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Assessing *Staphylococcus aureus* in ready to eat (RTE) food and risk assessment of food premises in Putrajaya

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Abstract

This cross sectional study was conducted to determine of the presence of *S. aureus* in ready-to -eat (RTE) food, food premise sanitation level and the relationship between *S. aureus* presence in RTE food and the sanitation levels of food premises in the locality of the study. A total of 106 samples of RTE food were analyzed by MPN-PCR and the results confirmed that 56 (53%) samples contained *S. aureus*. Surveyed by type, cooked RTE food had 50 (45%) of the sample having *S. aureus* presence compared to raw RTE with only 56 (55%). As for risk assessment, a checklist containing nine (9) main parameters consisting of 40 variables as a basis, was conducted on 53 food premises to determine their sanitation levels. The results revealed that 49 premises (92%) are sanitary while the remaining 4 (8%) were categorized as unsanitary. In terms of risk level, 4 premises (8%) were categorized as 'high risk', 26 (49%) 'moderate risk', and the remaining 23 (43%) are at 'low risk'. The study found no significant relationship between the presence of *S. aureus* in RTE food with the level of sanitation of premises in Putrajaya.

Introduction

Food borne diseases caused by microbiological agents are major problems faced by developing countries such as Malaysia (Satcher, 2000). As a developing country, this problem is one of the main public health problems in Malaysia (Satcher, 2000), and happens due to a probable change of decision making involving food production, storage, and consumption, as well as in the globalization and liberation of food trade and the importance of food (Abdelgadir *et al*., 2009).

The incident of foodborne diseases especially food poisoning is on the rise worldwide and becomes an important issue in public health because of its fast contagiousness plus lethality (Meldrum *et al*., 2006; Nguz, 2007). In Malaysia, statistic for the year 2011 reported 1629 cases of food poisoning and zero fatalities (MOH, 2012).

S. aureus is one of the major bacterial agents causing foodborne disease in humans worldwide (Le Loir *et al*., 2003; EFSA, 2010). The lethal enterotoxin produced by *S. aureus* are common causes of food poisoning (Dinges *et al*., 2000), pneumonia, wound infections and nosomial bacteraemia (Tiemersma *et al*., 2004). *S. aureus* produces staphylococcal enterotoxins (SEs) in contaminated food, and ingestion of SEs containing food can induce severe symptoms, including vomiting and high fever with/ without nausea and diarrhea, with rapid onset in typically less than 8 hours (usually between 3 and 4 hours) (Jett *et al*., 2001).

Hosts of *S. aureus* are food handlers (Cogan *et al*., 2002), especially at nasal areas and hands which is the vehicle of enterotoxigenic *S. aureus* by food handlers are important source of staphylococcal food contamination in restaurants and food outlets (Colombari *et al*., 2007). According to Taylor *et al*. (2000), there is an evidence to show that microorganisms are transferred to the hand in the process of handling food and through poor personal hygiene among food handlers. The presence of

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S. aureus or its enterotoxins in processed food is generally an indication of poor sanitation (Reginald and Gayle, 2001).

The objective of this study was to identify the association between the presences of *S. aureus* in RTE food and the sanitation level of food premises in Putrajaya. This study will provide the new baseline data, which will build and extend the index of *S. aureus* food contamination and relieve the insufficient data on microbiological hazards. The data can be used to establish the implementation of control measures as well as to improve the sanitation level of food premises and specially the food handlers' practices. This was also conducted to survey on whether the food premises which sell the ready to eat food complied with the legal requirements.

Materials and Methods

Risk assessment checklist

Risk assessment of food premises was carried out using a checklist which was adapted from food premises evaluation form issued by the Ministry of Health (MOH) and the Ministry of Urban Wellbeing, Housing and Local Government (UHLG). The form has been modified and developed further to fit the format scale of three (3) levels which are determined as low, moderate and high risk depending on which of the descriptions provided on the form best matches the appearance or practices observed. Pre test of assessment form was carried out and found to be consistent and practical to be use in the study. The full score of the checklist is 27. Low risks represent the premise with the score between 23 – 27, moderate risk (16 – 22) and the range of high risk score is 9 – 15. Meanwhile the determination of the sanitary and unsanitary status will be based on the total marks in the form of percentage. The total mark is 40 and the premises will be categorized as 'sanitary' with the marks of 20 and above. Meanwhile, the premises with marks below than 20 will be categorized as 'unsanitary'.

This form is used for assess nine (9) parameters that have 40 variables within it. Among the parameters that are assessed are raw materials (8 variables), food preparation (4 variables), food handlers (4 variables), equipment (4 variables), storage and serving (4 variables), pest control (4 variables), water supply (4 variables), sanitation facilities, drainage and waste disposal (4 variables), and building premises (4 variables). This assessment aims to evaluate or measure the level of sanitation of the premises and the safety of food sold. In this study of 53 food premises around Putrajaya were assessed level of sanitation. Those premises are serving RTE food. The premises evaluation was conducted by two (2) researchers within the time between 9.00 am to 2.00 pm. The assessment was done according to the schedule and the premises were not informed before the assessment.

Sample collection

The sampling determination was conducted according to the total number of food premises which serving RTE food in Putrajaya. Collection of food samples were carried out on 53 food premises with two (2) samples were purchased from any premises designated by a cumulative total of 106 samples were collected from the field. Samples obtained from food that is RTE either cooked or uncooked (raw material). Examples of samples that have been cooked are fried chicken, cooked meat curry and so on while examples from vegetables that are served raw (salad vegetables). Samples was purchased and sealed into a clean zip locked plastic bag and kept in an ice box. The samples were sent to the Food Safety and Quality 2 Laboratory, University Putra Malaysia (UPM) on the same day to be analysed.

Sample processing

Samples were first cut into small portions of food before it were put into stomacher bag (Interscience, France). Then 10g of the sample was weighed before it is put on top of the weighing scale as much as 90 ml trypticase soy broth (TSB). The sample bag is placed into stomacher machine for the purpose of blending the sample and produce samples that were homogenize for 60 seconds.

MPN test

The MPN test is run to calculate the number of probable numbers *S. aureus* in food samples. In this technique, samples are serially diluted to 1:10, 1:100 and 1:1000 and transferring subsamples of each dilution to three tubes. For the three tubes MPN method, 1ml aliquot from each dilution was transferred into triplicate MPN tubes, and then incubated at 37ºC for 48 hours. The content of each tube was checked for turbidity after 2 days of incubation. The turbid tube was selected and the DNA was extracted using boiling cell method for PCR detection *S. aureus*.

Extraction of genomic DNA

Genomic DNA from the MPN tubes was extracted using boil cell method, as described by Kawasaki *et al*., (2005). MPN tubes that turned cloudy after incubation were centrifuged at 13,400 x g for 1 minute. The supernatants were discarded and

500 µl of distilled water was added to the tubes to resuspend the pellet. Next, the suspension was boiled for 10 minutes and after that, the suspension was immediately cooled for another 10 minutes. Finally, the tubes were centrifuged again at 13,400 x g for 3 minutes. The clear supernatants were transferred to sterile new microcentrifuge tubes to be kept at -20°C for further study purposes.

Polymerase chain reaction

PCR detection of *S. aureus* was performed on a thermocycler (Applied Biosystems 2720 Thermal Cycler, USA). The lists of primers and their oligonucleotide sequences are as shown in Table 1. Two µl of DNA boiled lysate from MPN tubes were added to PCR mixture which made up into a 25 µl reaction mixture; 5 µl of 5× PCR Buffer, 1.5 mM of $MgCl_2$, 0.5 mM of deoxynucleoside triphosphate mix, 0.8 µM of *S. aureus* forward and reverse primers, and 1 U/µl of Taq polymerase. Amplification condition used was 4 min at 96°C for pre-denaturation; following that, was 35 cycles of denaturation at 94°C for 45 s, annealing at 59°C for 45 s, extension at 72°C for 45 s and a final round of extension at 72°C for 7 min. The PCR products were electrophoresed on 1% (w/v) agarose gel in 5× TBE Buffer for 23 minutes at 100 V and visualized under ultraviolet light using computer software (Gel Documentation System, SynGene, UK).

Statistical analysis

Statistical Package for the Social Sciences (SPSS) software (Version 18.0) was applied to determine whether there is any significant association between the presence of *S. aureus* in RTE food and the sanitation level of food premises in Putrajaya. The significant level was set at $p < 0.001$ and value of association was carried out depend on R's value. The association is assume strong or more if value of $r > 1$.

Results and Discussion

Presence of S. aureus *in RTE Food*

During the sampling period between August to September 2013, a total of 106 food samples were taken from 53 RTE food premises in the locality of the study. The result showed that 50 (amounting to 47%) samples consist of raw RTE food, while 56 (53%) were cooked RTE food (Table 2). Of the 50 raw RTE food sampled, 31 (55%) was found to be contaminated with *S. aureus* by MPN-PCR method, while 25 out of 56 samples of cooked RTE food from food premises in Putrajaya harboured *S. aureus* (Table 2). In generally, 36 out of 53 food premises (68%) served *S. aureus* contaminated food to the consumer. The MPN-PCR enumeration method detected 705.1 MPN/g of *S. aureus* in the contaminated RTE food. The bacterial load of *S. aureus* was lower in raw RTE food (220.2 MPN/g) than cooked RTE food (484.9 MPN/g) (Table 2).

Table 1. Base sequence of oligonucleotide primers

Target Group	Primer	Oligonucleotide Sequences (5'? 3')	References
S. aureus	nuc F (forward)	GCGATTGATGGTGATA CGGTT	Subhankari *et* al., 2011; Biswajit Saha *et al.*, 2008; Brakstad *et al.*, 1992.
	nuc R (reverse)	AGCCAAGCCTTGACGA ATAAAGC	

Table 2. Number of samples taken, detection and bacterial load of *S. aureus*

RTE Food	No. of Sample Taken		No. of *S. aureus* Detected		No. of *S. aureus* Non-Detected		Bacterial Load of *S. aureus* (MPN/g)
	n	%	n	%	N	%	
Raw	50	47	31	55	19	38	220.2
Cooked	56	53	25	45	31	62	484.9
Total	106	100	56	100	50	100	705.1

A study conducted by Sazidah (2006), found out that total coliform is the major cause of microbiological contamination in food samples followed by *Esherichia coli*, total plate count, *Bacillus cereus, Staphylococcus aureus* and *Salmonella* spp. The results recording the presence of *S. aureus* in food samples studied, were similarly obtained and supported in studies conducted in Southeast Asia, e.g. in Thailand there was detection in 10.8% of food sample taken (Chomvarin *et al.*, 2006), and in Vietnam, the detection was 21.2% out of 212 of samples (Bui *et al.*, 2010).

In the East Asian region, studies in Korea have shown that RTE foods are regularly contaminated with *S. aureus*. Study by Normanno *et al.*, (2005) shown that the most frequently contaminated food by *S. aureus* are cream cakes involving 31.6% of analysed samples. This may be due to the content of dairy products such as cream which are often the main cause of the occurrence of staphylococcal food poisoning.

According to Colombari *et al.*, (2007), RTE food, especially salads and sandwiches are among the main causes of the incident or outbreak of foodborne illness because this category of food is often prepared by hand and served cold, which may increase the

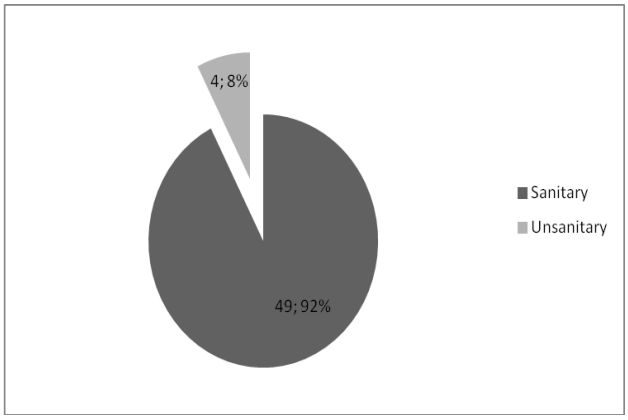

Figure 1. Level of sanitation of food premises

incidence of contamination with potential foodborne pathogens. This coupled with the *S. aureus* bacteria that is opportunistic pathogen, most likely to grow on food with reduced water activity and increased percentage of water phase salt i.e. ingredients of sandwiches such as cooked meat products, dry fermented sausage and cheese.

The results of this study also recorded both types RTE sample of raw and cooked detected the presence of *S. aureus*. This is in line with studies conducted by Sospedra *et al.*, (2013) which involved 14 samples, including both cooked and fresh vegetables (11 lettuce, 2 french beans and 1 potato) found to be contaminated by *S. aureus*. However in this study, analysis discovered that among the food samples positive for the presence of *S. aureus*, 55% were raw RTE food samples while the remaining 45% were cooked RTE food. This shows that raw RTE foods sample were more contaminated by *S. aureus* and is posing higher risk as compared to cooked RTE food. The study done by Shou-kui *et al*., (2013), found out that 10% *S. aureus* was detected in cooked meat compared to raw meat. The contamination of raw meat was significantly higher than cooked meat, indicating that the high temperature cooking can kill *S. aureus* effectively and contamination levels could be effectively reduced by improving sanitation and hygiene procedures. Furthermore, studies by Claudia and Maria, (2013), found the incidence of *S. aureus* ranged from 2.3% for 'fully cooked food for immediate sale or consumption' to 10.8% for 'raw fruits and vegetables ready for consumption'. These results could be due to inadequate cooking, improper holding temperatures, or poor personal and equipment cleanliness.

Sanitation level of food premises

The result of level of sanitation showed that (92% out of 53) premises obtained enough marks to be classified in the 'sanitary' category and only 4 (8%) premises are in the 'unsanitary' level according to the study's marking scale (Figure 2). This study also showed the number of food premises categorized according to their risk levels based on assessed parameters. From the total of all premises assessed, 4 (8%) premises are of high risk, 26 (49%) moderate risk and the rest 23 (43%) are of low risk.

Assessment of all food premises in the locality of the study, found that 49 premises (92%) are in hygienic condition and satisfactory, but only four (4) of the premises (8%) scored below a specified level or unsanitary which showed that the sanitary level of premises in the study locality is high. The same condition was done in a study by Tebbut (1991). The study was done towards retail premises which were recorded as much as 91% out of 1339 premises. These premises were categorized as 'good' and 'satisfactory' in condition. Zaid and Jamal (2011), in their study done in Melaka, Malaysia found that 99% premises achieved high merit marks. The scores were equal or more than 50 (mean score for food premise inspection) which was 77.21 ± 10.32.

Therefore, the most important objective is to ensure the sanitation of food premises is improved which is closely related to hygiene practices among food handlers as it can reduce the level of contamination by bacteria effectively on the premises (Shou-kin *et al.*, 2013). Moreover, according to Meldrum *et al.,* (2009), the proposed enforcement action by the the Authority Officer should be implemented seriously involving aspects such as assessment and sampling food premises to prevent the incidence of the disease occurring. Next, new sterilization or microbial inactivation techniques optimized for end products without quality deterioration (e.g. antimicrobial gas or supercritical carbon dioxide treatment) also need to be developed and practically applied to assure the microbiological safety (Lee *et al*., 2006; Jung *et al*., 2009; Kim *et al*., 2010).

Association between the presence of S. aureus *in RTE food and the sanitation level of food premises*

The result shows there is no significant correlation between the presence of *S. aureus* in RTE food with the level of sanitation of food premises in the locality of study ($r = -0.113$, $n = 106$, $p > 0.001$). Meanwhile, determination of the correlation between the presences of *S. aureus* in RTE food with nine (9) parameters which were contributed to the level of sanitation of food premise was conducted. The results show that there is no a significant correlation between the presence of *S. aureus* in RTE food with seven (7) parameters. They are raw material ($r = -1.73$, $n = 106$, $p > 0.001$), food preparation ($r =$

-0.28, n = 106, p > 0.001), building premises (r = -0.49, n = 106, p > 0.001), equipment (r = -1.85, n = 106, p > 0.001), storage and serving (r = -1.61, n = 106, p > 0.001), pest control (r = -0.013, n = 106, p > 0.001) and sanitation, drainage and waste disposal (r = -0.178, n = 106, p > 0.001). Otherwise the other two (2) parameters show that there are a significant correlation between the presences of *S. aureus* in RTE food. The parameters are water supply (r = -0.244, n = 106, p < 0.001) and building premise (r = -0.299, n = 106, p < 0.001).

The result shows no significant correlation between the presence of *S. aureus* in RTE food with the level of sanitation of food premises, it is supported by the study by Zaid and Jamal (2011) which stated that the microbiological contamination in food samples and food handlers practice are not related to the health status and level of sanitation operators of food premises. Furthermore Powell and Attwell (1995) stated that there are no consistent trend in the relation between microbial examination of food sampled and total rating inspection of food premises. In the study by Tebbutt (1991), there was no significant strong relationship between the variables in food microbiology with visual assessment of food premises.

However, the statement is contrary to Zaliha's study (2003) which found that food premises with low ratings of less than 50 marks were significantly associated with microbiological contamination of food samples obtained. Zaid and Jamal (2011) stated that the premises which have a good level of sanitation (clean assessment) have no correlation with the status of the cleanliness of food handlers practice or hygiene of the premises. This is because in his study, healthy food handlers had *S. aureus* detected in nasal swab tests. Compared to the past research, this study's analysis has suggested a correlation between the microbiological contamination in food samples with poor handling practices and the status of food handlers' personal hygiene.

In terms of food handling practices among food handlers, Sazidah (2006), found no significant relationship between the factor and the microbiological contamination of food samples. On the other hand, this study found that microbiological contamination of food samples was not significantly associated with food handler's healthy carrier status of pathogenic food bacteria and food premise sanitation level.

Conclusions

The study in the Putrajaya locality did find significant presence of *S. aureus* in RTE food, namely in 53% (56 out of 106) of samples collected. A total of 49 premises (92% out of 53) had their cleanliness level at 'sanitary', but only 4 premises (8%) is at 'unsanitary' i.e. below the specified level of cleanliness. There is no significant relationship between presence of *S. aureus* in RTE food and sanitation level of food premises.

Finally, to establish safer food chain in global market, assuring the microbiological safety of RTE food and protecting consumers against outbreaks of food poisoning is imperative. This requires monitoring of cleanliness of food premises and food safety on a regular and more active basis, then carrying out a more comprehensive and effective law enforcement activities by the authorities.

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