an adult patient presenting with septic shock from severe leptospirosis infection confirmed by real-time PCR assay on a blood sample taken at admission and by the successful isolation of pathogenic *Leptospira* strains from blood culture bottles. The isolate belonged to ST50 which had not been previously identified in Thailand. We conclude that

routine aerobic blood culture bottles can be a valuable source for the isolation of pathogenic *Leptospira* spp. from human leptospirosis cases.

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