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Generation of Methicillin-Resistant *Staphylococcus Aureus* Biofilm Infection in an Immunosuppressed Rat Model

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a common pathogen responsible for many related infections, and immunosuppressed individuals are more susceptible. Its pathogenicity is associated with its virulence factors, resistance to antibiotics, and ability to form biofilm (BF). MRSA-BF infections in immunosuppressed patients pose great difficulties to clinical treatment.

Material/Methods: The study aimed to establish a model of MRSA-BF infection in rats with cyclophosphamide (CTX)-induced immunosuppression. For this, rats were administered CTX on days 1 and 4. White blood cells (WBC) were counted, then rats were inoculated with a clinical MRSA 17546 (t037) on day 5. Rats were sacrificed on days 6–10 and tissue samples were examined by scanning electron microscopy.

Results: Using the dose of CTX: 150 (mg/kg) + 100 (mg/kg) is better than the other 2 programs as the survival rates of the immunocompromised rats were higher than in the other 2 immunosuppressive groups. The survival rate was not different between rats in the clean environment and in the SPF environment. However, the survival rate was affected by the sample acquisitions. Importantly, WBC counts started to decline on day 4, and then started to rise on day 9. Moreover, MRSA-BFs were formed earlier in immunosuppressed rats compared to the normal rats, as shown by scanning electron microscopy.

Conclusions: The study successfully established an immunosuppressed rat model of MRSA-BF infection, which provides methodological and data support for establishment of such animal models and is useful reference for related research. Our results may help further investigation of MRSA-BF infection.

MeSH Keywords: **Animal Experimentation • Antibiotic Prophylaxis • Cyclophosphamide • Methicillin-Resistant *Staphylococcus aureus***

Abbreviations: **MRSA** – methicillin-resistant *Staphylococcus aureus*; **BF** – biofilm; **CTX** – cyclophosphamide; **SEM** – scanning electron microscopy; **CMC** – carboxymethyl cellulose; **SD** – Sprague-Dawley; **CFU** – colony-forming units; **SPF** – clean barrier versus specific pathogen-free

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Background

Staphylococcus aureus is a common pathogen responsible for nosocomial infection and community-acquired pulmonary infection. The emergence of increasingly resistant S. aureus strains has raised concern regarding clinical infections [1], which tend to form biofilms (BFs) on medical implants or damaged tissue [2]. Several studies have confirmed that MRSA-BF formation is an important cause of drug resistance in *S. aureus* [3]. In particular, biofilm (BF) formation not only enhanced antibiotic resistance by inhibiting drug penetration and the host immune response, but also led to the intermittent release of the planktonic bacteria that continually propagated to cause chronic and persistent infection [3]. Indeed, the increasing drug resistance of *S. aureus* BFs has become a major concern for clinicians and a critical threat to human health.

The use of broad-spectrum antibiotics and glucocorticoids to treat patients with cancer or chronic diseases has increased in recent years, resulting in immunocompromised status and elevated susceptibility to MRSA infection. MRSA is a primary cause of hospital-acquired infection and high mortality [4], emphasizing the urgent need for new antibiotics to control the spread of MRSA infection. As such, there is an increasing need for reliable animal models to test the efficacy of MRSA antibiotics. Correspondingly, several experimental bacterial BF models have been developed that reproduce those found in human patients. For example, the carboxymethyl cellulose (CMC) pouch infection model in rats has been used to test the efficacy of antimicrobial agents against BF-forming bacteria [5]. Therefore, the present study sought to generate an MRSA-BF infection model in immunosuppressed rats to further research on MRSA-BF formation, virulence, drug resistance, and pharmacodynamics.

Material and Methods

Rats and reagents

Male Sprague-Dawley (SD) rats, age 6–8 weeks, and weighing 250–300 g (Laboratory Animal Center of Guangxi Medical University, Guangxi, China) were acclimated for 5–7 days prior to use in an environment with controlled temperature and relative humidity, and a 12-h light-dark cycle. All animal studies and experimental protocols were approved by the Guangxi Medical University Institutional Animal Care and Use Committee. Cyclophosphamide (CTX) was purchased from Sigma (St. Louis, MO, USA).

Bacterial strain and culturing

The MRSA strain 17546 (t037) was selected for its ability to form BFs from a group of 21 clinical *S. aureus* isolates characterized in a previous study by our group [2]. The standard strain *S. aureus* ATCC 29213 served as a control. MRSA 17546 (t037) was pre-cultured in Luria-Bertani broth (LB broth) at 37°C for 20 h before use in BF formation assays. Cultures were diluted in LB broth to 1×10^7 colony-forming units (CFU)/mL for use in the rest of the study. Bacterial colony counts were repeated 3 times.

Immunosuppression protocol

Rats were randomly divided into 16 groups (n=10 per group) based on housing environment (clean barrier versus (*vs.*) specific pathogen-free [SPF]), immune inhibitor treatment, and sampling times (Table 1). CTX was administered by intraperitoneal (i.p.) injection to induce immunosuppression in rats based on previous reports and preliminary experiments [6–13]. Specifically, CTX were given i.p. on the first day and the fourth day. The CTX dose was divided into 3 groups: high-dose group, 200 (mg/kg, on day 1) +150 (mg/kg, on day 4) (A1, A2, A3 and A4), middle-dose group, 150 (mg/kg, on day 1) +100 (mg/kg, on day 4) (B1, B2, B3 and B4), and low-dose group, 100 (mg/kg, on day 1) +75 (mg/kg, on day 4) (C1, C2, C3 and C4). All of the CTX dose groups had a blank control group (D1, D2, D3, and D4), which was i.p. injection of an equal volume physiological saline. It was to chosen as the best immunosuppressive regimen for use in the rest of the study.

Blood analysis

Rats were anesthetized with i.p. injection of 10% chloral hydrate (St. Louis, MO, USA) at 0.1 mL/100 g before collecting of whole-blood samples. Whole-blood samples (20 µL per sample) were collected from the posterior vena orbitalis of rats. In the continuous sampling group, whole-blood samples were collected from day 1 before CTX administration to days 2–10, which lasted 10 days. Blood samples were collected on day 1 before CTX administration, and then on day 4 and day 9 in the intermittent sampling group. There was no blood collection performed in the no-sampling group. After that, a 20-µL blood sample was added to 0.38 mL of white blood cell (WBC) dilution (2% glacial acetic acid) and mixed well. We used a small dropper to take 10 µL of the suspension and drop it into the Neubauer counter plate for 2–3 min. WBCs were counted using a low-magnification microscope. According to the counting principle, count proceeds from up and left, not down and right, when the WBC is on the Neubauer counter line. The WBC numbers were calculated by the formula: WBC/L=the total number of WBCs in the 4 squares (N)/4×10×10^6×20 (=N/20×10^9/L). In the formula, 1/4 shows the average number of WBCs in a large grid, ×10 indicates the number of WBCs in 10 squares, ×10^6 represents the number of WBCs in 1-liter dilution, and ×20 is the dilution factor. WBC counts were repeated 3 times.

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Table 1. Rat observation groups.

Group	Housing	Treatment		MRSA infection (CFUs)	Sampling
		Day 1	Day 4		
A1	Clean barrier	CTX (200 mg/kg)	CTX (150 mg/kg)		Continuous
B1	Clean barrier	CTX (150 mg/kg)	CTX (100 mg/kg)		Continuous
C1	Clean barrier	CTX (100 mg/kg)	CTX (75 mg/kg)		Continuous
D1	Clean barrier	Physiological saline			Continuous
A2	SPF	CTX (200 mg/kg)	CTX (150 mg/kg)		Continuous
B2	SPF	CTX (150 mg/kg)	CTX (100 mg/kg)		Continuous
C2	SPF	CTX (100 mg/kg)	CTX (75 mg/kg)		Continuous
D2	SPF	Physiological saline			Continuous
A3	Clean barrier	CTX (200 mg/kg)	CTX (150 mg/kg)		Intermittent
B3	Clean barrier	CTX (150 mg/kg)	CTX (100 mg/kg)		Intermittent
C3	Clean barrier	CTX (100 mg/kg)	CTX (75 mg/kg)		Intermittent
D3	Clean barrier	Physiological saline			Intermittent
A4	Clean barrier	CTX (200 mg/kg)	CTX (150 mg/kg)		None
B4	Clean barrier	CTX (150 mg/kg)	CTX (100 mg/kg)		None
C4	Clean barrier	CTX (100 mg/kg)	CTX (75 mg/kg)		None
D4	Clean barrier	Physiological saline			None
Immunosuppressive group	Clean barrier	CTX (150 mg/kg)	CTX (100 mg/kg)	1×10^7	None
Normal control group	Clean barrier	Physiological saline		1×10^7	None

MRSA infection

Another 30 rats served as the immunosuppressive group and normal control group for MRSA (n=15 each group) infection, as described in Table 1. Briefly, pouches were created in the intra-scapular area on day 4 by a subcutaneous injection of 10 mL sterile air with 21-gauge needles after trimming the hair with clippers. On the next day (day 5), the air was aspirated and replaced with 10 mL of sterilized 1.5% CMC in saline with 1×10^7 CFU MRSA 17546.

Scanning electron microscopy (SEM)

BF formation was confirmed in rats by SEM, as previously described (Hitachi, Japan) [14]. Briefly, membranous structures, including subcutaneous tissues, were carefully extracted on days 6–10 by making a small incision in the CMC pouches. Samples were immediately processed for SEM by fixing at room temperature with 2.5% glutaraldehyde for 24 h. The next day, the samples were rinsed 3 times in fresh PBS (pH 7.4), passed through an ethanol gradient for 15 min each, dehydrated in 100% ethanol (3 times for 10 min), dried, coated with gold, and examined by SEM [2,15].

Statistical analysis

Data were analyzed using SPSS 20 (SPSS, Inc., IBM Software, Armonk, NY, USA) and are expressed as the mean ± standard deviation (SD). Differences between groups were evaluated using the chi-square test and analysis of variance (ANOVA). $P<0.05$ was considered statistically significant.

Results

Effect of the doses of CTX, the environment, and sampling times on the survival rates of various immunocompromised rat models

Analysis of rats after i.p. injection of CTX revealed decreased physical activity and body weights in all 3 CTX-treated groups. During the observation period, the survival rate of the rats in

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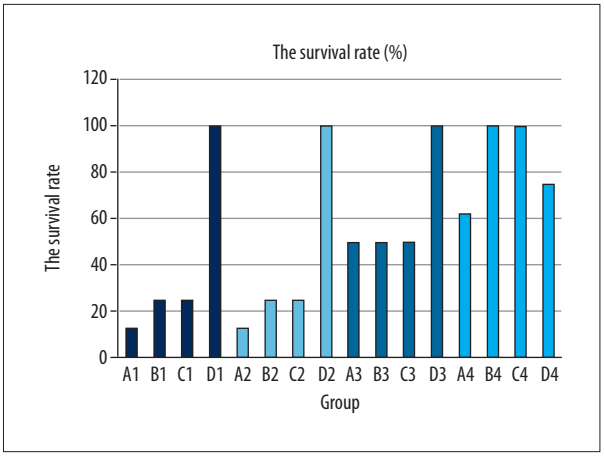

Figure 1. Survival rates of various immunocompromised models. The survival rate of the rats was 12.5% in group A1, 25% in groups B1 and C1, and 100% in group D1. The same result was found in groups A2, B2, C2, and D2. The survival rates in groups A3, B3, and C3 were 50%, whereas 100% of D3 rats survived. In comparison, 62.5% of rats survived in group A4, 100% in groups B4 and C4, and 75% in group D4.

clean barrier environment and with continuous sampling was 12.5% in group A1, 25% in groups B1 and C1, and 100% in group D1 (Figure 1). The same result was found in groups A2, B2, C2, and D2 in an SPF environment with continuous sampling. Similar findings were observed in rats who were housed in a clean barrier environment, with intermittent sampling. The survival rates in groups A3, B3, and C3 were 50%, whereas 100% of D3 rats survived. In comparison, 62.5% of rats survived in group A4, 100% in groups B4 and C4, and 75% in group D4 (Figure 1).

The high-dose CTX group had lower survival rates than other 2 groups. No differences in survival rates were observed between clean barrier environments and SPF with continued blood sampling, suggesting that environment has little effect on rat survival, although animals with intermittent blood sampling or non-sampled controls displayed a lower mortality rate compared to counterparts subjected to continuous sampling. These findings show that the survival rates were associated with the dose of CTX and the frequency sampling over a period of several days.

WBC counts in immunocompromised rats

Regardless of being in the clean environment or in the SPF environment with continuous sampling or intermittent sampling, there was a significant reduction in peripheral WBC counts [16], especially after the second administration on day 4 (Tables 2–4). On days 5–8, the WBC counts in the A1, A2, B1, and B2 groups fell below 1.5×10^9/L and subsequently increased to 1.32×10^9/L on day 10. However, more obviously, the counts of A1 and A2 were reduced more than counts of B1 and B2, which were less than 0.5×10^9/L at the beginning of the decrease. In contrast, WBC counts in the C1 group reached 1.74×10^9/L, more than 1.5×10^9/L; whereas the WBC counts of D1 group were almost 1×10^{10}/L. On the other hand, WBC counts in different groups using the same dose of CTX (Table 5) showed that the WBC counts may fall more in the SPF-environment group than in the clean-environment group in a few days, but counts were not as stable as in the other 2 groups.

Combining the survival rates with the WBC counts, we demonstrated the B4 immunosuppression rat model (the dose of CTX: [150+100] mg/kg, in the clean environment without sampling) was the best for use in our experimental study.

Table 2. Effect of cyclophosphamide (CTX) on peripheral white blood cell (WBC) counts per L from days 1–10 post-treatment in clean barrier environment.

Group	CTX (mg/kg)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
A1	200+150	10.59± 2.27	5.68± 2.22	2.86± 1.07	1.49± 0.87#&	0.64± 0.48	0.31± 0.18	0.16± 0.078	0.18± 0.058	0.30± 0.00	0.70± 0.00
B1	150+100	12.4± 3.76	4.87± 1.98	3.88± 1.40	2.76± 1.50&	1.14± 0.62	0.58± 0.26	0.58± 0.60	0.24± 0.16	0.52± 0.20@	1.32± 0.12
C1	100+75	12.08± 2.10	6.25± 1.68	4.98± 3.14	3.50± 1.92&	1.74± 1.00	1.33± 0.65	0.56± 0.21	0.66± 0.58	0.54± 0.24	1.53± 0.061
D1	Saline	10.61± 3.21	10.11± 3.79*	11.31± 4.58*	11.77± 1.91*	11.10± 3.42*	10.40± 4.32*	14.98± 5.08*	9.97± 3.32*	11.75± 7.94*	11.93± 3.80*

Mean ± standard deviation (SD); *vs.* B1 on the same day, * P<0.05; *vs.* C1 and D1 on the same day, # P<0.05; in the same group on Day 3 *vs.* on Day 4, & P<0.05; in the same group on Day 8 *vs.* Day 9, @ P<0.05. A1, B1, C1 – immunosuppressive group with using different dose of CTX; D1 – normal control group: with saline-treated.

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Table 3. Effect of cyclophosphamide (CTX) on peripheral white blood cell (WBC) counts per L from days 1–10 post-treatment in specific pathogen-free (SPF) environment.

Group	CTX (mg/kg)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
A2	200+150	11.11± 2.95	3.56± 0.81	1.89± 0.82	0.48± 0.34#&	0.22± 0.17	0.19± 0.06	0.44± 0.61	0.19± 0.06	0.60± 0.00	0.67± 0.00
B2	150+100	11.51± 2.44	3.94± 1.44	1.54± 0.55	1.24± 0.64#&	0.45± 0.25	0.18± 0.73	0.35± 0.19	0.23± 0.09	0.81± 0.37@	1.17± 0.54
C2	100+75	11.11± 3.32	4.23± 1.38	1.53± 0.26	2.19± 0.83	0.70± 0.33	0.30± 0.17	0.83± 0.65	0.40± 0.24	0.71± 0.48	1.29± 0.44
D2	Saline	12.28± 3.92	8.48± 2.01*	11.71± 4,63*	7.67± 3.55*	11.01± 5.01*	7.80± 4.44*	11.26± 5.56*	8.50± 4.24*	6.69± 2.46*	8.83± 3.55*

Mean ± standard deviation (SD); *vs.* B2 on the same day, * P<0.05; *vs.* C2 and D2 on the same day, # P<0.05; in the same group on Day 3 *vs.* on Day 4, & P<0.05; in the same group on Day 8 *vs.* Day 9, @ P<0.05. A2, B2, C2 – immunosuppressive group with using different dose of CTX; D2 – normal control group: with saline-treated.

Table 4. Effect of cyclophosphamide (CTX) on peripheral white blood cell (WBC) counts per L after CTX administration on days 1 and 4 in clean barrier environment with intermittent sampling.

Group	CTX (mg/kg)	Day 1	Day 4	Day 9	Day 10
A3	200+150	11.27±2.37	1.30±0.66#	0.47±0.11	0.95±0.06
B3	150+100	10.56±1.60	1.09±0.37#	0.58±0.14	0.92±0.06
C3	100+75	10.19±1.87	1.17±0.49#	0.73±0.18	1.04±0.11
D3	Saline	10.20±2.50	10.81±2.74*	11.52±3.45*	12.10±3.51*

Mean ± standard deviation (SD); *vs.* B3 on the same day, * P<0.05; in the same group on Day 1 *vs.* on Day 4, # P<0.05.
A3, B3, C3 – immunosuppressive group with using different dose of CTX; D3 – normal control group: with saline-treated.

Development of the MRSA-BF infection model

In preliminary experiments, 5 groups of normal rats were inoculated with 10^9, 10^8, 10^7, 10^6, or 10^5 CFU/mL each along with the injection of CMC. After infection, the rats gradually appeared moribund, resulting in survival rates of 0%, 25%, 100%, 75%, and 100%, respectively, at 7 days post-infection. Colony counts from pouch exudates isolated 4 days post-inoculation ranged from 30 to 300 per plate, which are considered good results (Figure 2). Similar results were also observed in immunosuppressed rats. Based these results, rats were inoculated with 10^7 CFU/mL for the remainder of the study.

Biofilm infections were established in the CMC pouch in the immunosuppressive group and normal control group on day 4. MRSA 17546 (t037) was pre-cultured in LB broth medium at 37°C for 20 h before inoculation. The bacterial suspension was subsequently diluted to 1×10^7 CFU/mL with LB broth and injected into a CMC solution on day 5. Similar results were observed in the immunosuppressed group and normal control group as in the preliminary study.

SEM analysis of MRSA-BF infected CMC pouches

SEM analysis of MRSA-infected normal control group rats on day 9 revealed uniform and dense accumulation of slightly mature BF on the CMC capsule surface, which grew denser on day 10. However, BF was sparse on day 8 and not detected on days 6 or 7 (Figure 3). In comparison, immunosuppressed group rats exhibited a more mature BF as compared to their normal control group counterparts on the corresponding day (Figure 4).

Discussion

Although *S. aureus* is part of the normal body flora, it can cause lung infection and easily induce infection in immunosuppressed patients, such as those with cancer or severe infections. Some studies indicate that about 2.1% of the population are colonized with MRSA, which was lower in orthopedic patients (0.9%) and higher in neurologic patients (3.7%) [17]. Notably, MRSA forms biofilms not only on indwelling foreign body infections, but also in bacteremia, endocarditis, soft-tissue infections, and osteomyelitis [18].

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Table 5. Effect of cyclophosphamide (CTX) on peripheral white blood cell (WBC) counts per L in different group.

Group	CTX (mg/kg)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
A1	200+150	10.59± 2.27	5.68± 2.22	2.86± 1.07	1.49± 0.87	0.64± 0.48	0.31± 0.18	0.16± 0.078	0.18± 0.058	0.30± 0.00	0.70± 0.00
A2	200+150	11.11± 2.95	3.56± 0.81	1.89± 0.82	0.48± 0.34*	0.22± 0.17	0.19± 0.06	0.44± 0.61	0.19± 0.06	0.60± 0.00	0.67± 0.00
A3	200+150	11.27± 2.37			1.30± 0.66					0.47± 0.11	0.95± 0.06
B1	150+100	12.4± 3.76	4.87± 1.98	3.88± 1.40	2.76± 1.50	1.14± 0.62	0.58± 0.26	0.58± 0.60	0.24± 0.16	0.52± 0.20	1.32± 0.12
B2	150+100	11.51± 2.44	3.94± 1.44	1.54± 0.55#	1.24± 0.64#	0.45± 0.25#	0.18± 0.73#	0.35± 0.19	0.23± 0.09	0.81± 0.37	1.17± 0.54
B3	150+100	10.56± 1.60			1.09± 0.37#					0.58± 0.14	0.92± 0.06
C1	100+75	12.08± 2.10	6.25± 1.68	4.98± 3.14	3.50± 1.92	1.74± 1.00	1.33± 0.65	0.56± 0.21	0.66± 0.58	0.54± 0.24	1.53± 0.061
C2	100+75	11.11± 3.32	4.23± 1.38	1.53± 0.26&	2.19± 0.83&	0.70± 0.33	0.30± 0.17&	0.83± 0.65	0.40± 0.24	0.71± 0.48	1.29± 0.44
C3	100+75	10.19± 1.87			1.17± 0.49&					0.73± 0.18	1.04± 0.11
D1	Saline	10.61± 3.21	10.11± 3.79	11.31± 4.58	11.77± 1.91	11.10± 3.42	10.40± 4.32	14.98± 5.08	9.97± 3.32	11.75± 7.94	11.93± 3.80
D2	Saline	12.28± 3.92	8.48± 2.01	11.71± 4.63	7.67± 3.55@	11.01± 5.01	7.80± 4.44	11.26± 5.56	8.50± 4.24	6.69± 2.46@	8.83± 3.55
D3	Saline	10.20± 2.50			10.81± 2.74					11.52± 3.45	12.10± 3.51

Mean ± standard deviation (SD); on the same day, *vs.* among A1, A2 and A3, * P<0.05; on the same day, *vs.* among B1, B2 and B3, # P<0.05; on the same day, *vs.* among C1, C2 and C3, & P<0.05; on the same day, *vs.* among D1, D2 and D3, @ P<0.05. A1, A2, A3, B1, B2, B3, C1, C2, C3 – immunosuppressive group with using different dose of CTX; D1, D2, D3 – normal control group: with saline-treated.

In this study, we successfully established a model of MRSA-BF infection in rats by CTX-induced immunosuppression. The model was based on previous rat models for bacterial BF and invasive MRSA [6,7,14,18–21]. To confirm that BF infection was consistent with that observed in humans, we assessed overall appearance, survival, WBC counts, and SEM images.

We previously selected SD rats as an experimental model due to their widespread use and the ability to evaluate various measures of drug pharmacology. Moreover, SD rats are more genetically stable than Wistar rats and have larger size and blood volume than mice, which makes rats better suited for the creation of back pouches and blood collections required for the present work.

As expected, our findings demonstrated that the chemotherapeutic CTX impaired the body's immune response to increase the chance of infection; thus, CTX can kill tumor cells and active immune cells at the same time. Consistent with our results, several studies showed that CTX inhibits the humoral and cellular immune responses in animals, resulting in immune suppression [6–13]. Moreover, changes in WBC counts – as the main immune effector cells – likely have important effects on immune status.

Several schemes have been reported to induce immune suppression status in experimental animal models; however, the timeline of these schemes was too long [8–11,16,22,23]. Moreover, previous studies demonstrated that rates of infection and death are positively associated with the number of traumatic operations. In our study, we opted to use the (150+100) mg/kg CTX dose administered on days 1 and 4. Thus, since our method only required 2 CTX administrations to result in successful immunosuppression, we believe it will be extremely useful in large-scale studies because of it is easy to use, reproducible, and expeditious.

The rat model was stable in groups D1, D2, and D3 with lower survival rates, but not in group D4. Before MRSA infection, the anesthesia procedure led to the death of 1 rat in group D4.

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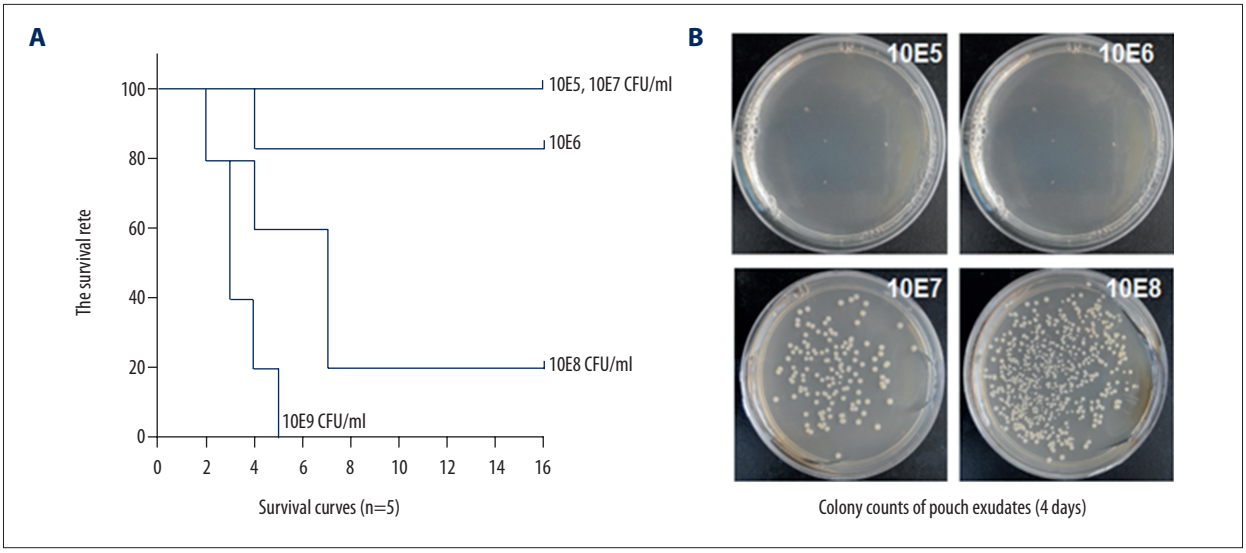

Figure 2. Survival curves and colony counts of pouch exudates. Rats were inoculated with 10^9, 10^8, 10^7, 10^6, or 10^5 CFU/mL in each pouch exudate. After infection for 7 days, the survival rates were 0%, 25%, 100%, 75%, and 100%, respectively. Colony counts from pouch exudates isolated 4 days post-inoculation ranged from 30 to 300 per plate. The concentration of 10E7 was considered to produce good results.

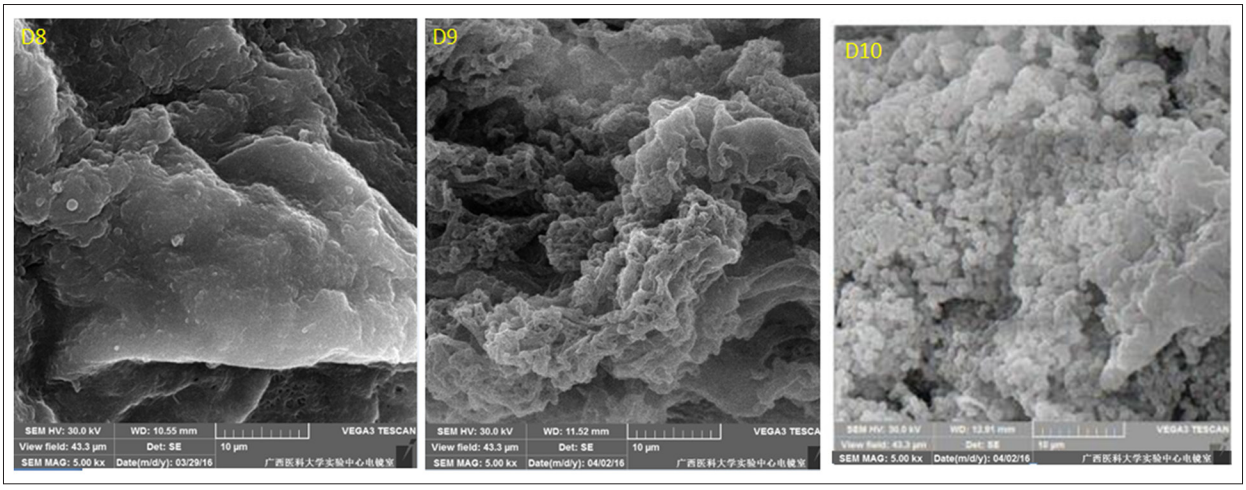

Figure 3. SEM of normal control group on days 8–10. SEM of normal control group on day 9 revealed uniform and dense accumulation of slightly mature BF on the CMC capsule surface, which grew denser on day 10. However, BF was sparse on day 8.

Therefore, we speculated that the trauma of blood sampling would decrease rat survival, since continuous blood sample collection can increase infection risk and lead to acute hemorrhagic anemia. Notably, we observed varying degrees of pallor in rat sclera, ears, and limbs (typically associated with acute blood-loss anemia), which was associated with the volume of blood removed [24]. Blood collection is a known traumatic stressor, and elicits heightened responses comparable to responses to stressors such as tension, fear, and cold. In our animal collection, rats monitored with continuous blood sampling showed impaired wound healing and associated complications, as well as higher mortality.

Based on our preliminary experiments data, we selected 1×10^7 CFU/mL MRSA for use in the immunosuppression experiments. As expected, all immunosuppressed rats displayed decreased energy and appetite, as well as weight loss, which were more pronounced than that in normal MRSA-infected rats, but it showed no effect on survival, confirming the absence of incidental mortality in our MRSA model.

We subsequently found that MRSA-BFs formed earlier in immunosuppressed rats than in normal counterparts. The planktonic MRSA bacteria aggregated and proliferated on a variety of extracellular polymers, and then gradually matured to form a BF. The high magnification of SEM allows for the visualization

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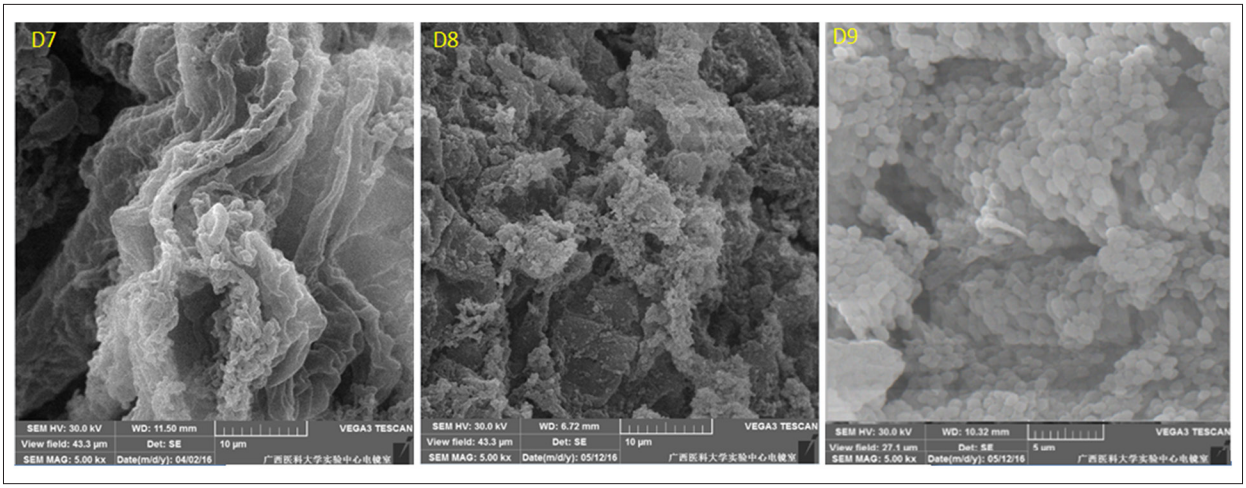

Figure 4. SEM of immunosuppression group on days 7–9. SEM of the immunosuppression group: immunosuppressed rats exhibited a more mature BF as compared to normal control group counterparts on the corresponding day. BF was sparse on day 7. At day 8, there was uniform and dense accumulation of mature BF, which grew denser on day 9.

of subtle structures; however, sample processing may have altered biofilm morphology to different degrees. This study provides a basis for additional studies on early and mature biofilms to determine whether early pharmaceutical intervention would be useful in immunocompromised patients.

Conclusions

We successfully established an immunosuppressed rat model of MRSA-BF infection, which provided methodological and data support for establishment of such animal models and a useful reference for related research. Of course, our model also has a few limitations that should be noted. For instance, SEM cannot provide quantitative data on BF formation, and it is unclear whether the duration of immunosuppression was sufficient to study treatment efficacy. While additional research is necessary to address these concerns, our immunosuppressed rat model may help further investigations on MRSA-BF infection. Furthermore, the immunosuppressed rat model could be used for the study of MRSA-BF virulence and drug resistance during MRSA infection.

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