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FULL PAPER

Multidrug-resistant *Staphylococcus aureus* (MDRSA) properties and their adherence ability on stainless steel surfaces at different temperature and time

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Abstract

Staphylococcus aureus is a pathogenic bacterium that capable to adhere on the processing surfaces that could cause a cross-contamination of foods. In Malaysia, *S. aureus* has been reported from foods and food-handlers hand at food service environment but the multidrug -resistant *S. aureus* (MDRSA) and their adherence on stainless steel were limited. This study was intended 1) to isolate *S. aureus* from food contact surfaces and characterize the isolates for MDRSA properties, and 2) to determine the adherence ability of the MDRSA strains. A total of thirty-eight *S. aureus* isolated from food premises in Sri Serdang were tested for the antibiotic resistance and it was carried out using five classes of antibiotics; Penicillin (I), Cephalosporins (II), Amino-glycosides (III), Quinolones Fluoroquinolone (IV), and Sulphonamide (V) by the standard procedures of Kirby-Bauer disc diffusion method. The adherence assay was performed on stainless steel disc at 25°C and 37°C on 24, 48 and 72 hrs incubation. As a result, twenty-three *S. aureus* were found as multidrug-resistant towards the antibiotics. All the MDRSA can adhere on stainless steel with a minimum 4.00 log CFU/mL. The adherence of MDRSA on stainless steel during 72 hrs were ranging from 4.11 to 6.55 log CFU/mL and 4.25 to 6.86 log CFU/mL at 25°C and 37°C, respectively. The highest adherence was found on 48 hrs at both temperatures. The MDRSA strains revealed high capacity to adhere on stainless steel at 37°C. As a conclusion, the MDRSA strains shows the strong adherence ability at their optimum growth temperature.

1. Introduction

Staphylococcus aureus known as a main human pathogen that has cause widespread infection worldwide. It is the most common enterotoxigenic staphylococcal species causing foodborne disease (Abdollah *et al*., 2014). Amongst the reported foodborne illnesses, *S. aureus* got the highest prevalence rate of resistant *S. aureus* infections reported in Asia (Sit *et al*., 2017). Recently, a massive *S. aureus* food poisoning outbreak occurred, involving primary school students in Bangi, Selangor, Malaysia, in 2017 (Baharudin and Mohd Ishak, 2017). As a pathogenic bacterium that able to have antimicrobial resistance properties and capable to adhere on the food processing surfaces, *S. aureus* could cause the food contamination and health threat once it conveys to human (Malheiros *et al*., 2010; Ciccio *et al.,* 2015).

Several studies have found the antibiotic resistant *S. aureus* on work surfaces, human and in food processing environment. *S. aureus* can adapt near developed protective mechanisms to reduce susceptibility against antibiotics (Hogberg *et al*., 2010). The antibiotic resistant genes are transferred among bacteria by carried on mobile genetic elements and spread via gene exchange processes. The indiscriminate usage of antibiotics offered the condition to mobilise gene resistant into pathogenic bacteria (Wise, 2002; Wright, 2007). Thus, resistance to each antibiotic used in medical work has now been reported (Payne *et al*., 2007). Additionally, *S. aureus* is a pathogen with combination of toxin-mediated virulence, and has gotten frighteningly resistant to numerous antibiotics (Beceiro *et al*., 2013; Kadariya *et al*., 2019). A multidrug-resistant *S. aureus* (MDRSA) are a growing global danger, consequently challenging the treatment of *S. aureus* infections.

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The lack of hygienic practices amongst food handlers particularly during preparation can contaminate food-contact surfaces such as cutting boards and these are the major factors causing *S. aureus* contamination in food products (DeVita *et al*., 2007). Several studies have found the adherence of *S. aureus* planktonic cells on labour surfaces including polypropylene, glass and stainless steel (Herrera *et al*., 2006; DeVita *et al*., 2007). However, the changes in environmental properties may lead to persistence of staphylococcal on food contact environment. More recent studies have suggested *S. aureus* to have a high capability of adhering and forming biofilm on stainless steel and polypropylene surfaces (Jeronimo *et al*., 2012; Meira *et al*., 2012; Souza *et al.,* 2014).

In Malaysia, the adherence of *S. aureus* has been reported in dairy products (Sasidharan *et al*., 2011), foods, as well as in the nostrils (Noor-Azira *et al*., 2012) and hands of food-handlers on foodservice premises (Tan *et al*., 2014). However, the findings on the survival of multidrug-resistant *S. aureus* (MDRSA) on food contact surfaces at different temperature and time are limited. Therefore, the objectives of this work are 1) to isolate *S. aureus* from food contact surfaces and characterize the isolates for MDRSA properties, and 2) to determine the adherence ability of the MDRSA strains on stainless steel surfaces at different temperature and time.

2. Materials and methods

2.1 Sample collection

A total of twenty-four isolates of *S. aureus* were obtained from a stock culture in the Food Safety and Microbiology Laboratory, Universiti Putra Malaysia, Serdang, Selangor (Shakira, 2016). Another 45 samples were collected from food contact surface at the food premises of Universiti Putra Malaysia (UPM), Serdang, Selangor. The samples were isolated from the surface of cutting board, tray, plate and wok by standard swabbing method. Sampling was done by swabbing these surfaces horizontally, vertically and diagonally of 10 cm x 10 cm.

2.2 Isolation of Staphylococcus aureus

The collected samples (n = 45) were enriched in Nutrient Broth (NB) (Oxoid, Basingstoke, UK) overnight. An aliquot of 0.1 mL was spread on a Nutrient Agar (NA) (Oxoid, Basingstoke, UK) plate and incubated at 37^{o}C for 18 to 24 hrs. The grown colonies were isolated using selective agar; Mannitol Salt Agar (MSA), and Baird Parker Agar (BPA) supplemented with egg yolk tellurite (Oxoid, Basingstoke, UK). The growth colonies on MSA and BPA were observed and the suspected colonies were identified using biochemical tests.

2.3 Identification of Staphylococcus aureus using biochemical test

The isolates were then confirmed by Gram-staining, a catalase test, and a coagulase test. A single colony of *S. aureus* revived from NA plates was identified. Standard method for Gram-staining (Lucia *et al*., 2017), catalase (Public Health England, 2014) and coagulase test (Thirunavukkarasu and Rathish, 2014) were performed accordingly. The reaction of the isolates upon these tests were shown in Table 1, indicate that the isolates tested positive for *S. aureus*.

2.4 Determination of Multidrug-resistant Staphylococcus aureus (MDRSA)

All thirty-eight isolates (stock cultures, n = 24; new isolates, n = 14) that have been identified as *S. aureus* (numbered as SA001-SA038) were tested for their resistance against the antibiotics. Eleven antibiotics; penicillin G (10 µg), amoxicillin (10 µg), ceftriaxone (5 µg), ceftazidime (30 µg), cephalothin (30 µg), cefotaxime (30 µg), gentamicin (10 µg), streptomycin (25 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), sulphafurazole (300 µg) (Oxoid, Basingstoke, UK) have been used. It was chosen according to their mechanism which are; β-Lactams and non β-Lactams (Table 2). Antibiotic tested comes from five antibiotic classes; Penicillin (I), Cephalosporins (II), Amino-glycosides (III), Quinolones Fluoroquinolone (IV) and Sulphonamide (V) which to indicate the presence of multidrug-resistant *S. aureus*. The test was carried by using the standard procedures of the Kirby-Bauer disc diffusion method. Inoculum was obtained from overnight fresh cultures on Tryptic Soy Agar (TSA) (Oxoid, Basingstoke, UK) adjusted to approximately log 10^8 CFU/mL, turbidity equivalent to a 0.5 McFarland standard (Kroning *et al*., 2016), and spread on Mueller

Table 1. Biochemical test results of *S. aureus*

Parameters	Characteristic of *S. aureus*
Selective media	
Baird-parker agar	Black and shiny with narrow white margins and surrounded by clear zone
Mannitol salt agar	Yellow colonies with yellow zones in the media
Gram-staining	Positive cocci in clusters
Catalase	Appearance of gas bubbles after emulsify with 3% hydrogen peroxide
Coagulase	Clumping cocci within 5-10 seconds resulted with rabbit plasma

Table 2. The antibiotics used to determine the resistance of *S. aureus* isolated from food contact surfaces.

Antibiotic agent	Group of antibiotics	Antibiotic
β-Lactams	Penicillin (I)	Penicillin G (P)
		Amoxicillin (AMC)
	Cephalosporin (II)	Ceftriaxone (CRO)
		Ceftazidime (CAZ)
		Cephalothin (KF)
		Cefotaxime (CTX)
Non β-Lactams	Aminoglycosides (III)	Gentamicin (CN)
		Streptomycin (S)
	Quinolone Fluoroquinolone (IV)	Nalidixic acid (NA)
		Ciprofloxacin (CIP)
	Sulfonamides (V)	Sulfisoxazole (SF)

Hinton Agar (MHA) surfaces. The antibiotic discs were then placed onto the inoculated MHA and incubated overnight at 37°C. The results were interpreted according to the standards of inhibition zone diameters for *Staphylococcus* spp. (CLSI, 2015). *Staphylococcus aureus* ATCC 13565 was used as the positive control for the tests.

2.5 Molecular identification of MDRSA

A total of fourteen presumptive *S. aureus* of new isolates were then confirmed by the molecular identification. Genotypic by DNA extracted from presumptive *S. aureus* isolates and the PCR were carried out by 16s ribosomal RNA gene sequencing method. These were confirmed by Bacterial DNA Barcoding, full -length of 16s rRNA (~1400 bp), 1st BASE MBS. This method is for identifying and confirmed all the staphylococcal isolates as *S. aureus*. In brief, for rapid DNA extraction, one to five colonies of each freshly subcultured strain were suspended in 50 µL sterile peptone water (SPW) and heated at 99°C for 10 mins. After centrifugation at 30,000 × *g* for 1 min, the supernatant was used as a DNA template and stored at -20ºC until PCR was performed.

2.6 Adherence of MDRSA at different temperature and time on stainless steel surface

2.6.1 MDRSA strains preparation

All twenty-three MDRSA strains were grown overnight (18 to 24 hrs) at 37°C with shaking 150 × *g* (gravity) in Tryptic Soy Broth (TSB) (Merck, Germany). The cells were harvested by centrifugation at 5000 × *g* for 3.5 mins and washed three times in phosphate buffered saline (PBS; 0.1M, pH 7.2). For the strains suspension, cell pellets were re-suspended in PBS and adjusted using a spectrophotometer to an A600 of approximately 0.5, corresponding to log 10^8 CFU/mL (Arellano, 2010; Cabeça *et al.,* 2012).

2.6.2 Test surfaces and experimental conditions

AISI-304 stainless steel disc (6 × 6 × 1 mm) were used as the test surfaces. The discs were individually cleaned, sanitized and sterilized according to the procedure described by Marques *et al*. (2007). The adherence of MDRSA strains on the stainless steel surfaces inoculated into Tryptic Soy Broth (TSB), were assessed at two different temperature, 25°C and 37°C for 24, 48 and 72 hrs incubation time.

2.6.3 Cell adherence and the quantification

A suspension of 50 µL (10^8 CFU/mL) was mixed with 100 µL of Brain Heart Broth (Merck, Germany). The mixture was pipetted to the centre of the stainless steel disc and incubated at 25°C and 37°C. After 24, 48 and 72 hrs of incubation, discs were withdrawn and immersed for 15 s in sterile peptone water (SPW, 0.1 g/100 mL) to release the non-adhered cells. The surface of the stainless steel disc was swabbed using a sterile moistened cotton swab for collecting the cell adherence. The cotton swabs were then re-suspended in 9 mL of sterile peptone water (SPW) by vigorously vortexing for 30 s. The peptone water was adjusted to a serially diluted (10^{-1} to 10^{-6}) in SPW, and 100 µL was spread plated onto a sterile Plate Count Agar (PCA). The plates were incubated at 25°C and 37°C for 24 – 48 hrs and the number of viable cells was counted. The results were expressed in log CFU/mL (Rode et al., 2007)

2.7 Statistical analysis

The experimental results were statistically analysed using the Microsoft Excel 2013 and Minitab 16 (State College, Pennsylvania) software packages. All experiments were performed in triplicate and the results expressed as an average. The data for cell adherence was normalized and expressed as log CFU/mL. A probability value ($p < 0.05$) was accepted to indicate a significant difference.

3. Results and discussion

3.1 Identification of Staphylococcus aureus

Among 45 new samples collected from various food contact surfaces, 14 (31.1 %) were found positive as a presumptive *S. aureus* after test on BPA, MSA, gram staining, catalase test and coagulase test (Figure 1). The positive result for the presumptive *S. aureus* were showed as in Table 1. The stock cultures of *S. aureus* isolates (n = 24, which is obtained from food contact surfaces) and the new isolates (n = 14) were combined (SA001-SA038) for multidrug resistant test.

3.2 Determination of MDRSA

The antibiotics resistant pattern of 38 *S. aureus* (SA001-SA038) were presented in Table 3. According to the result, *S. aureus* were more resistant to a group of Penicillin (I), Cephalosporin (II) and Quinolones fluoroquinolone (IV). Resistance rates against Penicillin G, Amoxycillin, Ceftazidime, Cephalothin, Ciprofloxacin, and Nalidixic acid were determined as 94.7%, 92.1%, 34.2%, 36.8%, 10.5% and 89.5% respectively. Penicillin G and Amoxicillin which belong to group Penicillin were determined to be antibiotics with the highest resistant. From the result, twenty-three (60.5%) (Stock culture = 13; new isolates = 10) were classified as a multidrug-resistant *S. aureus* (MDRSA) (Figure 2) since the isolates were resistant to three or more category of antibiotic group (Magiorakos *et al*., 2012). The isolates displayed resistant to Penicillin, Cephalosporins, and Quinolones Fluoroquinolone classes of antibiotics. A relatively result was found in the study of Deyno *et al*. (2017), revealed that the overall rate of MDR was 100%; all of the isolates were found to be resistant to three and more tested antibiotics. Recently, there were 44.2% and 23.9% MDRSA isolated from human were reported in Nepal and Northeast Ohio (NEO) (Kadariya *et al*., 2019). This result support that the study of MDRSA was important since it became high occurrence in worldwide. In addition, the positive MDRSA from new isolates (SA14-SA23) were then tested for their confirmation as *S. aureus* by molecular identification, since the isolates from stock culture (SA01-SA13) were already confirmed as *S. aureus* in previous study.

3.3 Confirmation of MDRSA by 16s rRNA sequences

All MDRSA for new *S. aureus* isolates were confirmed by 16s rRNA sequences. A total of ten MDRSA were showed positive as *S. aureus* in gene sequencing. These isolates proved to be *S. aureus* after the genetic detection of *S. aureus* by 16s rDNA. The forward and reverse sequencing results are edited and assembled into one full-length sequence. Table 4 showed blast results against NCBI 16S ribosomal RNA sequences.

3.4 Adherence ability of MDRSA at 25°C and 37°C

The adherence ability of the twenty-three MDRSA strains at 25°C and 37°C on 24, 48 and 72 hrs were tested, and the results were expressed in log CFU/mL. All 23 strains showed the capability to adhere to the stainless steel disc with a minimum cell count of 4.00 log CFU/mL. Figure 3 shows the number of viable cell adhered on stainless steel disc at temperature 25°C and 37°C during 24, 48 and 72 hrs were ranging from 4.11 to 6.55 log CFU/mL and 4.25 to 6.86 log CFU/mL, respectively. At 24 hrs incubation, 5 and 13 strains have high adherence with 6.01 to 6.81 log CFU/mL at 25°C and 37°C, respectively while at 48 hrs, 6 and 9 strains have high adherence with ranging from 6.05 to 6.86 log CFU/mL at 25°C and 37°C, respectively. At 72 hrs incubation, only SA21 and SA05 was good in adhered on stainless steel disc at 25°C and 37°C, with 6.28 log CFU/mL and 6.12 log CFU/mL, respectively. SA01 show poor adherence with the lowest cell growth (4.11 log CFU/mL and 4.25 log CFU/mL) for both temperature at 24 hrs. These results revealed the MDRSA strains have high capability to adhere on stainless steel surfaces at 48 hrs incubation compared to 24 hrs and 72 hrs, as the strains adhered with exceeding 6.00 log CFU/mL were mostly found at the incubation of 48 hrs. From this result, four trends; Trend I, Trend II, Trend III and Trend IV were found in the adherence of MDRSA strains at 25°C and 37°C as shown in Table 5. Trend III has the highest strains with 34.8% and 39.1% of MDRSA at both temperatures, 25°C and 37°C, respectively. It could be the adhesion of bacterial cell is extreme on 48 hrs than 72 hrs, which might be the bacterial cell start to remove from the stainless steel disc.

The result present in this study are similar to those reported by Souza *et al*. (2014), studied of *S. aureus* from food processing plants surface, found that a population density of 5.00 to 6.00 log CFU/mL for the strains after a 24, 48, and 72 hrs contact with stainless steel surface. As discussed regarding the substrate and the extrinsic characteristic have been reported to interfere on the bacterial adherence (Ista *et al*., 2010; Jeronimo *et al*., 2012). It was tested regarding the common temperature used in the preparation and processing of food. According to outcome from Meira *et al*. (2012) and Jeronimo *et al*. (2012), the study revealed a clear two-phase of adherence; 1) increasingly the highest numbers of cell adherence after 48 hrs, followed by 2) decreasing number of cell adherence at 72 hrs. The finding suggests, the adhered cells could present in high numbers but do not continuously increase over time

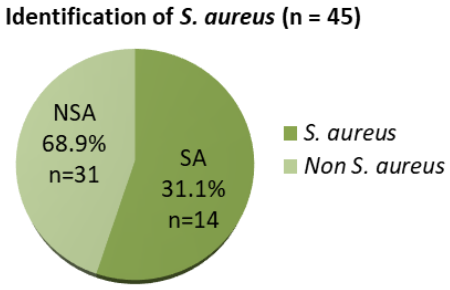

Figure 1. Identification of *S. aureus* isolates collected from food contact surfaces of food premises in Sri Serdang, Selangor.

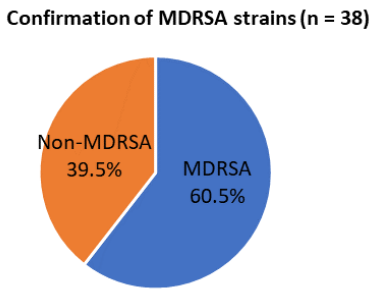

Figure 2. Confirmation of MDRSA collected from food contact

Table 3. Antibiotics resistant pattern of *S. aureus* obtained from food contact surfaces in Sri Serdang

S. aureus	Antibiotics classes (µg)											R
	Penicillin (I)		Cephalosporin (II)				Aminoglycosides (III)		Quinolones Fluoroquinolone (IV)		Salphonamides (V)	
	P 10	AML 10	CRO 5	CAZ 30	KF 30	CTX 30	CN 10	S 25	CIP 5	NA 30	SF 300	
SA001	R	R	-	-	R	-	-	-	-	R	-	3
SA002	R	R	-	-	-	-	-	-	-	R	-	2
SA003	R	R	-	-	R	-	-	-	-	R	-	3
SA004	R	R	-	-	-	-	-	-	-	R	-	2
SA005	R	R	-	-	R	-	-	-	-	R	-	3
SA006	R	R	-	R	-	-	-	-	-	R	-	3
SA007	R	R	-	R	-	-	-	-	-	R	-	3
SA008	R	R	-	-	-	-	-	-	R	R	-	2
SA009	R	R	-	-	-	-	-	-	-	R	-	2
SA010	R	R	-	R	-	-	-	-	-	R	-	3
SA011	R	R	-	R	R	-	-	-	-	R	-	3
SA012	R	R	-	-	R	-	-	-	-	R	-	3
SA013	R	R	-	-	-	-	-	-	R	R	-	2
SA014	R	R	-	-	-	-	-	-	R	R	-	2
SA015	R	R	-	-	-	-	-	-	-	R	-	2
SA016	R	R	-	-	R	-	-	-	-	R	-	3
SA017	R	R	-	R	-	-	-	-	-	R	-	3
SA018	R	R	-	-	-	-	-	-	-	R	-	2
SA019	R	R	-	-	R	-	-	-	R	R	-	3
SA020	R	R	-	-	-	-	-	-	-	R	-	2
SA021	R	R	-	-	-	-	-	-	-	R	-	2
SA022	R	R	-	-	-	-	-	-	-	R	-	2
SA023	R	R	-	-	R	-	-	-	-	R	-	3
SA024	R	R	-	-	R	-	-	-	-	R	-	3
SA025	R	-	-	-	R	-	-	-	-	-	-	2
SA026	R	R	-	R	-	-	-	-	-	R	-	3
SA027	-	-	-	-	-	-	-	-	-	-	-	-
SA028	R	R	-	R	-	-	-	-	-	R	-	3
SA029	-	-	-	-	-	-	-	-	-	-	-	-
SA030	R	R	-	-	R	-	-	-	-	R	-	3
SA031	R	R	-	R	-	-	-	-	-	R	-	3
SA032	R	R	-	R	-	-	-	-	-	R	-	3
SA033	R	R	-	R	-	-	-	-	-	R	-	3
SA034	R	R	-	-	R	-	-	-	-	-	-	2
SA035	R	R	-	R	-	-	-	-	-	R	-	3
SA036	R	R	-	R	R	-	-	-	-	R	-	3
SA037	R	R	-	-	R	-	-	-	-	R	-	3
SA038	R	R	-	R	-	-	-	-	-	R	-	3
Total R/N (%)	36/38 (94.7)	36/38 (92.1)	0/38 (0)	13/38 (34.2)	14/38 (36.8)	0/38 (0)	0/38 (0)	0/38 (0)	4/38 (10.5)	34/38 (89.5)	0/38 (0)	

R, Resistant; (-), No resistant; AML, Amoxycillin; P, Penicillin G; CRO, Ceftriaxone; CAZ, Ceftazidime; KF, Cephalothin; CTX, Cefotaxime; CN, Gentamicin; S, Streptomycin; SF, Sulphafurazole; CIP, Ciprofloxacin; NA, Nalidixic Acid; R, Resistant *S. aureus*; N, Number of *S. aureus*.

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Table 4. Blast results against NCBI 16S ribosomal RNA sequences (Bacteria only) Database, excluding uncultured Bacteria bacterium (taxid: 77133)

Isolates	Species/Subspecies	Base pair	Per Ident
SA14	*Staphylococcus aureus* strain 16S ribosomal RNA	1489 bp	99.87%
SA15	*Staphylococcus aureus* strain ATCC 12600 16S ribosomal RNA, complete sequence	1490bp	98.46
SA16	*Staphylococcus aureus* strain S33 R 16S ribosomal RNA, complete sequence	1552 bp	99.87%
SA17	*Staphylococcus aureus* strain ATCC 12600 16S ribosomal RNA, complete sequence	1490 bp	99.80%
SA18	*Staphylococcus aureus* strain NBRC 100910 16S ribosomal RNA gene, partial sequence	1477 bp	100%
SA19	*Staphylococcus aureus* strain S33 R 16S ribosomal RNA, complete sequence	1552 bp	99.87%
SA20	*Staphylococcus aureus* strain ATCC 12600 16S ribosomal RNA, partial sequence	1476 bp	100%
SA21	*Staphylococcus aureus* subsp. anaerobius strain MVF-7 16S ribosomal RNA, partial sequence	1476 bp	99.93%
SA22	*Staphylococcus aureus* strain S33 R 16S ribosomal RNA, complete sequence	1552 bp	99.59%
SA23	*Staphylococcus aureus* strain 16S ribosomal RNA	1492 bp	99.93%

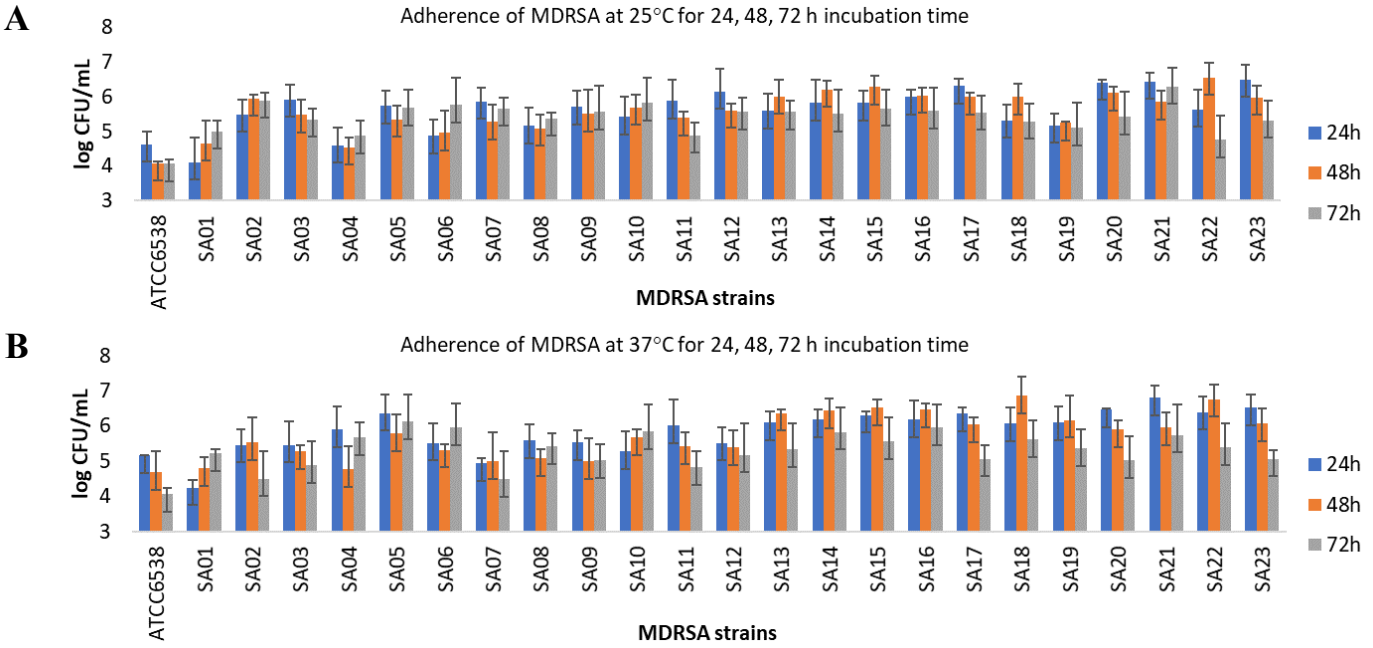

Figure 3. The adherence of MDRSA strains (n = 23, SA01-SA23) on stainless steel surfaces at 25°C (A) and 37°C (B) incubation temperature in 24, 48 and 72 hrs incubation time. The adherence ability of each stains was expressed in terms of viable cell growth (log CFU/mL) in BHI.

Table 5. The pattern of trends obtained in adherence of MDRSA strains at 25°C and 37°C.

Trend	Pattern of the trend	Description	Percentage of MDRSA (n=23)	
			Temperature	
			25°C	37°C
I	↗↗	Linear increasing	13.0 % (3)	8.7 % (2)
II	↘↘	Linear decreasing	26.1 % (6)	30.4 % (7)
III	↗↘	Increase and decrease	34.8 % (8)	39.1 % (9)
IV	↘↗	Decrease and increase	26.1 % (6)	21.7 % (5)

under the static conditions (Meira *et al*., 2012; Souza *et al*., 2014). This might be correlated with the irreversible adhesion stage as mentioned in the review of Garrett *et al*. (2008), a reversibly of the adsorbed cells number keep on restrained and become irreversibly adsorbed. This behavior could be correlated to the cell division process in forming the biofilm (Stoodley *et al*., 2002; Meira *et al*., 2012).

According to Onyango *et al*. (2012), the change in temperature is an important factor affecting bacterial growth and determines whether or not the bacteria can stay viable in potentially harsh and prolonged transmission situations between hosts. Moreover, temperature is the most common barrier used to control the growth of microorganisms. When *S. aureus* incubated in synthetic media, Malheiros *et al*. (2010) and Rode *et al*. (2007) noted the adherence on stainless steel surfaces and the highest adherence ability at 25°C - 30° C, respectively. They noted an increase in the count of adhered cells at 37°C (the optimum temperature for bacterial growth) in comparison to lower incubation

temperatures (25°C). The bacterial adhesion process will only occur at maximum growth when the bacteria are kept next to their optimum temperature.

3.5 Morphology observation of MDRSA colonies as an affected by temperature and time.

The MDRSA colonies showed different sizes and colours at 25°C and 37°C (Figure 4). The colony growth after 24–48 hrs at 25°C was smaller in size and more faded compared to 37°C. These differences in colony presence show that a lower temperature affected the growth of all MDRSA strains. Onyango *et al*. (2012) stated the changes in temperature are an important factor affecting bacterial growth particularly for pathogens like staphylococci, as the temperature is the most common barrier used to control the growth of bacteria.

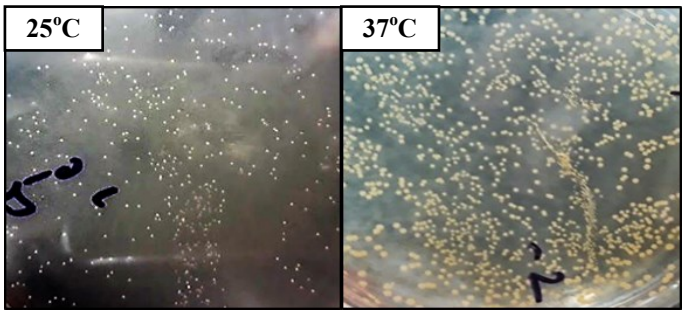

Figure 4. The colony formation of *S. aureus* on PCA at 25°C and 37°C.

Abdollah *et al*. (2014) and Schulte (2015) believed that possibly the most significant effect of temperature on microbial growth is on the shape of its enzymes, which are commonly required for metabolism. The enzymes will have a good shape only within a relatively slight range of temperatures. It may fit a lower metabolic activity when it is at a lower temperature since the cell itself grows in a stressed condition (Somero, 2004; Angilletta, 2009). Although this work aimed to investigate the adherence of viable MDRSA cell growth at 25°C and 37°C on stainless steel surfaces, the result also shows a difference in the colony formation between these two temperatures. The result presents the consequence of MDRSA when exposed to a low temperature (25°C) for elongated periods, and how this factor influences their subsequent growth and colony morphology.

3.6 Presence of small colony variants (SCV)

The exposure of all twenty-three MDRSA strains to prolonged low temperature resulted in the formation of presumptive small colony variant (SCV) phenotypes. Onyango *et al*. (2008) stated that these variants are characterised by mostly non-haemolytic and non-pigmented tiny colonies, about 1/10 the size of their wild -type (WT) counterparts. These represent subpopulations of bacteria that exhibit atypical growth features from those seen in their WT counterparts (Looney, 2000; Onyango *et al*., 2008). Staphylococci must have the ability to survive on inanimate objects through the transition processes from one host to another (Prescott *et al.,* 2002; Tuchscherr *et al*., 2010).

Prescott *et al*. (2002) mentioned that a temperature of 25°C is often applied during food processing. Although staphylococci can grow over a wide temperature range (6.5 – 46°C), their optimal range is 30 – 37°C. The ability of staphylococci to rapidly adapt to low and high temperatures is particularly crucial for their pathogenic strains (Singh *et al.,* 2008). Onyango *et al*. (2012) discovered the rates of change in SCV numbers in terms of viable bacterial populations over a week of exposure time and observed associated changes in cell-wall morphology and composition with the naked eye under a light plate counter. Based on colony characterization, the colony was physically assessed and categorised into two groups based on size and pigmentation. The colonies were characterised as SCVs if they presented pinpoint colonies (<1 mm in diameter) with decreased haemolytic activity and pigmentation colonies 24–48 hrs post-incubation, as described in previous studies (Kipp *et al*., 2004; Seifert *et al*., 2005; Von Eiff *et al*., 2006).

4. Conclusion

From this study, the isolation, identification and multidrug resistant properties of *S. aureus* from food contact surfaces were determined. The adherence of multidrug-resistant *S. aureus* (MDRSA) on stainless steel surfaces at temperatures of 25°C and 37°C was assessed in 24 hrs, 48 hrs and 72 hrs of incubation time. The result showed the strains had a better capacity to adhere on stainless steel surfaces at 37°C compared to 25°C. Therefore, MDRSA shows strong adherence ability at its optimum growth temperature.

Conflict of interest

The authors declare no conflicts of interest.

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