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Prevalence and antimicrobial resistance of *Staphylococcus aureus* isolated from vegetables sold in Malaysian wet markets

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ABSTRACT

Aims: *Staphylococcus aureus* is the most common pathogen found in humans, animals and foods worldwide. Vegetables contain essential vitamins, minerals, and fibres that may aid in protecting humans from chronic diseases and promote good health. This investigation aimed to find the prevalence of *S. aureus* contamination and antimicrobial resistance pattern of isolates from leafy vegetables in Peninsular Malaysia.
Methodology and results: A total of 397 samples, comprised of 16 different vegetables, were obtained from vendors in selected wet markets. Of the 397 samples, *S. aureus* was detected in 42 samples (10.6%) in which 9 (21.4%) were positive for methicillin-resistant *Staphylococcus aureus* (MRSA). The *S. aureus* isolates showed 52.3% resistance to tetracycline, followed by kanamycin (40.5%), penicillin G (35.7%), streptomycin (33.3%), oxacillin (21.4%) and cefoxitin (19.0%). The multiple antibiotic resistance indexes of *S. aureus* isolates varied from 0.21 to 0.50. The predominant antimicrobial resistance profiles of *S. aureus* were PDaOxSTeL (n=5), PDaSTeCe (n=4), TeKCnSxtC (n=3) and TeKCipQd, respectively.
Conclusion, significance and impact of study: The findings of this study revealed the consumption of raw or minimally prepared fresh leafy vegetables could be a possible source of infection with antimicrobial-resistant *S. aureus*.

Keywords: Staphylococcus aureus, antibiotic resistance, vegetables

INTRODUCTION

Leafy vegetables are well known as essential parts of a healthy and nutritious diet. Several countries, including Malaysia, have begun initiatives to inspire consumers to increase their consumption of these products (Denis *et al*., 2016). However, fresh vegetables have been increasingly recognised to be among the leading food vehicles of foodborne microbial pathogens or hazards (Lynch *et al*., 2009). Besides, fresh vegetables are also considered to harbour significant numbers of epiphytic microorganisms, mainly non-pathogenic (Alegbeleye *et al*., 2018). In recent years, there have been a rising number of outbreaks of fresh produce-related foodborne illnesses in many parts of the world, and attempts are being made to resolve these food safety harms (Jongman and Korsten, 2018).

Staphylococcus aureus is a major foodborne pathogen causing food intoxication worldwide (Kadariya *et al*., 2014). This pathogen can contaminate numerous food products, including fresh vegetables and produce several kinds of enterotoxins, causing diseases with differing severity from superficial skin diseases to severe and potentially fatal, invasive ones (Bennett *et al*., 2013). Studies published in Korea have shown that leafy vegetables such as Lettuce, sprouts, and perilla leaves, could be contaminated by *S. aureus* (Moon *et al*., 2007; Seo *et al*., 2010). In Malaysia, also, *S. aureus* is a well-known pathogen associated with food poisoning (Fluit, 2012). Although some studies reported the bacterial contamination of raw leafy vegetables (Wadamori *et al*.,

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2017), not much has been done in the area of *S. aureus* contamination (Puah *et al*., 2016).

There has been an alarming surge in antibiotic-resistant *S. aureus* in both developed and developing countries over the past few decades (Fair and Tor, 2014; Kashani *et al*., 2018). The excessive use of antimicrobial agents as prophylactic, therapeutic and growth promoters in food animals has steered to the emergence of resistant strains and remains a significant public health challenge (Medina and Pieper, 2016). In many countries, Penicillin has remained the antibiotic of choice to treat *S. aureus* infections (Kali, 2015). However, the production of an enzyme called penicillinase or beta-lactamase causes resistance in several strains of *S. aureus* to a penicillin (Jamali *et al*., 2015). Most isolated *S. aureus* strains from foods and animals are increasingly resistant to many frequently used antibiotics including methicillin, penicillin, streptomycin, macrolides, gentamicin and tetracycline (Alenizi, 2014; Jamali *et al*., 2015). Nevertheless, the resistance patterns of isolated *S. aureus* from fresh leafy vegetables in recent years show the emergence of multi-drug resistance (MDR) *S. aureus* strains which may cause a substantial threat to human health in Malaysia (Seo *et al*., 2010; Dhama *et al*., 2014). Therefore, this study aimed to examine the prevalence and antimicrobial resistance of *S. aureus* isolated from raw leafy vegetables obtained from wet markets in Malaysia.

MATERIALS AND METHODS

Sampling

In this study, a total of 397 samples comprised of 16 different raw leafy vegetables were purchased from selected wet markets located in four cities of Malaysia from May 2015 to March 2016. The selected vegetables were Indian pennywort (Pegaga), amaranth red (Bayam), sweet basil (Selasi), bean sprout (Kecambah), coriander (Ketumber), lettuce salad (Daun salad), spring onion (Daun bawang), Japanese parsley (Selom), winged bean (Kacang botol), laksa leaves (Kesom), amaranth green (Bayam), iceberg lettuce (Lettuce ais), mint (Daun pudina), wild parsley (Ulam raja), water spinach (Kankong) and Chinese flowering cabbage (Sawi bunga). The samples were collected in sterile plastic bags and transported instantly to the laboratory for analysis.

Isolation and detection of *S. aureus*

For the detection and isolation of *S. aureus*, 25 g (raw leafy vegetable) of each sample was added 225 mL of buffered peptone water (Merck, Germany) and homogenised. The samples were streaked onto Baird-Parker Agar (Merck, Germany) with an added 5% egg yolk and tellurite (Merck, Germany). The plates were then incubated at 37°C for 24-48 h. The suspected colonies with a typical black display in white circles on the Baird-Parker Agar were enumerated as *S. aureus* (Loncarevic *et al*., 2005; Jamali *et al*., 2015). The API Staph identification system was used to confirm suspected colonies (bioM-erieux, Marcy-l'-Etoile, France).

Antimicrobial susceptibility assay

The resistance to antimicrobial agents was performed by the Kirby-Bauer disc diffusion method using ready antibiotic disks (Oxoid, UK) on Mueller-Hinton agar (Merck, Germany) according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2006). The following fourteen panels of antimicrobial agents (Oxoid, UK) were used: tetracycline (30 mg), kanamycin (30 mg), gentamicin (10 mg), penicillin G (10 U), oxacillin (1 mg), chloramphenicol (30 mg), erythromycin (15 mg), tobramycin (10 mg), lincomycin (15 mg), ciprofloxacin (5 mg), quinupristin-dalfopristin (15 mg), clindamycin (2 mg), trimethoprim-sulfamethoxazole (1.25/23.75 mg) and streptomycin (10 mg). Escherichia coli ATCC 35218 were used for quality control.

PCR for the amplification of the *mecA* gene

DNA templates were prepared using crude cell lysates as specified by Zhang et al. (2012). A PCR using the primers *mecA1* (5 AAAATCGATGGTAAAGGTTGGC) and *mecA2* (5-AGTTCTGCAGTACCGGATTTGC), was applied for the determination of *mecA* gene using conditions as reported by Murakami *et al*. (1991).

Table 1: Prevalence of *S. aureus* in various raw leafy vegetable samples obtained from wet markets.

Samples	No. of Samples	Samples positive (%)	Prevalence (%)
Amaranth green	25	6	24.0
Amaranth red	25	2	8.0
Bean sprouts	25	3	12.0
Coriander	25	4	16.0
Water spinach	25	3	12.0
Winged bean	25	2	8.0
Laksa leaves	25	1	4.0
Iceberg lettuce	25	2	8.0
Mint	25	3	12.0
Spring onion	25	1	4.0
Indian pennywort	25	5	20.0
Wild parsley	25	2	8.0
Lettuce salad	23	1	4.3
Chinese flowering cabbage	25	3	12.0
Sweet basil	24	3	12.5
Japanese parsley	25	1	4.0
Total	397	42	10.6

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Statistical analysis

Statistical analysis was performed using the Chi-square test to find the relationship between contaminated samples and several types of vegetable. The variations were regarded as significant at $p \leq 0.05$.

Table 2: Antimicrobial susceptibility profile of *S. aureus* (n=42) isolated from various raw leafy vegetable samples obtained from wet markets.

Antimicrobial agents	Susceptible N (%)	Intermediate N (%)	Resistant N (%)
Penicillin G	19 (45.2)	8 (19.0)	15 (35.7)
Cefoxitin	34 (80.9)	0 (0)	8 (19.0)
Tobramycin	35 (83.3)	5 (11.9)	2 (4.7)
Ciprofloxacin	37 (88.1)	0 (0)	5 (11.9)
Clindamycin	25 (59.5)	5 (11.9)	12 (28.6)
Oxacillin	30 (71.4)	3 (7.1)	9 (21.4)
Tetracycline	20 (47.6)	0 (0)	22 (52.3)
Kanamycin	25 (59.5)	0 (0)	17 (40.5)
Erythromycin	32 (76.1)	0 (0)	10 (23.8)
Gentamicin	36 (85.7)	0 (0)	6 (14.3)
Lincomycin	25 (59.5)	5 (11.9)	12 (28.60
Streptomycin	28 (66.6)	0 (0)	14 (33.3)
Quinupristin-Dalfopristin	35 (83.3)	2 (4.7)	5 (11.9)
Trimethoprim-Sulfamethoxazole	37 (88.1)	0 (0)	5 (11.9)
Chloramphenicol	36 (85.7)	0 (0)	6 (14.3)

Table 3 Antibiogram of *S. aureus* isolated from various raw leafy vegetable samples obtained from wet markets.

Antibiogram	No. of isolates	Source	MAR index
PDaOxSTeL	5	Amaranth green (2), Mint (2), Laksa leaves	0.42
PDaSTeCe	4	Coriander, Japanese parsley, Water spinach, Indian pennywort	0.35
PDaEOxS	2	Bean sprouts, Chinese flower Cabbage	0.35
KPDaEOxSTe	1	Amaranth green	0.5
KPEOxTeL	1	Coriander	0.42
PSLCeK	2	Indian pennywort	0.42
KCeTor	2	Amaranth green, Indian pennywort	0.35
TeKCipQd	3	Amaranth red, Spring Onion, Sweet basil	0.28
TeKCnSxtC	3	Amaranth green (2), Lettuce salad	0.35
TeKCnSxtCQd	2	Sweet basil, Wild parsley	0.42
TeKCnC	1	Water spinach	0.28
TeKCip	2	Winged bean, Bean sprouts	0.21

RESULTS AND DISCUSSION

A total of 397 samples of raw leafy vegetables obtained from wet markets were investigated in this study. The overall prevalence of *S. aureus* isolated from the vegetables was 10.6% (42/397) as shown in Table 1, an indication that vegetables can be a possible cause of *S. aureus* infection in Malaysia. *S. aureus* was isolated from all the various types of vegetables examined.

The prevalence of *S. aureus* significantly varied between samples ($P < 0.05$). Incidentally, Amaranth green had the highest incidence of 24.0% (6/25), followed by Indian pennywort 20.0% (5/24) and coriander 16.0 % (4/25). Other studies also found a prevalence of *S. aureus* in fresh produce and raw leafy vegetables. In Korea, a survey of fresh fruits and vegetables such as lettuce, mixed salads and green onions reported contamination with *S. aureus* (Seo *et al*., 2010) while another study published by Nguz *et al*. (2005) isolated it from vegetables such as radish, lettuce, and seed sprouts. According to Olaimat *et al*. (2016), vegetables can become contaminated with such pathogens while growing, during harvest, during post-harvest handling, or distribution. This could be due to improper human handling and storage, contaminated irrigation water, containers, animal waste fertilisers and post-harvest washings (Mritunjay *et al*., 2015).

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S. aureus contamination can also be found at the dining point. *S. aureus* is known to be carried by food handlers (Faour-Klingbeil *et al*., 2016). High levels of *S. aureus* were detected in Russian salad (25.0%), and vegetable salad (12.0%) served in Turkish dining facilities (Aycicek *et al*., 2005).

Because *S. aureus* is well known for the tendency to become resistant to some antimicrobials, this study also looked into the resistance pattern of *S. aureus* to some antimicrobial agents, as shown in Table 2. There was a high prevalence of resistance to tetracycline, kanamycin, penicillin G, and to a minor extent, quinupristin-dalfopristin, trimethoprim-sulfamethoxazole, ciprofloxacin and tobramycin. The antimicrobial resistances demonstrated by the isolates were as follows; tetracycline 22/42 (52.3%), kanamycin 17/42 (40.5%), penicillin G 15/42 (35.7%), streptomycin 14/42 (33.3%), oxacillin 9/42 (21.4%) and cefoxitin 8/42 (19.0%). Furthermore, nine isolates (21.4%) were identified as Methicillin-resistant *S. aureus* (MRSA) by antibiotic susceptibility test, Oxacillin-resistant and confirmed by detection of the *mecA* gene. This result was like a study by Hong *et al*., (2015) in which eleven *S. aureus* isolates from leafy vegetables were MRSA, which showed the presence of the *mecA* gene and phenotypic resistance to oxacillin.

The high prevalence of tetracycline, kanamycin and penicillin G resistant *S. aureus* noticed in the present study, was similar to earlier studies reported by Jamali *et al*. (2015); Seo *et al*. (2010). Hong *et al*. (2015) also found almost 15 of 19 (78.95%) of *S. aureus* isolates isolated from perilla leaves being multidrug-resistant. This high prevalence of *S. aureus* resistance could be due to the wide use of animal manures as fertiliser in vegetable farming. Tetracycline, sulfonamides, and quinolone antibiotics have been detected in animal manures (chicken, cattle and swine) from China (Gao *et al*., 2012). Moreover, MRSA has increasingly been found in farm animal population and food of animal origin (Vicca *et al*., 2008). Other factors play a significant role in increasing the burden of environmental exposure to resistant bacteria. These come from several human activities, including emissions of antibiotic residues, fish production, animal production, wastewater treatment, and antibiotic manufacturing. Furthermore, antibiotics could be taken up by many vegetables if they were planted in soil contaminated by antibiotics (Hu *et al*., 2010). The results we obtained in this study showed that the most predominant antibiogram of *S. aureus* isolated from raw leafy vegetables were PDaOxSTeL (n = 5), PDaSTeCe (n = 4), TeKCnSxtC (n = 3) and TeKCipQd, respectively (Table 3). The MAR index of *S. aureus* isolated from vegetables ranged from 0.21 to 0.50 and was resistant to 2–7 various types of antibiotics. When isolates have a MAR index of 0.2 and above it indicates that strains were exposed to several antibiotics or originated from the environment where antibiotics were frequently used (Krumperman, 1983).

CONCLUSION

In summary, the presence of *S*. *aureus* from raw leafy vegetables retailed in wet markets is worrying. It showed that the consumption of fresh or minimally cooked vegetables might be a possible risk of foodborne bacterial pathogens. Increased antibiotic resistance amongst the *S. aureus* isolated from wet markets vegetables is also of great concern to the society. More importantly, the isolation of MRSA from vegetables obtained from wet markets is a public health concern for the consumers**.** The findings here highlighted the need for strict hygienic standards in wet markets to reduce the prevalence of *S. aureus*. The use of treated animal manures in agricultural practice also needs strict monitoring to curtail the emerging antimicrobial resistance bacteria in vegetables. The findings of this study highlighted the likely problems of the spread of *S. aureus* through raw vegetables and the need to curtail them.

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