Clinical Methicillin-Resistant *Staphylococcus aureus* May Transfer from Hospital to the Community Through Foods

Pharanai Sukhumungoon PhD[1*]
Sunisa Dangsriwan MSc[1]
Pattamarat Rattanachuay PhD[2]

[1] Department of Microbiology, Division of Biological Science, Faculty of Science, Prince of Songkla University, Hat-Yai, Thailand
[2] Department of Science, Faculty of Science and Technology, Prince of Songkla University, Pattani campus, Pattani, Thailand
* Corresponding author, e-mail address: pharanai82@gmail.com
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Abstract

Objective: To investigate the capability of methicillin-resistant *Staphylococcus aureus* (MRSA) and *mecA*-carrying *Staphylococcus aureus* (MCSA) transfer from hospital to community through foods due to the *mecA* gene is responsible for various antimicrobials resistance.

Methods: We investigated four MRSA from patients and healthy carriers and one *mecA*-carrying *S. aureus* (MCSA) from food, whether they were capable of surviving through acidic condition and simulated gastrointestinal system. All bacterial strains were examined in green papaya salad's liquid portion (GPL), pH 2.0 and pH 3.0 to test their toleration ability in acidic food. Bacterial toleration to gastrointestinal system was investigated using 0.3% (w/v) pepsin-supplemented phosphate buffer saline (PBS) (pH 2.0 and pH 3.0), and different concentrations bile salt-supplemented tryptic soy broth (TSB). T-test was used to compare the bacterial survival rates at room temperature and 4ºC, before exposure to GPL, pH 2.0 and pH 3.0.

Results: The results revealed that MRSA and MCSA could tolerate in GPL, pH 3.0 and pH 2.0 for 2 h and 1 h, respectively. Bacterial exposure to 4ºC for 3 h before incubated in GPL, pH 2.0, significantly prolonged bacterial survival ($P < 0.05$). Toleration to simulated gastrointestinal system demonstrated that clinical MRSA strain PSU20 well tolerated to simulated gastric juice, pH 3.0 [0.3% (w/v) pepsin] for at least 1 h with the bacterial survival populations of 4.40 log CFU/ml. In addition, this PSU20 well tolerated to all concentrations of bile salts.

Conclusion: This study suggests that clinical MRSA has potential to transfer from hospital to community through foods and is able to break the gastrointestinal innate immunity establishing infection in human. This is crucial for public health stand point.

Keywords: MRSA, hospital, gastrointestinal tract, *mecA*, bile salt

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แสตฟฟิโลคอคคัส ออเรียส จากโรงพยาบาลที่ดื้อต่อยาเมธิซิลิน อาจถูกถ่ายทอดไปยังชุมชนผ่านทางอาหาร

ภารนัย สุขุมังกูร ปร.ด.[1*]
สุนิษา แดงศรีวัลย์ วท.ม.[1]
ปฐมารัตน์ รัตนช่วย ปร.ด.[2]

[1] ภาควิชาจุลชีววิทยา สาขาวิทยาศาสตร์ชีวภาพ คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ วิทยาเขตหาดใหญ่ หาดใหญ่ ประเทศไทย

[2] ภาควิชาวิทยาศาสตร์ คณะวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยสงขลานครินทร์ วิทยาเขตปัตตานี ปัตตานี ประเทศไทย

* ผู้ติดต่อ, อีเมล: pharanai82@gmail.com

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บทคัดย่อ

วัตถุประสงค์: เพื่อศึกษาความสามารถของ methicillin-resistant *Staphylococcus aureus* (MRSA) และ *mecA*-carrying *Staphylococcus aureus* (MCSA) ในการถ่ายทอดจากโรงพยาบาลไปยังชุมชนผ่านทางอาหารเนื่องจากยีน *mecA* ก่อให้เกิดการดื้อยาต้านจุลชีพอย่างหลากหลาย

วิธีดำเนินการวิจัย: การศึกษาทำเพื่อตรวจสอบความสามารถของ MRSA และ MCSA ว่ามีความสามารถรอดชีวิตในอาหารที่เป็นกรดและในสภาวะระบบทางเดินอาหารเทียมที่สร้างขึ้นได้หรือไม่ แบคทีเรียทั้ง 5 สายพันธุ์ ได้รับการทดสอบความทนกรดใน green papaya salad's liquid portion (GPL) ที่ pH 2.0 และ 3.0 ส่วนความทนทานต่อสภาวะในระบบทางเดินอาหาร ทดสอบโดยการใช้ phosphate buffer saline (PBS) ที่มี pepsin ความเข้มข้นร้อยละ 0.3 (w/v) (pH 2.0 and pH 3.0) และใช้ tryptic soy broth (TSB) ที่มี bile salt ความเข้มข้นต่าง ๆ ส่วนการรอดชีวิตของแบคทีเรียใน GPL pH 2.0 และ 3.0 ที่อุณหภูมิห้องเมื่อเปรียบเทียบกับที่ 4°C ได้รับการวิเคราะห์ทางสถิติโดยวิธี T-test

ผลการวิจัย: ผลการทดลองแสดงให้เห็นว่า MRSA และ MCSA สามารถทนทานสภาวะความเป็นกรดของ GPL pH 3.0 และ 2.0 ได้เป็นเวลา 2 ชั่วโมง และ 1 ชั่วโมง ตามลำดับ การให้แบคทีเรียสัมผัสอุณหภูมิต่ำที่ 4°C เป็นเวลา 3 ชั่วโมงก่อนทดสอบความเป็นกรดใน GPL pH 2.0 พบว่าสามารถทำให้แบคทีเรียมีการรอดชีวิตได้ยาวนานขึ้นอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) การทดสอบความทนทานต่อสภาวะในระบบทางเดินอาหาร พบว่า MRSA สายพันธุ์ PSU20 ที่แยกได้จากผู้ป่วยในโรงพยาบาล สามารถทนต่อน้ำย่อยกระเพาะอาหารสังเคราะห์ที่ pH 3.0 ได้อย่างน้อย 1 ชั่วโมงโดยที่ยังสามารถคงปริมาณเชื้อรอดชีวิตได้ถึง 4.40 log CFU/ml นอกจากนี้ สายพันธุ์ PSU20 ยังทนต่อ bile salt ในทุก ๆ ความเข้มข้นที่ทดสอบอีกด้วย

สรุป: การศึกษานี้แสดงให้เห็นว่า MRSA จากโรงพยาบาล มีความสามารถในการถูกถ่ายทอดไปสู่ชุมชนได้ผ่านทางอาหารและสามารถผ่านระบบภูมิคุ้มกันชนิด innate immunity ในระบบทางเดินอาหารและอาจก่อโรคได้ในมนุษย์ ซึ่งสิ่งเหล่านี้ส่งผลต่อระบบสาธารณสุขโดยรวม

คำสำคัญ: MRSA โรงพยาบาล ระบบทางเดินอาหาร *mecA* เกลือน้ำดี

วันที่รับบทความ 24 พฤศจิกายน 2565 **วันแก้ไขบทความ** 14 ธันวาคม 2565 **วันตอบรับบทความ** 14 ธันวาคม 2565

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a nosocomial pathogen that was observed in the last quarter of 1960[1]. It is frequently found to be the crucial causative agent of human skin abscesses. Phenotypic characteristic of methicillin resistance is contributed by the presence of *mecA* gene coding for penicillin-binding protein2a (PBP2a), responsible for low β-lactam antibiotic affinity[2]. It can cause a wide range of severity upon human infections and its incidence is increasingly reported in hospitals worldwide. In United States, 2003, approximately 400,000 inpatients were reported to be infected by MRSA[3]. In addition, in 2005, the causation of approximately 19,000 hospital deaths in United States was resulted from MRSA infections[4].

Despite that MRSA is able to cause nosocomial infections, the potential MRSA strains carrying important virulence factors are also reported from foods[5-7]. Recent report has described the role of MRSA in acute gastroenteritis outbreaks acquired from the community[8]. The report described the link between the infection of staphylococcal enterotoxin C (SEC)-producing MRSA and the consumption of food from a delicatessen by pulsed-field gel electrophoresis (PFGE). This outbreak was subsequently found to clearly show that foods can act as the important MRSA vehicles.

Our previous report has pointed the high degrees of MRSA and *mecA*-carrying *S. aureus* (MCSA) contamination rates in the ready-to-eat foods sold in the hospital area. MRSA and MCSA strains from the foods and clinical sources were also genetically compared and showed high degrees of genetic similarity[6]. In addition, MCSA strains were found to survive in acidic food such as a green papaya salad (GP) which is consumed worldwide. Therefore, this study aims to investigate the survival capability of MRSA and MCSA strains in acidic food and simulated gastrointestinal system. This may help understanding the transfer of pathogens from hospital to community.

Methods

Bacterial strains

Four important MRSA strains and one MCSA strain isolated from patient, healthy carriers, and ready-to-eat foods, were collected from our previous studies[6,23] using Baird Parker agar, and selected as the surrogates in this study. Characteristics of bacterial strains were described in Table 1.

Survival of MRSA and MCSA in acidic food

Due to in our previous study, MRSA was found in GP in high rate, thus GP was employed as a model to investigate the acid toleration of MRSA and MCSA. Briefly, GP samples were purchased and 99 ml of the green papaya salad's liquid portion (GPL) was separated and kept into the glass bottle (Duran, Germany). The pH of GPL was adjusted to be 3.0 and 2.0 using 1.0 M citric acid (Sigma-Aldrich, USA). Afterwards, GPL were sterilized by autoclave. Tested bacteria were prepared as previously describe[6]. In brief,

Table 1 Characteristics of 4 MRSA strains and a MCSA strain used in this study

Strain	Date of isolation	Virulence genes							Source of isolation	Enterotoxin genes	Reference
		mecA	*luk-PV*	*vWbp*	*spa*	*coa*	*femB*	*sea*			
PSU20	24 Jan 2011	+	-	-	+	+	+	+	Hospital patient	*seg*, *sei*	23
PSU24	25 Jan 2011	+	-	-	-	-	-	-	Healthy carrier	*seg*	23
PSU83	25 Jan 2011	+	-	-	-	-	-	-	Healthy carrier	-	23
[a]PSU109	9 Sep 2013	+	-	-	+	-	-	-	Seasoned rice	*sec*, *sed*	6
PSU172	12 Dec 2013	+	-	-	-	-	-	-	Seasoned rice	-	6

[a]PSU109 carried *mecA* gene but was susceptible to cefoxitin by disk diffusion method, classifying as a MCSA strain.

an individual colony was grown in 5 ml of tryptic soy broth (TSB) at 37°C for 6 h with aeration at 150 rpm. Bacterial cells were washed using 0.85% NaCl solution (NSS) and adjusted to be 0.5 McFarland turbidity standards (approximately 1.5×10^8 cfu/ml) in NSS by Densitometer (Biosan, Latvia). Ten-fold dilution was performed to obtain a working culture (1.5×10^7 cfu/ml) using NSS as the diluent. One milliliter of a working culture was spiked into a 99 ml of GPL, pH 3.0 or pH 2.0, thoroughly mixed, and incubated statically at room temperature for 6 h. Survival of bacteria was monitored at 7 time points (0 to 6 h) by surface plate count on mannitol salt agar (MSA). The experiment was performed in triplicate. Moreover, to test that the cold exposure to bacteria can increase bacterial survival, the effect of cold temperature was also investigated using the same protocols as described above except that a working culture was kept at 4°C for 3 h before adding to GPL, pH 3.0 or pH 2.0.

Survival of MRSA and MCSA in simulated gastric juice

To simulate the condition of gastric system, phosphate buffer saline (PBS), pH 2.0 and pH 3.0, supplemented with 0.3% (w/v) pepsin (Sigma-Aldrich, USA) were used. The experiment was carried out as described by Wang et al[9] with slight modifications. Briefly, a 1 ml of 1.5×10^6 cfu/ml bacterial culture was added into 9 ml of 0.3% (w/v) pepsin-supplemented PBS (pH 2.0 and pH 3.0) and incubated at 37°C for 1 h. Bacterial survival was assessed by surface plate count on MSA. A 0.3% (w/v) pepsin-supplemented PBS, pH 6.2 was used as a control. The experiment was performed in triplicate.

Survival of MRSA and MCSA in bile salt

To simulate the condition of human intestinal tract, bacteria were tested for their toleration in various concentrations of bile salts. The experiment was performed as previously described[10] with slight modifications. In short, a working bacterial culture of 1.5×10^7 cfu/ml was prepared as described above. One milliliter of working culture was spiked into a 99 ml of sterile TSB supplemented with 0.1%, 0.3%, and 0.5% (w/v) of bile salt (Sigma-Aldrich, USA) and incubated statically at 37°C for 4 time points, 0 min, 30 min, 60 min, and 90 min. Bacterial survival was assessed by surface plate count on MSA. Sterile TSB was used as a control. The experiment was performed in triplicate.

Statistical analysis

Data were analyzed using SPSS for Windows software, version 11.0 (SPSS, Chicago, IL). T-test was used to compare the survival rates among bacteria at room temperature and at 4°C, before exposure to GPL, pH 3.0 and pH 2.0. Level of significance was set as $P < 0.05$.

Results

Survival of MRSA and MCSA in GPL

Due to the previous study has shown that MRSA and MCSA strains could well tolerate to GP, pH 4.0[6]. Thus, in this study, GPL was also employed as a model to assess the acid toleration capability of bacteria. At pH 3.0, the results revealed that PSU24 isolated from the throat of a healthy carrier, could withstand this degree of acidity for 3 h with the survival population of 3.16 log CFU/ml (mean value). However, it was at the undetectable limit after 4 h of incubation (figure 1). PSU20 isolated from hospital patient was also able to tolerate with the survival populations at 2 h of 3.11 log CFU/ml. All strains were not detected after 4 h.

The incubation of bacterial culture at 4°C before exposing them to the acidic pH in this current study was carried out since some raw materials are stored at low temperature. Bacterial survival ability at 4°C (pH 3.0) prolonged the bacterial survival time. PSU24 could survive for longer until 4 h with bacterial survival of 2.09 log CFU/ml, compared to the condition at room temperature which was under detection limit. Similar fashion was found in PSU20 and PSU83 (Figure 1). At 4°C (pH 2.0), it was found that cold temperature was capable of elevating the survival rates. At 0 h of incubation, we found the bacterial

population around 3.14 to 3.68 log CFU/ml in all strains. PSU24 also exhibited the highest rate of acid toleration expressing 2.48 log CFU/ml at 3 h of incubation. Furthermore, all strains could survive longer than those stored at room temperature with undetectable limit since 2 h of incubation (figure 1).

Survival of MRSA and MCSA in simulated gastric juice

Low pH of gastric system plays an important role as an innate immunity to destroy pathogens. The results in this experiment showed that MRSA strains from healthy carriers and foods could not tolerate to the simulated gastric juice, pH3.0 and pH 2.0, with 0.3% (w/v) of pepsin supplemented. They were under undetectable limit at 1 h of incubation. However, clinical MRSA strain PSU20 demonstrated a relatively strong toleration to simulated gastric juice, at pH 3.0 with the bacterial survival populations of 4.40 log CFU/ml after 1 h of incubation (figure 2). This may pose the health risk to the humans with its ability to break through an important innate immunity because PSU20 is a Staphylococcal enterotoxin-producing MRSA.

Survival of MRSA and MCSA in bile salt

Bile salts are antibacterial compounds that play the important roles in disruption of several bacterial components, e.g., destroying bacterial cytoplasmic membrane, denaturing proteins, and causing oxidative damage to bacterial DNA[11]. Therefore, we assessed the degree of bile tolerance in MRSA and MCSA in this current study. At 0% bile salt, the well-growth of all strains was

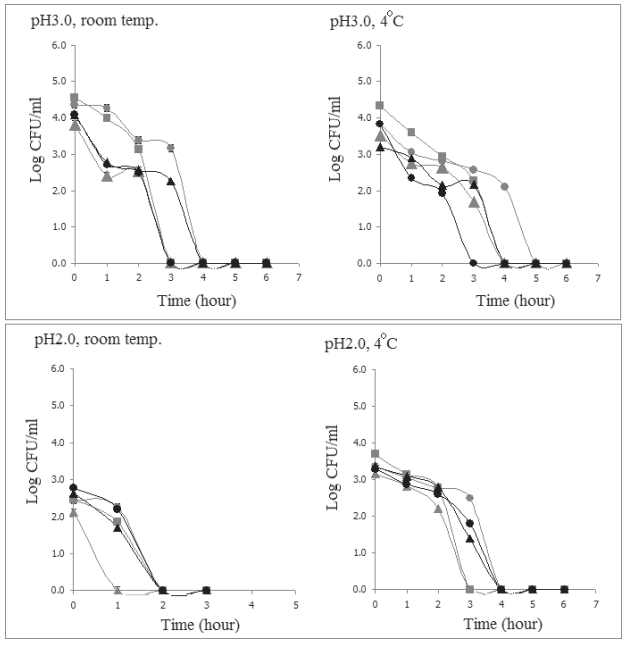

Figure 1 Survival of 4 MRSA strains and a MCSA in 4 storage conditions of GPL, storage at room temperature and expose at pH 3.0, storage at 4°C before storage at pH 3.0, storage at room temperature and expose at pH 2.0, and storage at 4°C before storage at pH 2.0. (■) PSU20. (●) PSU24, (▲) PSU83, (●) PSU109, (▲) PSU172

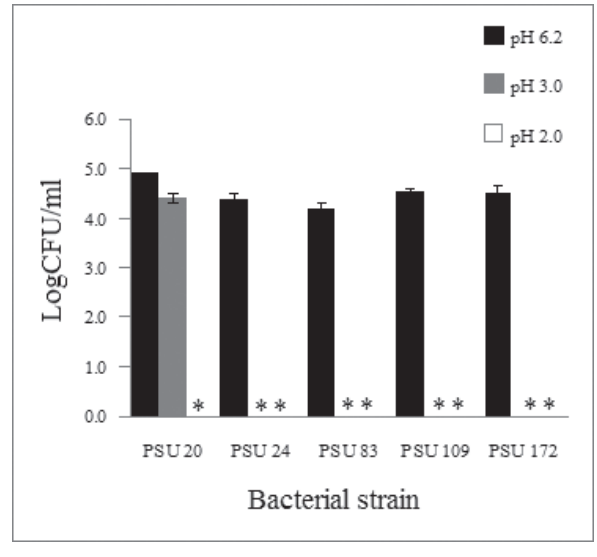

Figure 2 Survival of 4 MRSA strains and a MCSA in the simulated gastric juice (0.3% pepsin), pH 2.0 and pH 3.0 for 1 h of incubation at 37°C. Simulated gastric system (0.3% pepsin), pH 6.2 was used as a control. Asterisk (*) represents the lack of bacterial survivors

observed at 90 min of incubation (figure 3). However, at 0.1% bile salt, PSU172 gradually decreased and exhibited its final bacterial population at 90 min of 2.68 log CFU/ml (Table 3). In addition, this PSU172 was undetectable at 60 min in 0.3% and 0.5% bile salt conditions. Clinical MRSA strain PSU20 was considered unaffected by bile salt throughout the experiment. This suggests that clinical MRSA strains PSU20 from a patient is possible to survive human intestine.

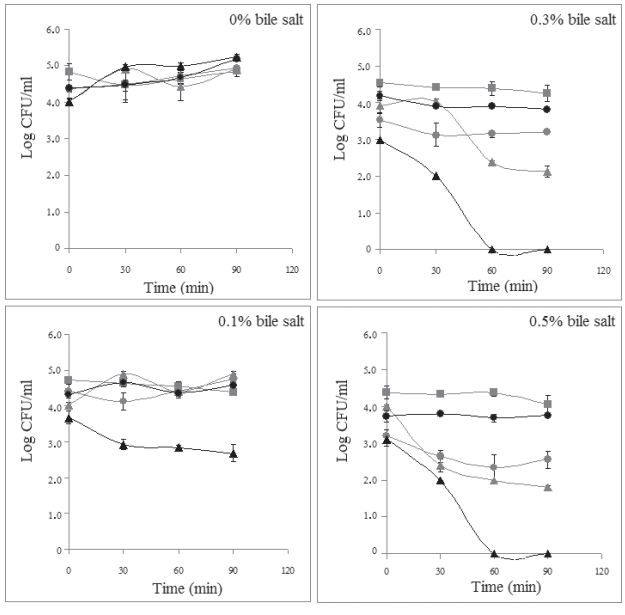

Figure 3 Survival of 4 MRSA strains and a MCSA in TSB supplemented with various concentration of bile salts (0.1%, 0.3%, and 0.5%). (■) PSU20. (●) PSU24, (▲) PSU83, (●) PSU109, (▲) PSU172

Discussion

In this current study, it was found that the bacteria that were exposed to low temperature were tougher in acid toleration than unexposed. There were some reasons that have previously been described. Anderson et al[12] investigated the induction of cold shock responses in *S. aureus* strain UAMS-1. It was grown at 37°C to the mid-log phase before incubated at 10°C for 30 min. It was shown that 46 genes were upregulated. Moreover, genes involved with anti-programmed cell death, *irgA*, *irgB* and several virulence genes were also induced. Cold shock gene, *cspB* (*csp* stands for cold shock protein) was induced 9.3 folds compared to the uninduced condition. Also, *cspA* was upregulated for 2 folds. This *cspA* was found to share the homology with other bacterial species and was shown to act as RNA chaperones to prevent RNA secondary structures, allowing the RNA to smoothly perform its biological roles in the bacterial cells[13]. Raju et al[14] also demonstrated that a stepwise adaptation of methicillin-susceptible *S. aureus* to oxacillin produced the greater resistance to lactic acid and citric acid, facilitating the survival of bacteria in gastric juice. It was thought that in the person who received antimicrobial agent whose mechanism of action was similar to or involved with oxacillin for treatment of infections (e.g. dicloxacillin), may be at higher risk in infection through consumption of acidic food contaminating MRSA or MCSA strains.

Although the favorable pH required for the growth of *S. aureus* was reported to be the range of 4.5-9.3[15], MRSA and MCSA strains in this present study were shown to be much higher tolerated to acidic pH and these bacterial species were also found in GP in high rates[6]. This result infers that MRSA and MCSA are able to retain their high number for at least 6 h at pH 4.0 (general pH level in GP) and can survive until they reach the human gastrointestinal tract. The acid toleration of *S. aureus* is not frequently reported. Most of *S. aureus* strains were reported to be suppressed in acidic condition. Abu-Ghaza et al[16] showed that 0.03% citric acid significantly inhibited clinical *S. aureus* growth. In addition, in one study, citric acid was shown to act as an effective permeabilizer killing Vancomycin Intermediate *S. aureus* (VISA) and MRSA isolated from orthopedics surgical site of infection[17]. Furthermore, one report from Nagoba et al[18] demonstrated the employment of 3% citric acid gel in the treatment of diabetic foot ulcers with *S. aureus* infections and this concentration of citric acid was effective in control of foot infection with the success rate more than 94% for Wagner grade I and II ulceration. These studies suggest that clinical *S. aureus* is sensitive to citric acid, which was opposite to our study that showed that a clinical MRSA was resistant to citric acid. Collectively, the finding that MRSA and MCSA in this current study tolerates to acidic condition results in the conclusion that GP can also be a potential vehicle of food-borne MRSA infections.

Lactic acid bacteria with the potential to be the probiotic bacteria can tolerate gastric juice in high rate[19] but for other group of bacteria it seems to possess the less ability in such a stringent condition. In Gram-negative bacteria, Zhu et al[20] demonstrated that the clinical *E. coli* strain 690 and *Helicobacter pylori* strain E5 were more susceptible to acidic condition with 1 mg Pepsin more than the control (without Pepsin). The toleration of simulated gastric juice of PSU20 in this experiment was thought to be crucial because it has high possibility to make an establishment of infection to the intestine. Furthermore, this PSU20 contains Staphylococcal enterotoxin G and Staphylococcal enterotoxin I genes (table 1), which is able to cause Staphylococcus food poisoning. These enterotoxins are found to be heat stable and resisted from proteolytic enzyme destruction such as pepsin, trypsin and also can keep their toxic activity[21].

Bile salt is secreted from the liver and acts as an innate defense mechanism of intestine[22]. Although *S. aureus*, a Gram-positive bacterium, is thought to be sensitive to bile salt, its resistant phenotype can be frequently observed. Recently, the work from Sannasiddappa et al[22] has uncovered the underlying mechanism by which *S. aureus* was able to tolerate to bile salts. The *mnhF* gene is found to confer such a tolerance by coding MnhF protein, a Na^+/H^+ antiporter subunit F1 responsible for sodium ion and proton ion secretions as well as bile salt efflux. The *mnhF* gene is in a *mnhABCDEFG* operon but only *mnhF* alone was shown to be enough to confer bile salt tolerance. We accessed the data in the National Center for Biotechnology Information (NCBI) and used the *mnhF* nucleotide sequence (294 nucleotides) for searching and found that *mnhF* gene was present in a wide variety of *S. aureus* and MRSA strains, for instance, MRSA strain DAR4145, MRSA USA300 (data not shown). These sequences exhibited 100% identity. Thus, it has a possibility that *mnhF* may equipped in MRSA strains in this present study and play a role in bile salt tolerance.

Conclusion

Although previous reports have frequently described the susceptibility of *S. aureus* including MRSA to organic acids including citric acid, MRSA and MCSA in this current study exhibited certain degrees of acid tolerance. More importantly, a clinical MRSA strain PSU20 from hospital could also survive in gastrointestinal system in high numbers. The MRSA equipped with Staphylococcal enterotoxins and other virulence factors including antimicrobial resistance, can lead to severe illnesses and can prolong the hospital stay. Thus, this study demonstrates the existence of a pathogenic MRSA strains that has potential to transfer from hospital to human through foods. This is crucial to the public health standpoint.

Conflicts of interest

The authors declare no conflicts of interest in this study.

Acknowledgement

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References

1. Jevons MP. "Celbenin" - resistant Staphylococci. Br Med J 1961;1(5219):124–5.
2. Utsui Y, Yokota T. Role of an altered penicillin-binding protein in methicillin- and cephem-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 1985;28(3):397-403.
3. Noskin GA, Rubin RJ, Schentag JJ, Kluytmans J, Hedblom EC, Jacobson C, et al. National trends in *Staphylococcus aureus* infection rates: impact on economic burden and mortality over a 6-year period (1998-2003). Clin Infect Dis 2007;45(9): 1132-40.
4. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA 2007;298(15):1763-71.
5. Bunnoeng N, Themphachana M, Pewleang T, Kongpheng S, Singkhamanan K, Saengsuwan P, et al. High prevalence and molecular characterization of methicillin-resistant

Staphylococcus aureus isolated from retailed meats, south Thailand. Int Food Res J 2014;21: 569-76.

6. Bunnueang N, Kongpheng S, Singkhamanan K, Saengsuwan P, Rattanachuay P, Dangsriwan S, et al. Methicillin-resistant *Staphylococcus aureus* from ready-to-eat foods in a hospital canteen, southern Thailand: virulence characterization and genetic relationship. Southeast Asian J Trop Med Public Health 2015;46:86-96.
7. Crago B, Ferrato C, Drews SJ, Svenson LW, Tyrrell G, Louie M. Prevalence of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) in food samples associated with foodborne illness in Alberta, Canada from 2007 to 2010. Food Microbiol 2012;32(1):202-5.
8. Jones TF, Kellum ME, Porter SS, Bell M, Schaffner W. An outbreak of community-acquired foodborne illness caused by methicillin-resistant *Staphylococcus aureus*. Emerg Infect Dis 2002;8(1):82-4.
9. Wang CY, Lin PR, Ng CC, Shyu YT. Probiotic properties of Lactobacillus strains isolated from the feces of breast-fed infants and Taiwanese pickled cabbage. Anaerobe 2010;16: 578-85.
10. Tulini FL, Winkelströter LK, De Martinis EC. Identification and evaluation of the probiotic potential of *Lactobacillus paraplantarum* FT259, a bacteriocinogenic strain isolated from Brazilian semi-hard artisanal cheese. Anaerobe 2013;22:57-63.
11. Urdaneta V, Casadesús J. Interactions between bacteria and bile salts in the gastrointestinal and hepatobiliary tracts. Front Med (Lausanne) 2017;4:163.
12. Anderson KL, Roberts C, Disz T, Vonstein V, Hwang K, Overbeek R, et al. Characterization of the *Staphylococcus aureus* heat shock, cold shock, stringent, and SOS responses and their effects on log-phase mRNA turnover. J Bacteriol 2006;188(19):6739-56.
13. Jiang W, Hou Y, Inouye M. CspA, the major cold-shock protein of *Escherichia coli*, is an RNA chaperone. J Biol Chem 1997;272(1):196-202.
14. Raju S, Rao G, Patil SA, Kelmani CR. Increase in cell size and acid tolerance response in a stepwise-adapted methicillin resistant *Staphylococcus aureus* mutant. World J Microbiol Biotechnol 2007;23:1227-32.
15. Lawley R, Curtis L, Davis J. The food safety hazard guidebook. 2nd ed. Cambridge: RSC Publishing; 2008. P7-126.
16. Abu-Ghaza BM. Effects of ascorbic acid, citric acid, lactic acid, NaCl, potassium sorbate and Thymus vulgaris extract on *Staphylococcus aureus* and *Escherichia coli*. Afr J Microbiol Res 2013;7:7-12.
17. Thool VU, Wadher BJ, Bhoosereddy GL. Citric acid: a prospective permeabilizer for treatment of VISA infection. Int J Curr Microbiol Appl Sci 2014;3:177-83.
18. Nagoba BS, Gandhi RC, Wadher BJ, Rao A, Hartalkar AR, Selkar SP. A simple and effective approach for the treatment of diabetic foot ulcers with different Wagner grades. Int Wound J 2010;7(3):153-8.
19. Lei M, Dai X, Liu M. Biological characteristics and safety examination of five enterococcal strains from probiotic products. J Food Saf 2015;35(3):324-35.
20. Zhu H, Hart CA, Sales D, Roberts NB. Bacterial killing in gastric juice-effect of pH and pepsin on *Escherichia coli* and *Helicobacter pylori*. J Med Microbiol 2006;55(Pt 9):1265-70.
21. Bhatia A, Zahoor S. *Staphylococcus aureus* enterotoxins: A review. J Clin Diagn Res 2007;1: 188-97.
22. Sannasiddappa TH, Hood GA, Hanson KJ, Costabile A, Gibson GR, Clarke SR. *Staphylococcus aureus* MnhF mediates cholate efflux and facilitates survival under human colonic conditions. Infect Immun 2015;83(6): 2350-7.
23. Sukhumungoon P, Hayeebilan F, Yadrak P, Kanobthammakul S, Nakaguchi Y, Saengsuwan P, et al. Molecular characterization and relationship of methicillin-resistant *Staphylococcus aureus* among strains from healthy carriers and University hospital patients, southern Thailand. Southeast Asian J Trop Med Public Health 2014;45:402-12.