GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

An experimental model for *Staphylococcus aureus* hepatic abscess in Bama minipig

R.G. Zhang*, X.D. Wang*, X.L. Zhang and Y.S. Yang

Department of Gastroenterology and Hepatology,
Chinese PLA General Hospital, Beijing, China

*These authors contributed equally to this study.
Corresponding author: Y.S. Yang
E-mail: rgdoccn@126.com

Genet. Mol. Res. 13 (3): 7113-7122 (2014)
Received June 7, 2013
Accepted October 3, 2013
Published February 21, 2014
DOI http://dx.doi.org/10.4238/2014.February.21.12

ABSTRACT. Pyogenic hepatic abscess (PHA) is a rare but potentially serious disease. Investigations of new therapeutic methods urgently need experimental support in corresponding animal models. However, to date, few studies have evaluated PHA in the minipig. The linear regression equation of the *Staphylococcus aureus* ATCC 25923 strain was established. PHA was successfully mocked, and *S. aureus* ATCC 29213 was the only pathogenic bacterium identified. The abscess formation stage was observed on the 21st day of the operation. This study will provide a baseline for further studies evaluating new treatment methods for PHA.

Key words: *Staphylococcus aureus*; Pyogenic hepatic abscess; Bama minipig

INTRODUCTION

Pyogenic hepatic abscess (PHA) is a rare but potentially life threatening condition. The annual incidence of PHA was estimated at 2.3 per 100,000 individuals in North America between 1999 and 2003 (Kaplan et al., 2004). The overall incidence of hospitalization was 3.6 per 100,000 individuals in the United States between 1994 and 2005 (Meddings et al., 2010) and was 32.2 per 100,000 individuals in Ireland between 1995 and 2007 (O'Farrell et al., 2010). The in-hospital case fatality rate was 9.1% in Taiwan between 1995 and 2007 (Chen et al., 2008). These figures indicate that a new treatment model for PHA should be investigated.

PHA animal models based on *Fusobacterium necrophorum* in male inbred A/J mice and male New Zealand white rabbits promote the development of a new therapeutic method (Hill et al., 1974; McDonald et al., 1984; Thompson et al., 1985; Weissleder et al., 1988). However, the applications are limited because of significant differences in anatomical structure and physiological function between these animals and humans.

Studies of new therapeutic methods urgently require corresponding animal models to provide experimental support (Alberti et al., 2002; Zatelli et al., 2005; Angelino et al., 2011). Although remarkable similarities exist between minipigs and humans (Thompson et al., 1985), few minipig models have been established to date.

Staphylococcus aureus was predominantly (8/10) isolated from the abscesses of disseminated lung lesions of slaughtered pigs (Liljegren et al., 2003). Acute pyemia was successfully induced in nine clinically healthy Yorkshire-Landrace-Duroc crossbreed pigs after 4 to 6 h by intravenous inoculation of *S. aureus* through the left ear vein. However, this method has not been applied for the development of a new therapeutic method owing to the presence of acute microabscesses of the liver in most animals analyzed (6/8) (Nielsen et al., 2009). This method provides a foundation to establish a PHA animal model in the minipig. The aim of this study was to establish a PHA animal model with *S. aureus* in the minipig.

MATERIAL AND METHODS

Relationship between optical density (OD) at 450 nm and viable bacterial counts

S. aureus ATCC 25923 was inoculated on a Luria-Bertani (LB) agar plate and incubated overnight at 37°C. A representative colony was suspended in 100-mL LB broth, and the suspension was incubated for 18 h at 37°C with agitation at 200 rpm. The suspension was diluted 8, 10, and 20 times, and the absorbance was monitored in a spectrophotometer (WFZ UV-2800AH; UNICO®) at a 450-nm wavelength in 10-mm diameter spectrophotometer tubes (Domínguez et al., 2001). The suspension with an OD between 0.9 and 1.0 was considered the original suspension and was further diluted 1.5, 2, 2.5, and 3 times. The corresponding OD_{450} was measured. The five suspensions were finally diluted 5 x 10^5 times, and 0.5-mL aliquots were inoculated on LB agar plates. Each dilution was inoculated on five LB agar plates. The colonies were counted by the naked eye after incubation overnight. The OD_{450} culture, fold-dilution, and corresponding colony counts were all recorded. The relationship of OD_{450} in the bacterial suspension to the viable bacterial count of *S. aureus* on the LB agar plate was

obtained by statistical analysis. The bacteria were quantified to 5 x 10^8 colony forming units/mL before use.

Establishment of PHA

Thirty clinically healthy Bama minipigs (numbered 1 to 30), weighing 21 to 25 kg (20.9 ± 3.0 kg) and aged 9 to 10 weeks, were purchased from Guangxi province, China. The pigs were allowed to acclimatize for 5 to 10 days before being subjected to the experiment. The pigs were randomly coded and food was withdrawn 12 h before the experiment.

The minipigs were sedated by intramuscular injection of a solution containing a mixture of 0.8 mg/kg body weight each zolazepam, tiletamine, xylazine, and ketamine and 0.17 mg/kg body weight butorphanol. A 22-G catheter was then inserted in the right ear vein to infuse the anesthetic, which consisted of a solution containing a mixture of 1 mg/mL xylazine, 2 mg/mL ketamine, 0.1 mg/mL butorphanol, and 48 mg/mL guaifenesine.

After exposing the abdominal cavity, 30 mL venous blood was obtained from the splenic vein and infused into 50-mL aseptic centrifuge tubes in 14- and 15-mL aliquots. Blood was thoroughly coagulated before the clot smoothly passed through a 16-G pinhead by shearing with scissors. One milliliter of *S. aureus* suspension and the 14 mL sheared clot were mixed and injected into the liver parenchyma (left lateral lobe) for the experiment. Simultaneously, the 15-mL clot was injected into the liver parenchyma (right lateral lobe) as a negative control. Bleeding was stopped by compression with an absorbable gelatin sponge. The minipigs were sent to a rearing room after the abdominal wall was sutured by using a three-tiered stitch. This study was conducted in accordance with the EU directive 86/609 and the Danish Animal Experimentation Act. The animal use protocol employed in this study was reviewed and approved by the Institutional Animal Care and Use Committee of the Chinese PLA General Hospital, China.

Post-mortem examination and abscess identification

After euthanasia, the longitudinal sections of the liver, spleen, kidneys, lungs, and heart were examined at 1-week intervals (Table 1). Liver abscess samples were spread on glass slides and subjected to Gram staining after drying, and were then inoculated into LB broth and incubated overnight. The 0.1-mL bacterial suspension was inoculated on an LB agar plate and incubated overnight.

The bacterial precipitate was generated by the centrifugation of 3-mL bacterial suspension in aseptic vials. The precipitates were stored at -80°C until chromatin DNA was extracted with a Bacterial DNA Kit (OMEGA) and lysostaphin. The primer pairs of the thermostable nuclease A gene (*nucA*) were 5'-GCG ATT GAT GGT GAT ACG GTT-3' and 5'-AGC CAA GCC TTG ACG AAC TAA AGC-3'. The cycling conditions were as follows: one cycle at 94°C for 45 s; 30 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 60 s; 72°C for 120 s; and 4°C. The desired fragment length was 279 bp after polymerase chain reaction (PCR) (Brakstad et al., 1992). PCR was performed in a 50-µL volume. The reaction was performed through the model 7000 sequence detection system (Applied Biosystems, Courtaboeuf, France). *S. aureus* strains ATCC 25923 and ATCC 29213 served as positive controls, and deionized water served as the negative control. All samples were analyzed in triplicate.

The liver abscess samples were fixed for 24 h with 4% formaldehyde in phosphate-buffered saline, and then processed through graded concentrations of ethanol and xylene, embedded in paraffin wax, cut into 3- to 5-μm sections, rehydrated, and finally stained with hematoxylin and eosin (HE).

Table 1. Related parameter in establishment of pyogenic hepatic abscess animal model.

Pig No.	*S. aureus*	Sex	BW (kg)	Injured or used location	Injured character, *S. aureus* counts, affix and animal post-mortem conditions	Abscess (in size)
1	ATCC 25923	M	22	Right inner lobe	CWF + bacteria 0.0002 mL (31 days)	No
2	ATCC 25923	M	24	Right inner lobe, right lateral lobe	CWF + bacteria 0.002 mL (31 days)	No
3	ATCC 25923	M	23	Right inner lobe, right lateral lobe	CWF + bacteria 0.02 mL (32 days)	No
4	ATCC 25923	M	21	Right inner lobe, left inner lobe	CWF + LB 1 mL (32 days)	No
5	ATCC 25923	M	22	Right inner lobe, left inner lobe	CWOF + bacteria 0.01 mL (7 days)	No
6	ATCC 25923	M	21	Right inner lobe, left inner lobe	CWOF + bacteria 0.002 mL (7 days)	No
7	ATCC 25923	F	21	Right inner lobe, left inner lobe	CWOF + bacteria 0.02 mL (7 days)	No
8	ATCC 25923	M	20	Right inner lobe, left inner lobe	CWOF + bacteria 0.2 mL (7 days)	No
9	ATCC 29213	M	22	Left lateral lobe	CWOF + bacteria 1 mL + VB 1 mL (15 days)	10*5mm (50 mm^2)
				Right lateral lobe	CWOF + bacteria 1 mL (15 days)	No
10	ATCC 29213	M	20	Right lateral lobe, left lateral lobe	CWOF + bacteria 2 mL (15 days)	No
11	ATCC 29213	F	20	Right inner lobe	CWOF + bacteria 2 mL + VB 2 mL (24 days)	4 x 4 mm (16 mm^2)
12	ATCC 29213	M	19	Right inner lobe	CWOF + bacteria 4 mL + VB 4 mL (24 days)	4 x 4 mm (16 mm^2)
13	ATCC 29213	F	20	Right inner lobe	CWOF + bacteria 6 mL+ VB 6 mL (cachexia, 24 days)	5 x 3 mm (15 mm^2)
14	ATCC 29213	M	21	Right inner lobe	CWOF + bacteria 8 mL + VB 8 mL (cachexia, 24 days)	7 x 3 mm (21 mm^2)
15	ATCC 29213	M	22	Right inner lobe (control), left inner lobe	Contused wound by fingers + bacteria 1 mL + HA 0.5 mL (hemorrhagic pneumonia, 20 days death)	18 x 11mm (198 mm^2)
16	ATCC 29213	M	16.5	Right inner lobe (control), left inner lobe	CWF + bacteria 1 mL + HS 0.5 mL (29 days)	10 x 3 mm (30 mm^2)
17	ATCC 29213	M	19	Right inner lobe	CWF + bacteria 1 mL + VB 6 mL + HS 1 mL (33 days)	4 x 4 mm (16 mm^2)
18	ATCC 29213	M	16	Right inner lobe	CWF + bacteria 1 mL + VB 6 mL + HS 1 mL (33 days)	13 x 10 mm (130 mm^2)
19	ATCC 29213	F	19	Right inner lobe	CWF + VB 8 mL + bacteria 1 mL + HA 1 mL (LA, pyothorax, TVCI, 9 days death)	12 x 8 mm (96 mm^2)
20	ATCC 29213	M	18	Left lateral lobe	contused wound by fingers + SL 8 mL + bacteria 1 mL (LA, endocarditis, TVCI, 13 days death)	31 x 23 mm (713 mm^2)
21	ATCC 29213	F	21	Left lateral lobe	CWF + SL 9 mL + bacteria 0.2 mL (anesthetic accident)	25 x 12 mm (300 mm^2)
22	ATCC 29213	F	19	Left lateral lobe	SL 13 mL + bacteria 0.4 mL (30 days)	34 x 22 mm (748 mm^2)
23	ATCC 25923	F	18	Right lateral lobe (control), left lateral lobe	SVBC 14 mL + bacteria 1 mL (33 days)	No
24	ATCC 29213	F	17	Right lateral lobe (control), left lateral lobe	SVBC 14 mL + bacteria 1 mL (LA, 33 days)	27 x 22 mm (594 mm^2)

Continued on next page

Table 1. Continued.

Pig	*S. aureus*	Sex	BW (kg)	Injured or used location	Injured character, *S. aureus* counts, affix and animal post mortem conditions	Abscess (in size)
				Left lateral lobe (post mortem)	SVBC 15 mL (jet injection)	25 x 10 mm (250 mm^2)
25	ATCC 29213	M	19	Right lateral lobe (control), left lateral lobe	SVBC 14 mL + bacteria 1 mL (15 days)	27 x 15 mm (405 mm^2)
26	ATCC 29213	M	22.5	Right lateral lobe (control), left lateral lobe	SVBC 14 mL + bacteria 1 mL (21 days)	No
27	ATCC 29213	M	27	Right lateral lobe (control), left lateral lobe	SVBC 14 mL + bacteria 1 mL (LA, 7 days)	35 x 20 mm (700 mm^2)
28	ATCC 29213	M	27	Right lateral lobe (control), left lateral lobe	SVBC 14 mL + bacteria 1 mL (14 days)	32 x 16 mm (512 mm^2)
29	ATCC 29213	F	29	Right lateral lobe (control), left lateral lobe	SVBC 14 mL + bacteria 1 mL (LA, 21 days)	32 x 20 mm (640 mm^2)
30		M	21	Right lateral lobe (post mortem)	Physiological saline solution 15 mL (jet injection)	32 x 5 mm (160 mm^2)

The contused wound by figure (CWF) means that the hepatic contused wound was caused by kneading liver parenchyma between thumb and middle finger, and the corresponding area contused wound is about 25 x 25 mm (625 mm^2). The contused wound by oval forceps means (CWOF) that the hepatic contused wound was caused by clamping liver parenchyma between two circellus of oval forceps and keeping on 5 min, and the corresponding area contused wound is about 19 x 19 mm (361 mm^2). The venous blood (VB) is from splenic vein and the self lipochondria (SL) is from self preperitoneal fat. Hemocoagulase atrox for injection (HA) is 1 U per 2 mL and heparin sodium (HS) injection is 1250 U per 2 mL. Days mean the day of anatomy after the operation and the 'death' means the day of natural death after the operation. The bacterium was quantified to 5 x 10^8 colony forming units/mL before utilization. BW = body weight; M = male; F = female; SVBC = self venous blood clot; LA = lung abscess; TVCI = thrombosis of vena cava inferior.

Statistical analysis

The SPSS 11.5 statistical software was used to analyze the data. Data are reported as means ± standard deviation (SD). The relationship between the OD_{450} in the bacterial suspension with the viable bacterial count on the LB agar plate was analyzed through linear regression analysis. The comparison of the size of PHA was analyzed through one-way analysis of variance (ANOVA) multiple comparisons. $P < 0.05$ was considered to be statistically significant.

RESULTS

Relationship between OD_{450} and viable bacterial count of *S. aureus*

The OD_{450} (X) in the bacterial suspension was significantly related to the viable bacterial count of *S. aureus* ATCC 25923 (Y x 10^8/mL) on the LB agar plate, which could be expressed by the linear regression equation: $Y = 1.15392X + 0.00768$ ($R^2 = 0.9763$; $P = 0.000$).

PHA establishment

First, PHA was investigated with *S. aureus* ATCC 25923. PHA could not be induced by injecting the bacteria into the superior mesenteric vein after the contused liver wound (Nos. 1, 2, 3, 6, 7, and 8) or by injecting bacteria to the area of the contused liver wound (No. 5).

Furthermore, PHA could not be induced by injecting the mixture of the bacteria and the self-venous blood clot into the liver parenchyma (No. 23).

Second, PHA was investigated with *S. aureus* ATCC 29213. PHA could not be induced by injecting bacteria into the superior mesenteric vein after the contused liver wound (No. 10) or by injecting the bacteria into the area of the contused liver wound (No. 9). However, PHA could be successfully induced by injecting the mixture of the bacteria and self-fresh venous blood into the area of the contused liver wound (Nos. 9, 11, 12, 13, and 14), although the abscess was small in size (23.6 ± 14.9 mm^2). The size of the abscess was not significantly changed after adding heparin sodium (Nos. 16, 17, and 18) or hemocoagulase atrox (Nos. 15 and 19) (58.7 ± 62.2 and 141 ± 72.1 mm^2, respectively) (F = 20.6, P = 0.698; F = 20.6, P = 0.245) into the mixture. The size was significantly increased after the mixture of the bacteria and self-lipochondria was injected (Nos. 20, 21, and 22) (587 ± 249.2 mm^2) (F = 20.6, P = 0.000). Percutaneous transhepatic echo-guided intervention was not feasible because the lipochondria was not thoroughly liquefied and the pus was too dry.

Finally, the PHA animal model was investigated by injecting a mixture of *S. aureus* ATCC 29213 and self-venous blood clot into the liver parenchyma (Nos. 24, 25, 26, 27, 28, and 29) (Figures 1 and 2). Typical PHA was found in 5 of the 6 minipigs (Nos. 24, 25, 27, 28, and 29) in the left hepatic lateral lobe but not in the right hepatic lateral lobe. The mean size of the abscesses was 570.2 ± 115.1 mm^2 (F = 20.6, P = 0.000). The abscess cavity contained pus and necrotic liver tissue. The outer layers of the necrotic liver tissue were covered with gray-yellow pus, and the stratum internum was composed of bolarious amorphous material.

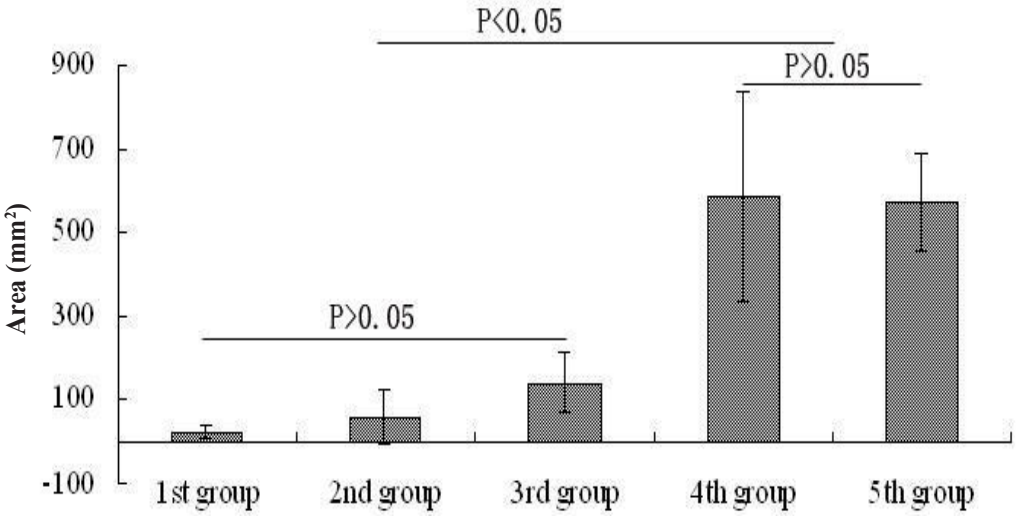

Figure 1. Comparison of pyogenic hepatic abscess (PHA) in size. PHA was induced in liver parenchyma by injection of the mixture. The mixture of *Staphylococcus aureus* ATCC 29213 and fresh venous blood was named as the 1st group. The mixture of ATCC 29213, heparin sodium and fresh venous blood was named as the 2nd group. The mixture of ATCC 29213, hemocoagulase atrox and fresh venous blood was named as the 3rd group. The mixture of ATCC 29213 and self-lipochondria was named as the 4th group. The mixture of ATCC 29213 and self-venous blood clot was named as the 5th group.

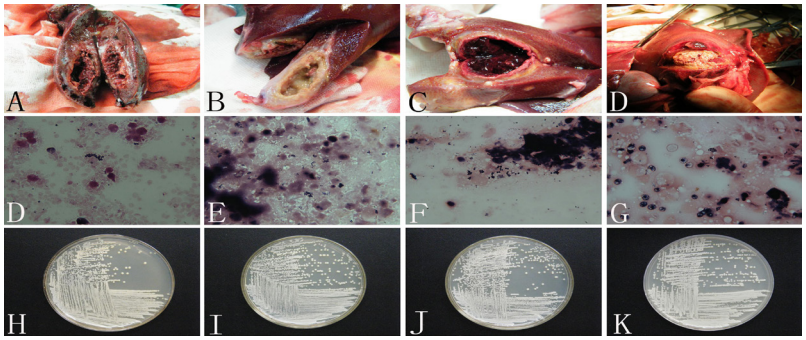

Figure 2. Longitudinal section, Gram stain and culture of pyogenic hepatic abscess (PHA). Typical PHA was found after post-mortem examination. Size: **A.** 35 x 20 mm (No. 27 pig, 7 days after post mortem); **B.** 32 x 16 mm (No. 28 pig, 14 days after post mortem); **C.** 32 x 20 mm (No. 29 pig, 21 days after post mortem); **D.** 27 x 22 mm (No. 24 pig, 33 days after post mortem). Numerous Gram-positive thyrsiform cocci were found (1000X). **E.** Pig 27; **F.** pig 28; **G.** pig 29; **H.** pig 24. Typical colonies of *Staphylococcus aureus* ATCC 29213 found after the bacterial suspension were inoculated on LB agar plate and incubated overnight. **H.** Pig 27; **I.** pig 28; 3; **J.** pig 29; **K.** pig 24.

PHA was not found in minipig No. 26. Multiple metastatic lung abscesses were found in 3 of the 5 minipigs (Nos. 24, 27, and 29); however, they were not found in the heart valves, spleens, or kidneys of the minipigs (Nos. 24, 25, 26, 27, 28, and 29).

The lacerated wound was 300 mm^2 after the mixture of 0.2 mL bacteria and 9 mL self-lipochondria was injected in the hepatic parenchyma (No. 21). The wound was 250 mm^2 after jet injection of 15 mL self-venous blood clot (No. 24), and was 160 mm^2 after jet injection of 15 mL physiological saline (No. 30).

PHA pathogenic identification

Many Gram-positive thyrsiform cocci were found after the liver abscess samples were smeared on glass slides (Figure 2). The suspension appeared gray-yellow in color after inoculated into the LB broth and incubated overnight with agitation. Typical *S. aureus* colonies were found after the bacterial suspension was inoculated onto LB agar plates and incubated overnight (Figure 2). The desired fragment was found after PCR amplification (Figure 3), which confirmed that the pathogenic bacterium of the PHA was *S. aureus* ATCC 29213.

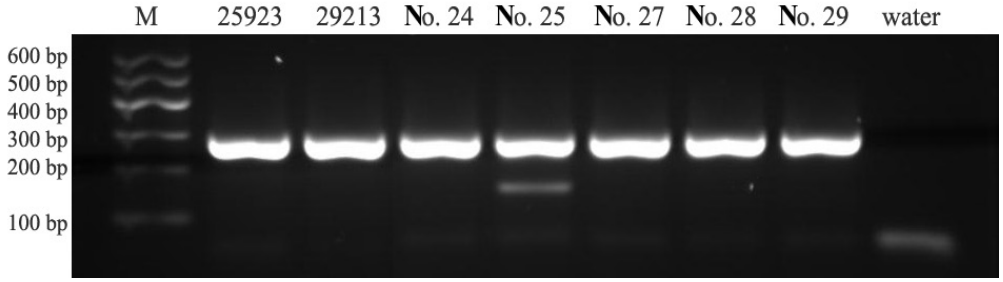

Figure 3. PCR identification of the pathogenic bacteria of pyogenic hepatic abscess (PHA). The pathogenic bacteria of PHA were identified by PCR with *nucA* oligonucleotide primers. From left to right, there were the standard molecular marker (M), *Staphylococcus aureus* ATCC 25923, ATCC 29213, PHA of No. 24 pig, PHA of No. 25 pig, PHA of No. 27 pig, PHA of No. 28 pig, PHA of No. 29 pig, and deionized water, respectively.

PHA pathological identification

The stained paraffin sections showed the evolution of the abscesses over the course of 33 days (Figure 4). An abscess containing pus and an incomplete wall was observed 7, 14, and 15 days later. An abscess containing pus and a complete wall was observed 21 days later. Early fibrosis developed within the abscess and around the liver parenchyma 33 days later. Abscess prophase (inflammation stage) was found 7, 14, and 15 days later, the abscess formation stage was observed 21 days later, and the abscess recovery phase was observed 33 days later.

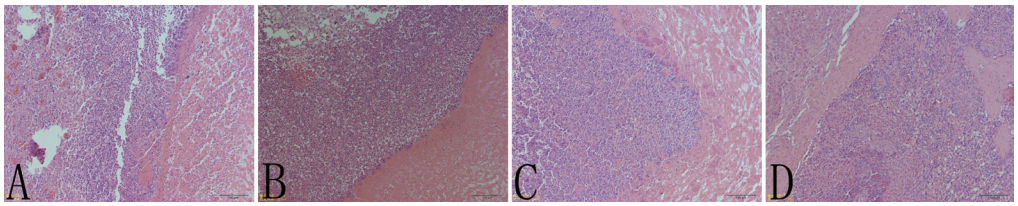

Figure 4. Pathological findings of PHA animal model. Paraffin section stain showed a cavity containing pus and necrotic liver tissue (100X). The capsule of PHA became clearer as the animal grew. **A.** Pig No. 27; **B.** pig No. 28; **C.** pig No. 29; **D.** pig No. 24.

DISCUSSION

Spectrophotometry is commonly employed to determine the concentration of bacterial suspensions, and plate count is considered a traditional way of measuring viable bacterial counts. The wavelengths of 450, 540, and 600 nm are usually employed to measure the OD of bacterial concentrations (Pienaar et al., 1994; Domínguez et al., 2001; O'Mahony and Papkovsky, 2006; Shin et al., 2007). Results will be more reliable if the wavelength of the incidental light is shorter. Thus, OD_{450} was employed to measure the bacterial concentration in this study.

Although hepatic hematoma was found after the contused wound in all cases, the blood and injected bacteria (*S. aureus* ATCC 29213) could be quickly absorbed into the blood circulation and further cleared by the animal's immune system, which might explain why PHA could not be induced by injecting bacteria into the superior mesenteric vein after the contused wound or into the area of the contused wound (Nielsen et al., 2009).

The addition of fresh venous blood could be one of the reasons for the successful delay of bacterial seepage into the blood circulation. Heparin inhibits blood coagulation, whereas hemocoagulase atrox promotes it; however, their addition could not significantly change the size of the abscess, which could reflect their low biological effects. The addition of self-lipochondria produced a larger abscess, but is not a feasible method of interventional therapy because the injected lipochondria was not thoroughly liquefied and became too dry.

PHA could be stably induced after injecting the mixture of *S. aureus* ATCC 29213 and self-venous blood clot into the liver parenchyma. The abscess was large and liquefied in nature, which was sufficient for interventional therapy.

Metastatic lung abscesses were found in high frequency (3/5) although no abscesses were found in the heart valves, spleens, or kidneys. This could be related to the large loading dose in the lungs during the establishment of PHA (Nielsen et al., 2009). PHA was not found in minipig No. 26, which is likely due to the fact that the bacterial suspension was not mixed before usage owing to carelessness.

Therefore, the thorough mixing of the bacterial suspension, sufficient clotting of the self-venous blood, and jet injection of the mixture of *S. aureus* ATCC 29213 and the self-venous blood clot could all play important roles in establishing PHA models.

The pathogenic bacterium of PHA was *S. aureus* ATCC 29213, which was confirmed by Gram staining, bacterial culture, and PCR amplification. The typical abscess was found 7 days later. The abscess prophase (inflammation stage) was found after 15 days, the abscess formation phase was observed on the 21st day, and the abscess recovery phase was observed on the 33rd day. Thus, the 21st day could be the optimal period for percutaneous transhepatic echo-guided intervention (Alvarez-Peréz et al., 2001; Balint et al., 2001; Alberti et al., 2002; Zatelli et al., 2005; Ferraioli et al., 2008; Meddings et al., 2010; Mezhir et al., 2010; O'Farrell et al., 2010; Angelino et al., 2011). To our knowledge, this is the first report of the establishment of a PHA animal model in the Bama minipig.

Our study has at least four limitations. First, the computation of the viable bacterial counts of *S. aureus* ATCC 29213 was determined through linear regression analysis of ATCC 25923, which might have resulted in a discrepancy. Second, the high frequency of metastatic lung abscesses during the PHA establishment might have delayed PHA recovery after interventional therapy. Third, the pus was too thick to be aspirated through an 18-G PTC needle, which possibly caused difficulty in the interventional therapy. Finally, the reasons why *S. aureus* ATCC 29213 could cause PHA while ATCC 25923 could not require further investigation. These shortcomings may be addressed in future studies.

In conclusion, Bama minipig PHA was successfully established by injecting the mixture of *S. aureus* ATCC 29213 and self-venous blood clot into the liver parenchyma. The abscess formation stage was observed on the 21st day of the injection.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by the China Postdoctoral Science Foundation (#20100471817) and the Army 11th Five Important Special Financial Assistance (#08Z037).

REFERENCES

Alberti D, Borsellino A, Locatelli C, Nani R, et al. (2002). Percutaneous transhepatic alcoholization: a new therapeutic strategy in children with chronic granulomatous disease and liver abscess. *Pediatr. Infect. Dis. J.* 21: 1081-1083.

Alvarez-Peréz JA, González JJ, Baldonedo RF, Sanz L, et al. (2001). Clinical course, treatment, and multivariate analysis of risk factors for pyogenic liver abscess. *Am. J. Surg.* 181: 177-186.

Angelino G, Natali GL, Falappa P, Folgori L, et al. (2011). Successful treatment with percutaneous transhepatic alcoholization of a liver abscess in a child with chronic granulomatous disease. *Pediatr. Infect. Dis. J.* 30: 819-820.

Balint TD, Bailey BM, Mendelson KG and Pofahl W (2001). Hepatic abscess: Current concepts in diagnosis and treatment. *Curr. Surg.* 58: 381-384.

Brakstad OG, Aasbakk K and Maeland JA (1992). Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. *J. Clin. Microbiol.* 30: 1654-1660.

Chen SC, Tsai SJ, Chen CH, Huang CC, et al. (2008). Predictors of mortality in patients with pyogenic liver abscess. *Neth. J. Med.* 66: 196-203.

Domínguez MC, de La Rosa M and Borobio MV (2001). Application of a spectrophotometric method for the determination

of post-antibiotic effect and comparison with viable counts in agar. *J. Antimicrob. Chemother*. 47: 391-398.
Ferraioli G, Garlaschelli A, Zanaboni D, Gulizia R, et al. (2008). Percutaneous and surgical treatment of pyogenic liver abscesses: observation over a 21-year period in 148 patients. *Dig. Liver Dis.* 40: 690-696.
Hill GB, Osterhout S and Pratt PC (1974). Liver abscess production by non-spore-forming anaerobic bacteria in a mouse model. *Infect. Immun.* 9: 599-603.
Kaplan GG, Gregson DB and Laupland KB (2004). Population-based study of the epidemiology of and the risk factors for pyogenic liver abscess. *Clin. Gastroenterol. Hepatol.* 2: 1032-1038.
Liljegren CH, Aalbaek B, Nielsen OL and Jensen HE (2003). Some new aspects of the pathology, pathogenesis, and aetiology of disseminated lung lesions in slaughter pigs. *APMIS* 111: 531-538.
McDonald MI, Lucore CL and Durack DT (1984). An experimental model for pyogenic liver abscess. *J. Infect.* 8: 129-136.
Meddings L, Myers RP, Hubbard J, Shaheen AA, et al. (2010). A population-based study of pyogenic liver abscesses in the United States: incidence, mortality, and temporal trends. *Am. J. Gastroenterol.* 105: 117-124.
Mezhir JJ, Fong Y, Jacks LM, Getrajdman GI, et al. (2010). Current management of pyogenic liver abscess: surgery is now second-line treatment. *J. Am. Coll. Surg.* 210: 975-983.
Nielsen OL, Iburg T, Aalbaek B, Leifsson PS, et al. (2009). A pig model of acute *Staphylococcus aureus* induced pyemia. *Acta Vet. Scand.* 51: 14.
O'Farrell N, Collins CG and McEntee GP (2010). Pyogenic liver abscesses: diminished role for operative treatment. *Surgeon* 8: 192-196.
O'Mahony FC and Papkovsky DB (2006). Rapid high-throughput assessment of aerobic bacteria in complex samples by fluorescence-based oxygen respirometry. *Appl. Environ. Microbiol.* 72: 1279-1287.
Pienaar AC, Coetzee L and Bragg RR (1994). A rapid method to quantify bacterial contamination on hatching eggs. 1. Correlation of optical density with initial bacterial count. *Onderstepoort J. Vet. Res.* 61: 341-349.
Shin SJ, Han JH, Manning EJ and Collins MT (2007). Rapid and reliable method for quantification of *Mycobacterium paratuberculosis* by use of the BACTEC MGIT 960 system. *J. Clin. Microbiol.* 45: 1941-1948.
Thompson WM, Stude RA, McDonald MI, Halvorsen RA, et al. (1985). Computed tomographic evaluation of an experimental model for pyogenic liver abscesses. *Invest. Radiol.* 20: 152-158.
Weissleder R, Saini S, Stark DD, Elizondo G, et al. (1988). Pyogenic liver abscess: contrast-enhanced MR imaging in rats. *AJR Am. J. Roentgenol.* 150: 115-120.
Zatelli A, Bonfanti U, Zini E, D'Ippolito P, et al. (2005). Percutaneous drainage and alcoholization of hepatic abscesses in five dogs and a cat. *J. Am. Anim. Hosp. Assoc.* 41: 34-38.