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Detection of enterotoxin gene (*sea*) and biofilm formation ability among multi-drug resistant *Staphylococcus aureus* isolated from shawarma sandwich sold at selected kiosks in Klang Valley, Malaysia

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Abstract

The occurrence of multi-drug resistant *Staphylococcus aureus* in food product of animal origin has increased the concern about their spread into the food supply chain. Presence of multidrug-resistant *S. aureus* in food products, including ready-to-eat foods imposes potential hazard for consumers. The objective of this research was to investigate the presence of multi-drug resistance of *S. aureus* in sixty ready-to-eat shawarma sandwiches. Agar-disc diffusion assay determined their resistance to 11 antibiotics. The *sea* and *sed* enterotoxin genes were detected by polymerase chain reaction method. Biofilm formation potential (BFP) was quantified by microtitre plate assay. The result revealed that thirty-six samples (60%) were positive for *S. aureus*. Majority of the isolates (n = 29; 80.6%) were resistant to at least one antibiotic. The isolates demonstrated highest resistance against ampicillin (69.4%) and penicillin (69.4%), while resistance to ciprofloxacin, tetracycline and kanamycin were 47.2%, 33.3% and 22.2%, respectively. Several isolates were resistant to trimethoprim (5.6%), trimethoprim-sulfamethoxazole- (2.8%), gentamicin (2.8%) and cephalothin (2.8%), while none exhibited resistance to chloramphenicol and nitrofurantoin. Out of the thirty-six isolates, twelve isolates (33.3%) were resistant to three or more classes of antibiotic (multidrug-resistant) and 50% had a Multiple Antibiotic Resistance index value more than 0.25. Of the multi-drug resistant isolates, four were positive for *sea* genes but no *sed* genes were present. All multi-drug resistance isolates were biofilm formers with five and six isolates were strong and moderate formers, respectively. Additionally, all the *sea* gene carrying multi-drug resistance isolates were strong biofilm formers. These findings revealed shawarma as a potential vehicle for the spread of multidrug-resistant *S. aureus*, suggesting more control measures for ready-to-eat food.

1. Introduction

Shawarma sandwiches are served in several fast-food restaurants in Europe, the Middle East, Canada, and most recently in Malaysia. Shawarma's origin can be traced back to Turkey, and to the Turkish word "çevirme" meaning to rotate (Sirkeci, 2016). It is however known in various countries by various names including chawarma, doner kebab, donair, and gyro (Ayaz *et al.,* 1985; Todd *et al.,* 1986; Nimri *et al.,* 2014). Shawarma is a famous sandwich made of chicken, lamb, or beef. The majority of restaurants serving shawarma use the breasts of chicken, as these cuts are cheaper and more easily digestible than red meat (Kayaardi *et al,* 2006).

Shawarma is mostly popular as street food, so it is at risk of contamination by food pathogens from the environment. Among the major problems facing developing nations including Malaysia are foodborne

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infections caused by microbial agents, likely occurring as a result of a change in decision-making involving the production, consumption, and storage of food brought about by the liberation and globalization of trade and the increased importance of food (Satcher, 2000; Abdelgadir *et al.,* 2009; Shafizi *et al.,* 2016). In previous study, an increasing incidence of foodborne infections had been observed in Malaysia between 2005 and 2008 (Rahman, 2019). Nevertheless, death rates due to these foodborne infections have reportedly remained the same with an equivalent rate of mortality to that obtained in 2008 (Abdul-Mutalib *et al.,* 2012). Additionally, in 2006, 3625 food premises out of a total available of 81,686 were shut down because they did not meet the requirements of the Food Act 1983, Section II, as enforced by the relevant authority (MOH, 2006; Sani *et al*., 2014). The foodborne infections in Malaysia can mainly be traced to unhygienic food handling procedures, which contribute to 50% of the cases (MOH, 2007).

Shawarma is a food that can potentially become contaminated with pathogenic bacteria during preparation and processing. Studies analyzing the pathogen contamination of shawarma in various countries revealed that the shawarma had high contamination of several microbes and certain foodborne pathogens that were multidrug-resistant including *S. aureus* (Harakeh *et al.,* 2005; Odu and Akano, 2012; Abdalhamid *et al*., 2013; Nimri *et al.,* 2014; Salem *et al*., 2016).

S. aureus is one of the most vital species of bacteria in food microbiology and has long been regarded as a potential threat to foodborne illnesses. It colonizes human skin and the front nares in a substantial fraction of the human population (Kluytmans *et al.,* 2005; Castro *et al*., 2016). Some of these pathogens are resistant to antimicrobials including the commonly used classes of antibiotics (Sharaf *et al*., 2012; Nimri *et al.,* 2014; Bantawa *et al*., 2019).

The main threat of food contamination to general public health stems from bacteria that are resistant to antibiotics since the determinants of the resistance could be transferred to other bacterial pathogens in food. Several reports have shown the occurrence of foodborne pathogenic microbes that are resistant to antimicrobials (Sharaf *et al*., 2012; Nimri *et al.,* 2014).

S. aureus is able to produce heat-stable enterotoxins responsible for several diseases (Bergdoll, 1983). There are five serological staphylococcal classical antigens identified including staphylococcal enterotoxins (SE) A, B, C, D and E out of which SEA and SED a responsible for about 95% of the global incidence of staphylococcal food poisoning (Sezer *et al*., 2015). The most common toxin detected in outbreaks of food poisoning in the United States was the SEA with almost 77% occurrence, followed by SED with 38% and SEB with 10%. SEA is the most commonly found enterotoxin among SFD outbreaks in Japan, France, and UK (Balaban and Rasooly, 2000; Argudín *et.,* 2010).

To the best of the author's knowledge, the current study is the first to determine the occurrence of multidrug-resistant *S. aureus* from chicken shawarma in Arabic kiosks in Malaysia. It further aims to investigate the prevalence of antimicrobial resistance in the isolates against some commonly used antibiotics. The findings of this research will provide the necessary information for relevant authorities to manage public health threats associated with the consumption of shawarma sandwiches.

2. Materials and methods

2.1 Sample collection

A total of sixty shawarma samples were purchased from kiosks at Arabic restaurants (n=20) in the Klang Valley area, Malaysia. The samples were obtained from three visits to each kiosk in the periods of July 2016 to Mac 2017. Samples were collected in a sterile plastic bag and kept in an icebox (4-8°C) immediately after collection and transported to the laboratory within 4 hours.

2.2 Sample processing

Shawarma sandwich samples were cut into smaller pieces using a sterile knife and 25 g of the samples were mixed with 225 mL of sterile buffered peptone water (Oxoid, United Kingdom) in a stomacher bag and homogenized for 60 s using a Stomacher Lab-Blender 400 (Seward Medical, UK). The homogenized samples were incubated overnight at 37ºC.

2.3 Isolation and identification of Staphylococcus aureus

An aliquot of 0.1 mL of the homogenized sample was spread onto a Baird-Parker agar (BPA) medium (Himedia, India) supplemented with Egg Yolk Tellurite Emulsion (Himedia, India). After incubated at 37°C for 24 hrs, a typical colony (black/dark gray colony surrounded by an opaque halo) was selected and subjected to Gram Staining, catalase test and coagulase reactions (Lancette and Tatini, 1992). The suspected *S. aureus* colonies were further tested by PCR for confirmation using specific primer pairs of *nuc* gene (Integrated DNA Technologies, Inc., Coralville, IA, USA) with the forward sequence of 5'-GCGATTGATGGTGATACGGTT-3' and reverse sequence of 5'-AGCCAAGCCTT GACGAACTAAAGC

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-3' (Brakstad *et al.,* 1992; Kuźma *et al.,* 2003; Yang *et al.,* 2007). These primers amplify 270 bp region of *nuc* gene fragment of *S. aureus*. A total of 25 µL reaction mixture was prepared for the amplification and it contained 1 µL of 50 ng of the genomic DNA, 1 µL each of 10 µM of the forward and reverse primers, 12.5 µl of 2x PCRBIO Taq Mix Red (PCR Biosystems Ltd, London, UK) and finally made up to 25 µL with sterile distilled water. The amplification was performed in a thermal cycler (Kyratec Super Cycler Thermal Cycler, Australia) with the following conditions: initial denaturation at 95°C for 10 mins followed by 37 cycles of denaturation at 94°C for 60 s, annealing at 55°C for 30 s, extension at 72°C for 90 s and a final extension 72°C for 5 mins. *S. aureus* ATCC 13565 was used as a positive control in this experiment. For PCR products visualization, 5 µL of the PCR products were loaded onto an ethidium bromide-stained 1.5% agarose gel for electrophoresis and visualization of the amplified PCR products. The amplicons were then measured at 270 bp using Gelpilot 100 bp DNA ladder (Qiagen, Hilden, Germany).

2.4 Antimicrobial susceptibility testing

Susceptibility of all the *S. aureus* isolates to antimicrobials was determined by an agar diffusion test using commercially available antibiotic disks (Oxoid, United Kingdom) as described by the Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI, 2017). An overnight Mueller Hinton broth (MHB) (Merck, Germany) culture of the test isolate (0.5 McFarland) was spread on a Mueller Hinton Agar (MHA) (Merck, Germany) plate. Antibiotic discs were evenly dispensed on the inoculated MHA plates and incubated at 37°C for 18–24 hrs. Eleven types of antibiotics used were Penicillin (10 µg), Ampicillin (10 µg), Gentamicin (10 µg), Kanamycin (30 µg), Tetracycline (30 µg), Chloramphenicol (30 µg), Trimethoprim (5 µg), Sulfamethoxazole-Trimethoprim (25 µg), Ciprofloxacin (5 µg), Cephalothin (30 µg), and Nitrofurantoin (300 µg). After incubation, the diameter of the inhibition zone was measured. Antibiotic susceptibility profile was determined based on the CLSI standard (CLSI, 2017). The resistance of a single isolate to three or more classes of antibiotics (Oteo *et al*., 2005; Geidam *et al*., 2012) was characterized as multidrug resistance (MDR). The values of multiple antibiotic resistance (MAR) index were calculated as the ratio of number of antibiotics to which the organisms were resistant to the total number of antibiotics to which the organisms were exposed and is mathematically represented thus;

$$MAR = A/B$$

Where A = the total number of antibiotics to which the organism is resistant, and B = the total number of antibiotics to which the organism was exposed

The MAR index is an important tool for the assessing the health risks that aids in identifying an isolate originating from a region excessive usage of the antibiotic (Davis and Brown, 2016). Values of MAR index greater than 0.2 are considered an important indicator for assessing the contamination risk (Krumperman, 1983).

2.5 Detection of enterotoxin genes (sea and sed)

Primers employed for SEA detection consisted the forward 5'-CCT TTG GAA ACG GTT AAA ACG-3' and the reverse 5'-TCT GAA CCT TCC CAT CAA AAA C-3' with estimated amplicon size of 127 bp, while primers used for SED detection consisted of the forward 5'-CTA GTT TGG TAA TAT CTC CTT TAA ACG-3' and the reverse 5'-TTA ATG CTA TAT CTT ATA GGG TAA ACA TC-3' with estimated amplicon size of 319 bp (Becker *et al.,*1998). PCR products were prepared in a 25 µL reaction volume that consisted of 1 µL of 50ng genomic DNA, 1 µL each of 10 µM forward and reverse primers, 12.5 µL of 2x PCRBIO *Taq* Mix Red (PCR Biosystems Ltd, London, UK) which composed of PCRBIO *Taq* DNA polymerase, 6 mM $MgCl_2$ and 2 mM dNTPs and 9.5 µL sterile distilled water. Amplification of SEA and SED PCR products was carried out in a thermal cycler (Model SC300, Kyratec Australia). The thermal cycler conditions of amplification include; 94ºC for 5 mins of initial denaturation, 30 cycles of 94ºC for 1 min of final denaturation, annealing at 55ºC for 1 min and extension at 72ºC for 1 min. Finally, extension followed at 72ºC for 5 mins.

2.6 Biofilm formation

The biofilm formation ability of all the multi-drug resistant isolates was estimated using the crystal violet staining method described by Beehan (Beehan *et al*., 2015) with minor modifications. An overnight culture of individual isolate grown in tryptic soy broth (TSB) (Oxoid) at 37°C was adjusted to 0.5 McFarland and were diluted in TSB supplemented with 0.25% glucose (1:40). An aliquot of 200 mL of the dilutions was dispensed in each well of a sterile polystyrene 96-well microtitre plate (SPL, Life Science, Korea) and the plates were incubated at 37ºC for 24 hrs. Following incubation, the wells were rinsed three times using phosphate buffered saline (PBS; pH 7.4±0.1; Thermo Fisher Scientific, USA), air-dried for 1 hr at 60ºC and stained with 0.25% crystal violet (Thermo Fisher Scientific, USA). The plates were then incubated at 25ºC for 15 mins and the biofilm formation

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capacity was characterized by measuring the optical density at OD_{570nm} with a microplate spectrophotometer (Benchmark Plus Microplate Spectrophotometer System, Bio-Rad, USA).

Interpretation of the results of the mean absorbance of the test wells was done in accordance with Stepanović *et al*. (2000) as non-adherent, weak, moderate or strong biofilm formers on the basis of comparison with the control wells (uninoculated wells) of the plate. Non-adherent were those with absorbance less than or equal to the control wells ($OD \leq OD_C$); the weakly adherent ($OD_C \leq OD \leq 2x\ OD_C$), moderately adherent ($2x\ OD_C \leq OD \leq 4x\ OD_C$) and the strongly adherent ($4x\ OD_C < OD$). Isolates were analyzed for the biofilm in triplicates. Growth medium without bacterial inoculum served as a negative control and *S. aureus* ATCC 6538 was used as a reference strain.

3. Results

3.1 Detection of Staphylococcus aureus in shawarma sandwich

Among the total 156 isolates obtained from sixty shawarma samples, thiry-six (23.1%) were positive for *S. aureus* (Table 1). More than 25.0% of the positive *S. aureus* isolates were detected from shawarma sold in nine (9) kiosks namely; R-16 (50.0%), R-7 (40.0%), R-6 and R-13 (33.3%), R-12 (30.0%), R-2 (28.5%), R-1, R-10 and R-19 (25%) while the remaining 11 kiosks had lower number of positive isolates (<25%).

3.2 Susceptibility of Staphylococcus aureus to antibiotics

Table 2 shows that all 36 isolates (100%) were susceptible to chloramphenicol and nitrofurantoin. The isolates demonstrated highest resistance (69.4%) against both antibiotics in the Penicillin class; namely ampicillin and penicillin, while resistance to ciprofloxacin, tetracycline and kanamycin were 47.2%, 33.3% and 22.2%, respectively. Only several isolates were resistant to trimethoprim (5.6%), trimethoprim-sulfamethoxazole (2.8%), gentamicin (2.8%) and cephalothin (2.8%). Intermediate resistance was only observed in 6 isolates (16.7%) against kanamycin. Of the 36 isolates, 29 were resistant to at least one (1) antibiotic (see Table 3). Twelve of the 36 isolates (33.3%) were resistant to three or more classes of antibiotic (MDR) and 50% had a MAR index value more than 0.25.

3.3 Characteristic of MDR Isolates of Staphylococcus aureus, the presence of sea and sed genes and their biofilm formation potential

Multidrug resistance associated with enterotoxigenic genes detection and biofilm formation ability is shown in Table 4. The main MDR profiles of the isolates were penicillin- fluoroquinolone-aminoglycosides-tetracycline (n=6), penicillin-fluoroquinolone-tetracycline (n = 2) and penicillin-aminoglycosides-tetracycline (n = 2). Four of the MDR were positive for *sea* genes but no *sed* genes were present in all MDR isolates (Figures 1 and 2). All MDR isolates were able to form biofilm. Five were strong formers, six were moderate and only one with

Table 1 Detection of *S. aureus* from shawarma sandwich from kiosks (n = 20) in Klang Valley, Malaysia.

Location	Kiosk Code	No. of Sample	No. of Isolate	No. of Positive Isolate (%)
Kajang	R-1	3	8	2 (25.0)
	R-2	3	7	2 (28.5)
	R-3	3	10	2 (20.0)
	R-4	3	12	2 (16.7)
	R-5	3	8	1 (12.5)
South City	R-6	3	6	2 (33.3)
	R-7	3	5	2 (40.0)
	R-8	3	12	2 (16.7)
	R-9	3	11	2 (18.2)
	R-10	3	12	3 (25.0)
One South	R-11	3	6	1 (16.7)
	R-12	3	10	3 (30.0)
	R-13	3	9	3 (33.3)
	R-14	3	6	1 (16.7)
	R-15	3	6	1 (16.7)
Kuala Lumpur	R-16	3	2	1 (50.0)
	R-17	3	9	2 (22.2)
	R-18	3	5	1 (22.2)
	R-19	3	8	2 (25.0)
	R-20	3	4	1 (25.0)
Total		60	156	36 (23.1)

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Table 2. Antibiotic resistance profile of *S. aureus* isolates obtained from shawarma sandwiches (n = 60).

Class of Antibiotics	Antibiotic (ug)*	Resistant (%)	Intermediate (%)	Susceptible (%)
	AMP	25 (69.4)	-	11 (30.5)
Penicillin	P	25 (69.4)	-	11 (30.5)
Fluoroquinolone	CIP	17 (47.2)	-	19 (52.7)
	CN	1 (2.8)	-	35 (97.2)
Aminoglycosides	K	8 (22.2)	6 (16.7)	22 (61.1)
Cephem	KF	1 (2.8)	-	35 (97.2)
Tetracycline	TE	12 (33.3)	-	24 (66.7)
Folate pathway	W	2 (5.6)	-	34 (94.4)
inhibitor	SXT	1 (2.8)	-	35 (97.2)
Phenicol	C	-	-	36 (100)
Nitrofurantoin	F	-	-	36 (100)

* AMP = ampicillin, P = penicillin G, CIP = ciprofloxacin, CN = gentamicin, K = kanamycin, KF = cephalothin, TE = tetracycline, W = trimethoprim, SXT = trimethoprim-sulfamethoxazole, C = chloramphenicol, F = nitrofurantoin.

Table 3. Multiple antimicrobial resistance (MAR) index value of *S. aureus* isolates.

Isolate	Antibiotic resistant profile*	No. of antibiotics**	MAR Index***
S1.1	P AMP W	3(2)	0.27
S1.2	P AMP	2(1)	0.18
S2.2	CIP	1(1)	0.09
S2.1	CIP	1(1)	0.09
S3.1	P AMP CIP	3(2)	0.27
S3.2	P AMP CIP TE	4(3)	0.36
S4.1	P AMP CIP TE K	5(4)	0.45
S4.2	P AMP CIP TE K	5(4)	0.45
S5.1	-	-	-
S6.1	P AMP CIP KF CN	5(4)	0.45
S6.2	P AMP	2(1)	0.18
S7.1	P AMP	2(1)	0.18
S7.2	P AMP	2(1)	0.18
S8.1	-	-	-
S8.2	P AMP CIP TE K	5(4)	0.45
S9.1	-	-	-
S9.2	P AMP CIP TE K	5(4)	0.45
S10.1	P AMP TE K	4(3)	0.36
S10.2	P AMP CIP TE K	5(4)	0.45
S10.3	P AMP	2(1)	0.18
S11.1	-	-	-
S12.1	-	-	-
S12.2	-	-	-
S12.3	P AMP CIP	3(2)	0.27
S13.1	P AMP	2(1)	0.18
S13.2	P AMP TE K	4(3)	0.36
S13.3	P AMP CIP TE	4(3)	0.36
S14.1	P AMP CIP	3(2)	0.27
S15.1	P AMP	2(1)	0.18
S16.1	-	-	-
S17.1	P AMP CIP	3(2)	0.27
S17.3	P AMP TE	3(2)	0.27
S18.3	CIP SXT W TE K	5(4)	0.45
S19.1	P AMP CIP TE K	5(4)	0.45
S19.3	CIP	1(1)	0.09
S20.2	P AMP	2(1)	0.18

AMP = ampicillin, P = penicillin G, CIP = ciprofloxacin, CN = gentamicin, K = kanamycin, KF = cephalothin, TE = tetracycline, W = trimethoprim, SXT = trimethoprim-sulfamethoxazole, C = chloramphenicol, F = nitrofurantoin.
**The number of antibiotics to which each isolate was resistant. The number in the parenthesis indicates the total number of the classes to which the antibiotics belong.
***Multiple antimicrobial resistances.

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Table 4. Characteristic of MDR isolates of *S. aureus*, the presence of *sea* and *sed* genes and their biofilm formation potential

Kiosk code	Isolate	MDR profile *	*sea* genes **	*sed* genes **	Biofilm formation ***
R-3	SA3.2	3 class of antibiotic (a, b, e)	+	nd	+++
R-4	SA4.1	4 class of antibiotic (a, b, c, e)	nd	nd	+++
	SA4.2	4 class of antibiotic a, b, c, e	+	nd	+++
R-6	SA6.1	4 class of antibiotic a, b, c*, d	nd	nd	++
R-8	SA8.2	4 class of antibiotic a, b, c, e	+	nd	+++
R-9	SA9.2	4 class of antibiotic a, b, c, e	nd	nd	++
R-10	SA10.1	3 class of antibiotic a, c, e	+	nd	+++
	SA10.2	4 class of antibiotic a, b, c, e	nd	nd	++
R-13	SA13.2	3 class of antibiotic a, c, e	nd	nd	+
	SA13.3	3 class of antibiotic a, b, e	nd	nd	++
R-18	SA18.3	4 class of antibiotic b, c, e, f	nd	nd	++
R-19	SA19.1	4 class of antibiotic a, b, c, e	nd	nd	++

* multi-drug resistance pattern based on antibiotic class; a = Penicillin, b = Fluoroquinolone, c = Aminoglycosides, d = Cephem, e = Tetracycline, f = Folate pathway inhibitor
** genes detected = +; genes not detected = nd
*** biofilm formation; strong = +++; moderate = ++; weak = +

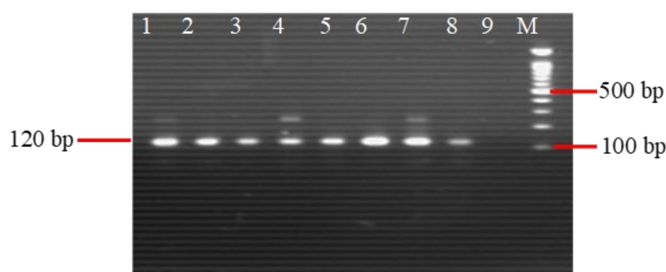

Figure 1. Visualized PCR products of Staphylococcal Enterotoxin A (*sea*). Lane 1-7= Positive sample (SEA), Lane 8 = Positive control of *S. aureus* ATCC 13565, Lane 9 = Negative control, Lane M =100 bp DNA marker

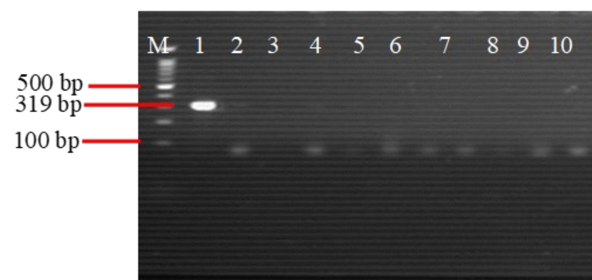

Figure 2. Visualized PCR products of Staphylococcal Enterotoxin D (*sed*). Lane 2-9= Negative sample (SED), Lane 1= Positive control of *S. aureus* ATCC 13565, Lane 10= Negative control, Lane M= 100 bp DNA marker

weak ability. Additionally, all the *sea* gene-carrying MDR isolates were strong biofilm formers. Moreover, shawarma samples that contained MDR isolates which carried *sea* genes and with strong biofilm formation ability were detected from four kiosks; namely R-3, R-4, R-8 and R-10.

4. Discussion

The occurrence of *S. aureus* in shawarma sandwich in the current study (60%) was relatively higher than those study reported for various Ready-to-Eat (RTE) foods in Malaysia (Shafizi *et al.,* 2016), China (Song *et al.,* 2015; Yang *et al.*, 2016), Trinidad (Syne *et al.,* 2013), and Bangladesh (Islam *et al.,* 2019), which generally had the occurrence of *S. aureus*. In Sri Lanka, 32.0% *S. aureus* was found in shawarma and ham (Wimalasekara, 2016).

In this study, the occurrence of *S. aureus* in shawarma samples may be due to three main factors; namely insufficient cooking of chicken meat,

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contamination by raw ingredients and poor hygiene. Raw meat of chicken has been reported contaminated with *S. aureus* (Vazgecer *et al*., 2004). *S. aureus* was also found isolated from 75% chicken and 52% turkey retail meats (Tang *et al*., 2017). It was observed during this study that piles of chicken meat were roasted on a rotating skewer, with the outer layer of meat cooked properly, while the inner part within the pile of meat remains raw and juicy. During serving, the outer part of the meat block was shaved, leaving the remaining block of meat to remain heated on the skewer. Based on this cooking method, shawarma samples may contain *S. aureus* cells that survived the method of cooking as roasting chicken meat block on the rotated skewer may cause insufficient or inconsistent cooking of the chicken meat.

Moreover, raw ingredients added to the shawarma; tahini sauce (from the oil of sesame seeds) and vegetables could also be possible sources of contaminants. *S. aureus* was found in vegetables such as cabbage, tomato and lettuce, all were found to have high contamination in ready-to-eat foods including shawarma (Okafor *et al*., 2003; Meldrum *et al*., 2009; Sospedra *et al*., 2013; Faour-Klingbeil *et al*., 2016). These vegetables may be contaminated with soil that is possibly transmitted to the hands of food handlers, and consequently to the shawarma sandwich (Okafor *et al*., 2003; Vazgecer *et al*., 2004; Harakeh *et al*., 2005; Meldrum *et al*., 2009; Odu and Akano, 2012).

Food handlers are the key players that prevent food contamination during food preparation by preventing cross-contamination and by using proper cooking methods and storage of cooked food (Soon *et al*., 2011). This is also supported by random observation by the authors during sample collection in this study, where the majority of the workers in the kiosks did not wear gloves during shawarma preparation and serving. Consequently, the *S. aureus* occurrence in shawarma sandwiches is likely a result of contamination by the food handlers due to their poor hygiene practices. Thus, the contamination of shawarma could be heavily related to the method of preparation, as *S. aureus* is transmitted through sneezing, coughing, and wounds in the hands of food handlers (Odu and Akano, 2012; Abdalhamid *et al*., 2013; Nimri *et al.,* 2014).

In this study, 80.6% of *S. aureus* isolates were resistant to at least one antimicrobial, with the substantially higher resistance to the Penicillin class of antibiotic; ampicillin (69.4%) and penicillin (69.4%). Similarly, Geidam *et al*. (2012) found that more than 50% of the *S. aureus* isolated from the skin of poultry in Malaysia exhibited resistance to penicillin and ampicillin. Similar resistance to penicillin (53.3%) and ampicillin (72.30%) was observed in *S. aureus* isolated from the food handler's hands in Malaysia (Tan *et al.,* 2014). This result is nearly analogous to that of Arfatahery *et al*. (2016), who found that all the strains of *S. aureus* isolated from shrimp and fish showed resistance to penicillin and ampicillin. *S. aureus* resistant to penicillin (99.3%) was also reported in a study investigating dairy products in north-western Greece (Papadopoulos *et al*., 2018). These results could be due to the fact that antibiotics have been used increasingly for the treatment of bacterial diseases in humans and animals. Several antibiotics, especially for penicillin are generally applied in veterinary medicine (Cháfer-Pericás *et al.,* 2010).

The resistance of the microorganisms to three or more classes of antibiotics considered as multidrug-resistant (MDR) (Gibbs *et al.,* 2006; Waters *et al.,* 2011). In this study, twelve isolates were MDR and six of them demonstrated MDR pattern that was dominated by four antibiotic classes (penicillin, fluoroquinolone, aminoglycosides and tetracycline). Multidrug-resistant bacteria are now increasingly common because of the indiscriminate use of antibiotics.

Chickens are possible sources of MDR bacteria because various antibiotics are used to treat infections in chickens and to promote their growth; hence, making it possible for 100% of isolates from chicken in Oman to exhibit multidrug resistance (Al-Bahry *et al.,* 2007; Al-Bahry *et al.,* 2015) as compared to an earlier study conducted in Oman in 1986 that reported only 30% of the isolates being MDR (Al-Bahry, 1999). Multiple drug resistance proves difficult to overcome especially by the commonly used antibiotics and this intensifies the ability of the bacteria to establish themselves in their hosts leading to the production of more virulent factors that enhance their survival (Tan *et al.,* 2014; Frieri *et al*., 2017).

The Emergence of multiple drug resistance among bacteria from food handlers, food premises and food products has also been a global concern (Altalhi *et al.,* 2010; Ryu *et al.,* 2012; Rasheed *et al.,* 2014; Tan *et al.,* 2014; Melo *et al.,* 2015) and could lead to food poisoning outbreaks that in turn leads to economic loss and loss of lives if people of compromised immunity are involved.

We targeted two classical SEs (*sea* and *sed)* genes and found that of the thirty-six *S. aureus* isolates that were resistant to at least one antibiotic, seven (19.4%) harbored the *sea* genes. No *sed* gene was found in any of the isolates tested. The *sea* gene is the most commonly reported in contaminated foods and also in staphylococcus-related food poisoning cases worldwide

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(Argudín *et al*., 2010; Gholamzad *et al.,* 2015). A study by Kérouanton *et al.* (2007) in France on *S. aureus* linked to food poisoning outbreaks also revealed the highest prevalence of SEA alone and in combination with SED but relatively low percentages 13.9% and 6.7% respectively. The presence of *S. aureus* toxins in foods is an indication for the potential to cause food intoxication thereby leading to food poisoning outbreak. Several studies involving the detection of *S. aureus* and its enterotoxins from foods and food products have been conducted worldwide. These include the presence of enterotoxigenic *S. aureus* in ready-to-eat foods in Korea (Kim *et al.,* 2011), retail aquatic food products in China (Rong *et al*., 2017), goat milk in China (Xing *et al.,* 2016), from food poisoning clone of *S. aureus* in Japan (Sato'o *et al*., 2014). This study is the first study in Malaysia and therefore there are not sufficient data to make comparisons.

Although the production of *sea* genes was not investigated in our study, the presence of *sea* genes carrying *S. aureus* isolates in shawarma samples could be of concern in terms of enterotoxin production. A study on retail foods in China (Wang *et al*., 2017) reported that more than 90% of the *sea* gene carrying *S. aureus* isolates have produced enterotoxins. Considering these MDR isolates which also carry *sea* gene may enter the food chain, and may be transferred to consumers, their ability to form biofilm was established to evaluate their persistence in the food environment. Our study demonstrated that five of the 12 MDR isolates were strong biofilm formers, and four of these MDR isolates harboured *sea* genes.

Conflict of interest

The authors declare no conflict of interest.

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