ORIGINAL ARTICLE

Assessment of Pathogenicity of Community-Acquired MRSA Isolates in Mice-Induced Peritonitis

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ABSTRACT

Introduction: Methicillin-Resistance *Staphylococcus aureus* (MRSA) is known as a major nosocomial pathogen in healthcare. However, it has now spread in the community known as community-acquired MRSA (CA-MRSA). Thus, the survival and pathogenicity of CA-MRSA isolates were assessed using *in vivo* peritonitis model with comparison to ATCC-MRSA. Two CA-MRSA isolates; CA-MRSA1 and CA-MRSA2 that were isolated from healthy population, were studied and compared. **Methods:** Mice were assigned into 4 groups and injected intraperitoneally with ATCC-MRSA, CA-MRSA1 or CA-MRSA2, respectively. Sterile Dulbecco's Phosphate-Buffered Saline (DPBS) represents negative control. Mice were observed twice daily, 0-72 hours of post-infection. Any signs of distress were recorded for severity score and survival analyses. Mice were euthanised at 72 hours post-inoculation or by referring to the Peritonitis Severity Scoring (PSS) system. Organs of interest, peritoneal lavage and abscess were processed for bacterial counts. Tissue samples were analysed for histopathological scores. **Results:** All mice inoculated with MRSA showed clear signs of illness with peritonitis symptoms of $p<0.001$ and comparable PSS scores were recorded in all infected mice groups. Intraperitoneal injection of lethal dose of MRSA resulted in significant death of ATCC-MRSA ($p<0.05$) and CA-MRSA-infected mice ($p<0.01$), compared to the un-infected. Bacterial burden was significantly high in all samples harvested from mice challenged with CA-MRSA2 compared to ATCC-MRSA except in abscess and lung. Significant liver necrosis and spleen inflammation were observed in CA-MRSA1, and lung inflammation in ATCC-MRSA-infected mice. **Conclusion:** Nasal carriage CA-MRSA isolates from a healthy population has the potential to cause peritonitis with comparable severity as ATCC-MRSA.

Keywords: Community-acquired; Methicillin-Resistance *Staphylococcus aureus*; Nasal Carriage; Peritonitis Model; Virulence

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) is known as a commensal pathogen which colonizes the human anterior nares. *S. aureus* has emerged as the vital cause of sepsis in developing countries, with growing resistance as the major issue (1). Methicillin-Resistant *S. aureus* (MRSA) is more problematic than other types of Staphylococcus because it cannot be treated with common antibiotics including methicillin (2). It is notorious as the prime cause of hospital-associated infections and has become a major public health problem worldwide. Although MRSA has been conventionally considered as a major nosocomial pathogen in clinical settings, the number of MRSA cases acquired in the community settings has also been rising. The early case of community-associated MRSA (CA-MRSA) infection was reported in 1980, in the United States, followed with a more-widespread in the 1990s (3). The rise in the number of patients with CA-MRSA infection was observed to be in parallel with the risk of inappropriate initial antimicrobial treatment. This leads to an increase in treatment failure and death (1). Besides, patients diagnosed with CA-MRSA infections usually associated with unidentified risk factors compared to patients associated with hospital-associated MRSA (HA-MRSA) infections (4).

Due to the increasing incidence of healthcare-associated infections, the emergence of CA-MRSA has been alarming. Besides, the epidemiology of MRSA has changed with classic HA-MRSA clones being replaced with the CA-MRSA clones. This is due to the introduction of these strains into the healthcare setting (5). Moreover, CA-MRSA composed of more-diverse clonal groups compared to HA-MRSA. It usually

contains a unique *SCCmec* type IV DNA element (4, 6) with higher potential in transmission and virulence than HA-MRSA clones (5). Additionally, the production of Panton-Valentine Leukocidin (PVL) enhanced the virulence of CA-MRSA compared to HA-MRSA, which only possesses *SCCmec* I, II and III (7). Recently, a study reported that CA-MRSA is easily transmissible compared to HA-MRSA (8). Thus, to manage CA-MRSA infections, there are several issues which need to be considered. First, CA-MRSA infections emerge in healthy individuals and currently circulate within a community setting. Secondly, CA-MRSA is becoming a highly potential cause of nosocomial infections (3).

Peritonitis is one of the complications led by the highest percentage of *S. aureus* infections. It has the potential to cause fatal inflammation of the peritoneum area and organs within it (9). In addition to antibiotic resistance, the severity of *S. aureus* infections is associated with virulence factors produced, including enzymes (coagulase, lipase, and nucleases) and multiple toxins with diverse activities. A serious complication of peritoneal dialysis (PD) is associated with poor outcome and high PD failure rates. In the United State, 18% of the infection-related mortality in PD and end-stage renal disease patients was due to peritonitis, with *S. aureus* being the greatest number of positive cultures within healthcare setting (10, 11). Meanwhile in Malaysia, it was reported that the total death rate due to peritonitis between 2009 and 2013 was about 20.3%, with *S. aureus* being the most commonly identified bacterial isolates (12). Although the basis for the increased virulence of peritonitis-potential CA-MRSA strains from a healthy population is yet to be understood (13), MRSA-peritonitis was postulated to be linked to the high risk of hospitalization (14).

Since it is common that patients infected with CA-MRSA have unidentified risk factors compared to those with HA-MRSA (4), this has created a continuous threat to the public health. The possibility of this infection to occur is considered high if there is a presence of nasal carriage of *S. aureus*. However, its eradication with local antibiotics decreases infectious catheter complications (15). This step is crucial since a single positive nose culture may increase the risk for *S. aureus* peritonitis (16, 17). Therefore, it is important to monitor the distribution pattern and the pathogenic status of CA-MRSA in the community. However, there is yet any research done to investigate the severity that may cause by MRSA isolated from healthy population or specifying on the original setting of the isolates despite several reports on the outcome of *S. aureus* peritonitis. Therefore, to assess the virulence status of the CA-MRSA nasal carriage isolated from healthy population, the pathogenicity, survival rate and the severity of peritonitis symptoms in mice infected with CA-MRSA isolates were compared with the reference strain ATCC 700699 MRSA (ATCC-MRSA) using mice model of peritonitis.

MATERIALS AND METHODS

Bacterial strains

Two MRSA of carriage origin previously isolated from nares of healthy students and preserved in -80oC freezer were assessed in this study (18), labelled as CA-MRSA1 and CA-MRSA2. Prior to confirmation of *S. aureus* and detection of *mec*A genes, these sample isolates were grown onto Tryptic Soy Agar (TSA, Merck, Germany). The identification of sample isolates was performed based on Mannitol Salt Agar (MSA, Merck, Germany) test, Gram stain colony morphology, then followed by coagulase and catalase tests. Then, sample isolates were cultured overnight in Luria Bertani broth (LB broth, Merck, Germany) and grown at 37°C with shaking (200 rpm) to achieve the exponential phase of growth. Once the desired O.D. and the exponential phase have been obtained, 10-fold (1:100) of serial dilution was performed, washed and resuspended in sterile PBS to obtain ~1x10^9 of colony forming units per mL (CFU/mL). ATCC 700699 MRSA (ATCC-MRSA) was used as a reference strain.

Detection of *mec*A genes

DNA extraction kit (Gene All®, Korea) was used to extract DNA from *S. aureus* isolates. The extracted DNA was amplified by Polymerase Chain Reaction (PCR) for the *mec*A genes detection using forward and reverse primers, *mec*A-F (5′-AAAACTAGGTGTTGGTGAAGATATACC-3′) and *mec*A-R (5′-GAAAGGATCTGTACTGGG TTAATCAG-3′) (19). The PCR product was subjected to electrophoresis in 2% agarose gel containing DNA safe stain and viewed under Molecular Image Gel Doc XR system (Bio Rad, United States).

Animal

Female BALB/c mice of six weeks of age were used in this experiment. For each group, mice were housed 6-8 per cage under room temperature of 29-32°C and humidity of 70-80% with 12 hours light-dark cycles. Mice were given *ad libitum* access to standard chow (Kaytee) and water. All mice were allowed to acclimatize to the new surrounding for a week prior to infection with MRSA. The experiments were conducted according to the ethical guidelines and approval from the Institutional Animal Care and Use Committee (IACUC) of Universiti Putra Malaysia (UPM/IACUC/AUP-R047/2018).

MRSA-induced peritonitis model

Mice were assigned into four groups; (i) un-infected control, (ii) ATCC 700699 MRSA (ATCC-MRSA), (iii) CA-MRSA1, and (iv) CA-MRSA2 with 6-8 mice each group. Mice were exposed to MRSA by intraperitoneal injection with 200 µL of 10^9 CFU/mL of ATCC-MRSA, CA-MRSA1 and CA-MRSA2, respectively. Un-infected mice were injected with sterile PBS instead. Mice were then monitored twice daily for the sign of morbidity or recovery for up to 72 hours. PSS was recorded and score

between 0 and 4 were given to each criteria. Mice were euthanised if the PSS at any given time point was greater than or equal to 21 (≥21), or if the points ascribed to respiratory rate or quality increased by more than or equal to 3 (≥3) or found dead, and of which the time of death was recorded. All mice were euthanised by cervical dislocation 72 hours post-infection and samples (peritoneal fluids, abscess, liver, spleen and lung) were collected for further analysis.

Bacterial burden

Peritoneal-derived cells were isolated by lavage of the peritoneal cavity using 2 mL of sterile PBS according to Meurer et al. (21), with minor modifications. Abscess, peritoneal fluid, liver, spleen and lung were aseptically isolated and further analysed for (i) bacterial load count, and/or (ii) histological analyses. For bacterial burden analysis, samples were homogenized in 3 mL of sterile PBS. Total bacterial burden in respective tissues was established via serial dilutions plating of peritoneal lavage or tissue homogenate on TSA plates for 24 hours at 37°C. Results were expressed as CFU per mL (CFU/mL).

Histopathological Scoring

For the histopathological analysis, tissue samples (i.e. liver, spleen and lung) were flattened in a tissue cassette, fixed by immersion in formalin, embedded in paraffin, sectioned at 5 µm of thickness and stained with Hematoxylin and Eosin (H&E). Tissue specimens were then histopathologically analysed and blind-graded by a professional pathologist from the Department of Pathology, Faculty of Medicine and Health Science, UPM. To score the pathological cases tissues, the tissue surface was analysed based on: (a) inflammation, (b) abscess, (c) necrosis, (d) edema, (e) haemorrhage, and (f) bacterial colonies. Each parameter was graded 0-3, with 0 being normal, 1 being mild, 2 being moderate and 3 being severe.

Statistical analysis

Differences between groups were analysed by Log-rank (Mantel-Cox) test, unpaired Student t test, one- or two-way ANOVA with Tukey's post-test comparison where indicated. Exact p values were determined using GraphPad Prism 7.0d statistical analysis software, with $p<0.05$ considered to be statistically significant.

RESULTS

Survival rate of CA-MRSA infected mice

Groups of 6-8 female BALB/c mice were intraperitoneally infected with nasal isolates of CA-MRSA from healthy community (18), labelled as CA-MRSA1 and CA-MRSA2. ATCC-MRSA, a clinical isolate, was used as a reference strain in this study. Compared to un-infected group, lethal dose of intraperitoneal injection containing approximately 10^9 CFU/mL ATCC-MRSA caused a significant death of infected mice ($p<0.05$) and resulted in more than 65% mortality over 3 days post-infection (Fig. 1). Interestingly, both CA-MRSA infected groups recorded higher mortality rate ($p<0.01$) when compared to un-infected mice, with 80% and 100% of mortality were recorded in CA-MRSA2 and CA-MRSA1, respectively. However, they were not statistically significant when compared to ATCC-MRSA, but both showed comparable virulence status. All un-infected (control group) mice survived throughout this study.

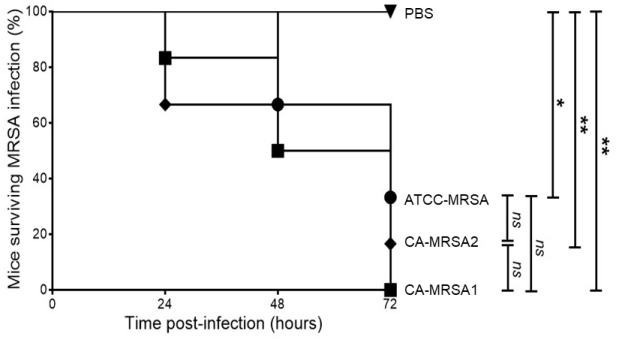

Figure 1: Challenge of mice by intraperitoneal injection of different isolates of CA-MRSA. Groups of 6-8 female BALB/c mice were assigned into four groups and intraperitoneally injected with 200 µL of 10^9 CFU/mL of ATCC 700699 MRSA (ATCC-MRSA), CA-MRSA1 and CA-MRSA2, respectively. Un-infected mice were injected with sterile PBS. The ATCC-MRSA strain was used as a reference strain in this study. Mice were then monitored twice daily for morbidity or recovery for up to 72 hours. PSS was recorded and mice were euthanised if the PSS at any given time point was greater than 21 (≥21), or if the points ascribed to respiratory rate or quality increased by more than 3 (≥3) or found dead. At 72 hours post-infection, all mice were sacrificed by cervical dislocation and samples were collected for further analysis. Survival rate was analysed by using Log-rank (Mantel-Cox) test, with mean ±SEM, * $p<0.05$, ** $p<0.01$, and *** $p<0.001$ are considered as significant.

Peritonitis Severity Scoring (PSS) of CA-MRSA infected mice

The severity of peritonitis symptoms was clinically evaluated by referring to the PSS system (20). Figures 2A-C show the PSS scores of mice infected with ATCC-MRSA and CA-MRSA isolates. Our findings demonstrated that all infected mice developed severe symptoms of peritonitis compared to the un-infected mice ($p<0.001$). After 24 hours post-infection, CA-MRSA2 recorded the highest PSS scores compared to CA-MRSA1 ($p<0.01$) and ATCC-MRSA ($p<0.05$) (Fig. 2A). Further observation showed that mice within this group demonstrated no mobility and decreased in respiration, as well as less response towards touching. With an average score of 20, CA-MRSA2-infected mice showed high PSS scores consistently throughout the 3 days observation (Fig. 2A-C). In contrast, both CA-MRSA1 and ATCC-MRSA recorded an average of less than 15 PSS scores at 24 hours post-infection. During 24 hours post-infection until 72 hour post-infection, there was a significant progression

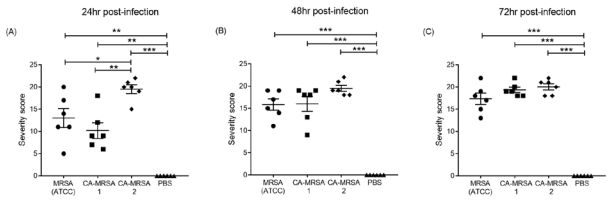

Figure 2 : Peritonitis Severity Scoring (PSS) of MRSA-infected mice. Mice were observed daily up to 72 hours post-infection and any signs of distress were observed and recorded by using PSS system. PSS was recorded daily based on its appearance, level of consciousness, activity, response to stimulus, eyes, and respiration rate and quality. (A-C) shows comparison of PSS scores within groups per day. These data were analysed using one-way ANOVA followed by Tukey's multiple comparisons test respectively, with mean ±SEM, * p<0.05, ** p<0.01, and *** p<0.001 are considered as significant.

of symptoms in CA-MRSA 1 (p<0.01) and ATCC-MRSA (p<0.05). By 72 hours post-infection, both CA-MRSA1 and ATCC-MRSA have developed severe peritonitis symptoms and achieved comparable PSS scores to CA-MRSA2. Meanwhile, all un-infected control mice were healthy with no symptoms of peritonitis.

Formation of abscess in the peritoneal cavity

With no significant difference observed between groups, both ATCC-MRSA and CA-MRSA groups demonstrated the presence of abscess formation in the peritoneal cavity of infected mice (Table I). Besides, the bacterial load recovered from the abscess of ATCC-MRSA and CA-MRSA groups also showed comparable numbers. Although the CA-MRSA1 infected group showed higher CFU count, size and number of abscess formation, it was statistically not significant (Table I). No formation of abscess was observed in un-infected mice.

Bacterial load in the abscess, peritoneal fluids and organs

To determine the level of MRSA dissemination, abscess formation, peritoneal fluids and organs of interest (i.e. liver, spleen and lung) of euthanised mice were collected and cultured on TSA agar for bacterial load analysis (CFU/mL) (Table I). It was observed that all infected groups of mice demonstrated the presence of bacteria in all collected samples. Apart from that, the peritoneal fluid of CA-MRSA2 recorded a significant number of bacteria counts compared to CA-MRSA1 (p<0.05) and ATCC-MRSA (p<0.05). Additionally, similar observation was also seen in liver sample of CA-MRSA2, but it was only significant when compared to ATCC-MRSA (p<0.05). Lung samples resulted in comparable bacterial load in all infected groups, for both ATCC-MSRA and CA-MRSA. While spleen recorded the highest bacterial load within CA-MRSA-2 group compared to other strains. No bacteria count was observed from samples of un-infected group.

Table I: Bacterial count (CFU/mL) in abscess, peritoneal fluid, liver, spleen and lung

	ATCC-MRSA	CA-MRSA1	CA-MRSA2	PBS
Abscess				
Bacterial count (CFU/mL)	1.5×10^3	1.8×10^3	1.4×10^3	Nil
Average number of abscess formation per mouse	0.8	1.5	1.4	Nil
Diameter (mm)	1.6	2.3	1.6	Nil
Peritoneal fluid	1.2×10^3	0.9×10^3	2.5×10^3*	Nil
Liver	0.3×10^3	1.0×10^3	1.5×10^3*	Nil
Spleen	0.5×10^2	0.25×10^2	5.0×10^2*	Nil
Lung	1.8×10^3	1.0×10^3	1.2×10^3	Nil

*p<0.05 is considered significant when compared to ATCC-MRSA

Histopathological analyses of organs from infected mice

In the histopathological analysis, liver, spleen and lung tissues were clinically scored and blind-graded with '0' being normal and '3' being severe. Further pathological aspects of the tissue structure were assesed based on inflammation, formation of abscess, necrosis, edema, hemorrhage and bacteria colonies (Fig. 3A-C). In the present study, significant necrosis in liver (p<0.05) and

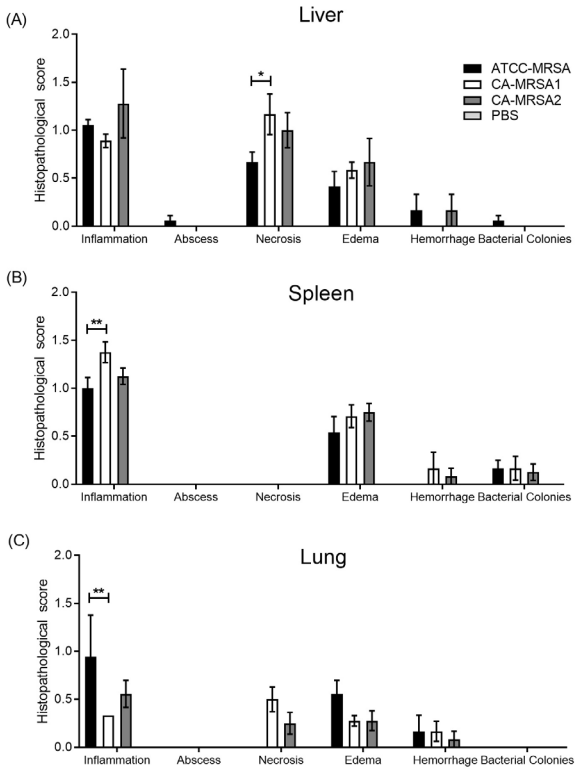

Figure 3: Histopathological Scoring. Prepared histology tissues on glass slides were stained by performing Hematoxylin and Eosin (H&E) staining method. Tissue specimens were histopathologically analysed and blind-graded. The tissue surface was analysed with the respect of the following parameters: a) inflammation, b) abscess, c) necrosis, d) edema, e) haemorrhage, f) bacterial colonies. Each parameter was graded 0-3, with 0 being normal, 1 being mild, 2 being moderate and 3 being severe. These data were analysed using two-way ANOVA followed by Tukey's multiple comparisons test respectively, with mean±SEM, * p<0.05, ** p<0.01, and *** p<0.001 are considered as significant.

inflammation in spleen ($p<0.01$) tissues were observed in CA-MRSA1-infected mice when compared to ATCC-MRSA. In contrast, ATCC-MRSA caused a significant inflammation in lung of infected mice when compared to CA-MRSA1 ($p<0.01$). Further observation indicated that tissues from all MRSA-infected groups demonstrated the presence of many megakaryocytes, which indicates the occurrence of inflammation. Abscess was only observed within liver tissues of ATCC-MRSA-infected mice.

DISCUSSION

Previous epidemiology findings have reported certain strains of MRSA are more commonly associated with the invasive infections. Despite being a common pathogen associated with healthcare setting, MRSA has also emerged as a problematic pathogen in the community setting within these past decades. Besides, CA-MRSA strains have increased virulence as well as fitness properties compared to HA-MRSA strains (22). Usually, CA-MRSA infections are mild. However, under severe cases, it can cause serious complications and is life-threatening. Nowadays, CA-MRSA has been one of the most common pathogens in the USA leading to 50% rate of infections in skin and soft tissue (23). Thus, to reduce the spreading of this infection, it is crucial to continue monitoring the distribution pattern as well as the pathogenic status of CA-MRSA in the community. This present study assessed the virulence status of CA-MRSA nasal carriage isolated from healthy population by looking at the survival rate, bacterial load and histopathological scores of peritonitis-induced mice model.

In the previous study, it has been suggested that the intraperitoneal injection of $6x10^9$ CFU of *S. aureus* Newman caused 95% mortality in C57BL/6 mice (24). This finding was consistent with our current data that demonstrated significant mortality in the intraperitoneally injected BALB/c mice with a lethal dose (10^9 CFU/mL) of ATCC-MRSA and CA-MRSA. Both CA-MRSA- and ATCC-MRSA-infected groups demonstrated high mortality rates when compared to the un-infected group. Similarly, Kockritz-Blickwede et al. (25) also suggested that BALB/c mice were susceptible to *S. aureus*, exhibiting 100% mortality within 1 week of infection. In addition, Rauch et al. (24) also proposed that the lethal outcome upon staphylococcal challenge was not influenced by the mouse strain, instead, by metabolically active and replicating Staphylococci.

Further evaluation on the symptoms severity of peritonitis developed in mice post-infection with MRSA isolates were clinically evaluated using the PSS system developed by Shrum et al. (20). Based on this scoring system, the changes in behaviours and appearance were systematically observed for peritonitis symptoms, disease outcomes, morbidity or mortality during experimental period. After the evaluation of certain variables, scores between 0 and 4 (peritonitis severity increases within the scores) were recorded. If the scoring at any given time points were greater than 21, or when the points described to respiratory rate or quality increased by more than 3, mice were immediately euthanised to avoid any chronic pain.

With an average score of 20, high PSS score was reported in the CA-MRSA2 group within 3 days observation as early as 24 hours of post-infection. On the contrary, the CA-MRSA1 and ATCC-MRSA infected mice have developed delayed symptoms of peritonitis, with PSS average score of less than 15 and eventually progressed to >20 within 48- and 72-hours post-infection. A significant progression of clinical symptoms in these groups was also observed throughout the study. In addition, mice within these groups were observed to be progressively manifesting additional symptoms, including decreased in respiratory rate, increased in laboured breathing with minimal response to auditory and tactile stimuli. Poor quality of respiratory (breathing) was also observed in all infected mice. However, at 72 hours post-infection, all MRSA-infected mice, including both CA-MRSA and ATCC-MRSA, showed comparable peritonitis symptoms and PSS scores between groups. From these outcomes, it is speculated that CA-MRSA isolates have similar possibility in causing peritonitis as the clinical isolate. In a nutshell, both groups recorded a comparable virulence status, which is consistent with the studies conducted in Australia and South Korea that reported no significant difference in the outcomes of CA-MRSA and HA-MRSA infections (26, 27).

However, our findings were found to be contradicting with the previous study, which reported a high virulence and capacity of CA-MRSA strains to evade the host's defense system compared to the common HA-MRSA strains (28). Additionally, it was also suggested that CA-MRSA has a faster multiplication compared to HA-MRSA strains with significant shorter doubling time frame (29). Thus, based on our outcomes, we suggested that although the inoculation of 109 CFU/mL of CA-MRSA did not increase the peritonitis symptoms compared to ATCC-MRSA, the symptoms were significant and comparable with ATCC-MRSA in all infected mice.

S. aureus infection starts by forming abscess and were enclosed by pseudocapsules, separating the pathogen from immune cells (30). The Staphylococci lumps were then sequestered within the peritoneal cavity through early fibrin deposits and followed by the subsequent formation of an outer layer of collagen (11). These outcomes were similar with our findings that showed MRSA to cause the formation of abscess in the peritoneal cavity in all infected groups. The size (diameter) and number of the abscess formation found in CA-MRSA infected mice were no different compared to the ATCC-MRSA infected mice. Similar finding

was reported by Wardenburg et al. (31), in which no difference was observed in term of size of the abscess formation between groups of mice infected with wild-type or mutant, LAC or LAC pvl strains.

Apart from the size and number of abscess formation, bacterial counts (CFU/mL) within the peritoneal fluid were also recorded. The bacterial count was found to be significantly higher in CA-MRSA2 compared to CA-MRSA1 and ATCC-MRSA. Although there is no explicit explanation, it is hypothesized that PVL might contribute to the peritoneal infection by colonizing and invading the peritoneal cavity (31). This might be due to several possibilities such as BALB/c lineage that is particularly susceptible to PVL, or the differences in cellular susceptibility to the toxin, or in the host immune response to toxin-expressing strains (31). Moreover, MRSA colonies were also seen deposited within all organs isolated from infected mice.

Analysis of bacterial colonies within the liver demonstrated an approximately 90% of the MRSA was sequestered from the circulation in the liver with being 10-folds more efficient than the spleen (32). Bacterial counts within the liver of both CA-MRSA groups were also higher compared to ATCC-MRSA. In contrast, the bacterial count of ATCC-MRSA within the lung was higher than CA-MRSA which may cause pneumonia. Moreover ATCC-MRSA has also been associated with bacteraemia, as well as other invasive infections in patients when exposed to health care settings. It is among the most frequently identified pathogens causing healthcare-associated pneumonia (HCAP), hospital-acquired pneumonia (HAP), and ventilator-associated pneumonia (VAP) worldwide (33). On the other hand, CA-MRSA also usually causes soft tissues infection in otherwise healthy individuals (34).

Significant necrosis was seen in liver tissues of CA-MRSA1 infected mice when compared to ATCC-MRSA. Moreover, necrosis was only observed in lungs from CA-MRSA groups. Previously, it has been reported that mice receiving injections of MRSA isolates demonstrated localized necrotic foci inside the liver (35). Meanwhile, edema was observed in all infected mice with no significant difference between groups. In this study, CA-MRSA infected mice recorded higher inflammation scores in spleen compared to ATCC-MRSA. In addition, further histological analysis on the spleen tissues of all infected groups also demonstrated the presence of megakaryocytes. These findings are consistent with a study reported that infection of CA-MRSA isolated from nasal cavity caused a significant number of megakaryocytes presence within the spleen tissue, which indicates inflammation (36). It is known that increased in platelet demand during acute systemic inflammation, warrants adaptation of megakaryocytes. We speculate that PVL gene might as well contribute to this finding, Cremieux et al. (37) have previously reported that 3-9 days after inoculation of CA-MRSA (PVL+) in mice has resulted in infections within spleens (bacteremia) with the highest expression of PVL detected at 72 hours post-infection. Unlike in spleen, ATCC-MRSA recorded significantly higher inflammation scores in lung. A study reported that migration of CA-MRSA into healthcare settings caused nosocomial infections and supplanted traditional HA-MRSA strains as a cause of nosocomial pneumonia (38). Thus, there is possibility for CA-MRSA to be associated with the healthcare setting pneumonia (39).

CONCLUSION

CA-MRSA is likely to be a continuous threat to the public health in the foreseeable future. The present study indicates that nasal carriage of CA-MRSA has the potential of causing severe peritonitis symptoms and has high mortality rate in infected mice, which is comparable to the reference strain. Thus, periodic screening of healthy individuals especially those with high risk for MRSA carriage will limit the spread of this pathogen and ease the burden of staphylococcal diseases. In addition, the use of drugs for treating MRSA should be managed properly to minimize the emergence of further antimicrobial resistance. Although this study is limited to small samples size, this research will serve as a basis for future studies. It is crucial to ensure consistent practices are adopted towards early diagnosis, appropriate intervention and widespread surveillance to increase awareness that are needed in terms of information or education on the risks, management and treatment of MRSA, especially the serious threat of CA-MRSA carried by healthy population.

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