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Microbial Contamination of Meat Contact Surfaces at the Selected Beef Processing Plants in Selangor and its Biofilm Formation Ability

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ABSTRACT

Cross-contamination of meat products with the surrounding contact surfaces does occur during slaughtering procedures. This study aimed to assess the microbial contamination level of meat contact surfaces at the selected local abattoirs. Swab samples of knives, splitting tools and air curtains were collected from two sites in Selangor. The presence of selected indicator and pathogenic microorganisms (total Aerobic Plate Count [APC], Enterobacteriaceae, *Escherichia coli* [*E. coli*] and *Salmonella* spp.) were determined using the plate count method. The isolates obtained were then tested in terms of the biofilm formation ability using the microtiter plate crystal violet assay. Overall results showed that the average total APC for all contact surfaces was 4.77±1.14 log CFU/cm^2 (mean±sd). Enterobacteriaceae was found on 75% of the samples at 3.31±1.14 log CFU/cm^2. *E. coli* was only detected on 11.36% of the total contact surfaces at 2.91±1.00 log CFU/cm^2 whereas 25% of the total samples were positive with *Salmonella* spp. Splitting tools were identified as the most contaminated meat contact surface. Variations in biofilm formation ability were observed between isolates although most of them formed weakly adherent biofilms, especially at

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4°C. The study findings help to enhance the systems used in the local abattoirs to ensure safe and top quality meat production.

Keywords: Abattoirs, beef, biofilm formation, meat contact surface, meat contamination

INTRODUCTION

Beef is one of the animal-based main diet for Malaysians and one of the important agro-based products in Malaysia (Arif et al., 2015). Even though 75% of the beef marketed in Malaysia is imported, the local beef production steadily increased from 48 835 metric tonnes in 2011 to 49 598 metric tonnes in 2017 (Department of Veterinary Services [DVS], 2018a). This is due to the increase of 1.7% p. a. in consumption per capita for this commodity over the 7 years' period (2011-2017) (DVS, 2018b). Malaysia is expected to have a total slaughter of more than 450,000 head (cattle) per year by 2020 (Arif et al., 2015). The upward trend of local beef production and demand increases the need to enhance the current ruminant industry performance in Malaysia.

Disease outbreak due to consumption of contaminated meat products with pathogenic microorganisms has been reported worldwide (Desmarchelier et al., 2007; Nørrung & Buncic, 2008). Pathogenic microorganisms are associated with meat contamination that include *E. coli*, *Listeria monocytogenes*, *Salmonella* spp. and *Campylobacter* spp. (Sofos, 2008; Sofos & Geornaras, 2010). Reports on microbial contamination of meat products in Malaysia involve pathogens such as *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp. (Fauzi et al., 2016; Fazlina et al., 2012; Sahilah et al., 2010; Son et al., 1998; Sukhumungoon et al., 2011; Tan et al., 2019; Thung et al., 2017; Wong et al., 2012; Zulfakar et al., 2017). However, the studies focused on retail meat sold at wet markets and hypermarkets. The status of the microbial contamination level in the local abattoirs especially in terms of meat contact surfaces is still limited (Chong et al., 2017a, 2017b).

Abattoirs are the first place of beef production before being distributed to retail markets. Hence, abattoirs play a role in early prevention of microbial contamination of meat products. Contamination of meat products occurs during the slaughter and after slaughtering in the abattoir. Besides direct contact with the cattle faeces and viscera during the dehiding and evisceration steps, meat products are exposed to cross-contamination with the surrounding contact surfaces throughout the slaughter and post-slaughter procedures (Giaouris et al., 2014; Jackson et al., 2001). Hence, cleaning and disinfection of the meat processing areas as well as maintaining good hygienic practice among the meat handlers are pivotal in minimising the risk of microbial contamination.

Biofilm is the assemblages of microbial cells attached on a surface, forming a sessile community embedded in an extracellular polymeric substance (EPS) matrix (Giaouris et al., 2015). In fact, microorganisms exist in the form of biofilm on the surfaces exposed to the environment (Frank, 2001). The formation of biofilm

on contact surfaces has become a serious issue in the food processing industry and it is an ongoing threat in the meat industry (Wang 2019). Meat contact surfaces in the abattoir may harbour meat residues and nutritious detritus if the premise sanitation practice is compromised, thus providing a suitable environment for biofilm production and contribute to a continuous source of contamination (Sofos & Geornaras, 2010). Biofilm has high tolerance to the commonly used sanitisers and has high transferability potential between surfaces (Wang, 2019). These can jeopardise the abattoir hygienic state, thus affecting the safety and quality of the meat products.

This study aimed to assess the hygienic level of meat contact surfaces at two selected abattoirs in Selangor, Malaysia. The contamination level of selected indicator and pathogenic microorganisms (total APC, Enterobacteriaceae *E. coli* and *Salmonella* spp.) and the biofilm formation ability were determined.

MATERIALS AND METHODS

Sample Collection

Samples were collected from two abattoirs in Selangor, Malaysia. One of them is a large scale abattoir with a more systematic production line whereas another one has a smaller scale operation that only supplies for the local district markets. Sampling activities were conducted four times at each abattoir within nine months. Meat contact surfaces include knives (used to dehide the carcasses), splitting tools (used to eviscerate the carcasses) and air curtains (located in front of the chillers). The meat contact surfaces were chosen based on their potential to be in contact with beef carcasses thus increasing the risk of microbial contact and availability in both abattoirs. Sample collection from the meat contact surfaces was done prior to slaughtering as well as after slaughtering (that involved one batch of cattles) on the same day. Five swab samples were taken from each meat contact surface (knives and splitting tools). However, for air curtain samples, only one composite sample was taken at each sampling time. Sampling activities were conducted four times at each abattoir within nine months.

Pre-moistened sterile cotton swabs with buffered peptone water (BPW) (Merck, Germany) were used to collect samples from the meat contact surfaces (Midura & Bryant, 2001). The whole blade area of the knives and splitting tools were swabbed whereas a sterile 100 cm^2 template was used to sample the air curtains. Delineated areas of all samples were then swabbed for the second time with a dry cotton swab. Sterile swabs were then kept in 20 mL sterile BPW and maintained at 4°C before being transported to the laboratory for further analysis.

Sample Preparation

Samples were homogenised using vortex for 30 seconds. Ten mL of the homogenised sample were used to enumerate total aerobic plate count (APC), Enterobacteriaceae and *E. coli* while the remaining sample were used to detect *Salmonella* spp. (da Silva et al., 2013).

Detection and Enumeration of Total Aerobic Plate Count (APC), Enterobacteriaceae and *E. coli*. Plate count method was used to enumerate the presence of APC, Enterobacteriaceae and *E. coli* using plate count agar (PCA; Merck, Germany), violet red bile dextrose agar (VRBD; Merck, Germany) and eosin methylene blue agar (EMB; Merck, Germany) respectively. Series of 10-fold serial dilutions were performed on the homogenised samples before plating 0.1 mL aliquot from each dilution on the specified media. The plates were then incubated aerobically at 37°C for 24 h. The colonies were counted and recorded as log CFU/cm^2. Bacterial colonies from APC samples were further characterised based on the Bergey's Manual of Determinative Bacteriology (Williams, 2000) and Gram staining. Presumptive *E. coli* colonies detected on EMB agar were then subjected to standard biochemical tests (indole, methyl red, Voges-Proskauer and citrate tests) (da Silva et al., 2013).

Detection of *Salmonella* spp. Ten mL of the homogenised sample was incubated at 37°C for 20 h as pre-enrichment. An aliquot of 0.1 mL of the incubated samples was then transferred to a 10 mL Rappaport-Vassiliadis soy broth (RVS broth; Merck, Germany) and vortexed thoroughly before being incubated for 20 h at 40°C. A loopful of enriched suspension was then streaked onto xylose lysine deoxycholate agar (XLD; Merck, Germany) and incubated at 37°C for 24 h. *Salmonella* spp. was detected by the presence or absence of red colonies with black centres. Presumptive colonies were then subjected to standard biochemical tests (triple sugar iron agar, indole, methyl red, Voges-Proskauer and citrate tests) (da Silva et al., 2013).

Identification and Confirmation of Bacterial Isolates via PCR

DNA extraction was done according to Pui et al. (2011) with slight modifications. Bacterial culture was grown overnight on nutrient agar. One loopful of bacteria culture was suspended in 500 µL of sterile deionised water and centrifuged at 1000 × *g* for 5 min at 4°C. The supernatant was discarded and the pellet was resuspended with 200 µL phosphate buffered saline (PBS) (OXOID, Hampshire, England) and mixed thoroughly. The mixture was boiled at 95°C for 15 min and cooled at -20°C freezer for 15 min and recentrifuged at 1000 × *g* for 10 min at 4°C. The supernatant suspended with genomic DNA was transferred to a new tube and the each sample quality was confirmed using nanodrop spectrophotometer (Thermoscientific, Model 2000). The supernatant was used as the DNA template solution and kept at -20°C before PCR analysis.

PCR Amplification and Sequence Processing

The 16S rDNA amplification was conducted based on Suardana (2014) with some modifications. The PCR programme was carried out in 50 µL reaction solution containing 5 µL DNA template, 25 µL PCR

master mix (Promega, USA) and 5 µL of each primer. The primers used in this study were 27f (5'-AGA GTT TGA TCC TGG CTC AG-3') AND 1429r (5'-GGT TAC CTT GTT ACG ACT T-3'). The PCR cycling profile started with initial denaturation at 94°C for 2 min, followed by 35 denaturation cycles at 94°C for 1 min, annealing at 45°C for 1 min, followed by extension at 72°C for 2 min and final extension at 72°C for 10 min. About 5 µL PCR products was analysed by electrophoresis in 1% agarose gel with 4 µL sybr green as the DNA gel stain at 70 V for 40 min. Gel was visualised by UV trans-illumination.

The DNA sequencing of the PCR products was performed by First Base Sdn. Bhd., Malaysia. The sequences obtained were compared with NCBI database through basic local alignment search tool (BLAST) at www.ncbi.nih.nlm.gov/BLAST. For comparison, isolates with closely related sequences were searched. Identification of the isolates was done via sequence similarity.

Preparation of Bacterial Isolates for Biofilm Assay

All bacterial isolates obtained were stored as stock culture in tryptone soy broth (TSB; Oxoid, Hampshire, England) with 20% glycerol at -20°C. Working cultures were maintained on tryptone soy agar (TSA; Oxoid, Hampshire, England) and stored at 4°C no longer than a month. For experiment, a single colony was extracted from the working culture plate and grown overnight in TSB for 18-20h at 37°C without shaking. Cell suspensions were prepared by centrifuging the TSB culture at 1000 × *g* for 15 min. Cells were washed once in PBS at pH 7.3 (Oxoid, Hampshire, England) before being resuspended in fresh TSB to achieve bacterial concentration of approximately 10^7 CFU/mL. The optical density (OD) of 0.08 for the initial inoculum levels was standardised using a spectrophotometer (Shimadzu UV Mini 1240, Australia) at 625 nm. About 100 mL of diluted cell suspension was also plated on TSA in duplicate to confirm the inoculum level. Plates were incubated at 37°C for 24 h before counting the colonies.

Measurement of Biofilm Formation using Crystal Violet Staining

The bacterial isolate biofilm formation ability was measured according to Stepanović et al. (2004) with slight modifications. In short, 100 µL of bacterial suspension and 100 µL of fresh TSB (control wells) were added to 96-well flat bottom plate (Greiner Bio-One, Germany) in triplicate. The plates were then covered and incubated aerobically without shaking for 24 h at three temperatures (4, 25 and 37°C). After incubation, bacterial suspensions were aspirated by pipette and the wells were washed for three times with 200 µL PBS to remove loosely attached cells. Attached cells were heat-fixed at 55°C for 15 min and then stained with 100 µL of filtered 1% crystal violet for 15 min at 37°C. Wells were rinsed five times with sterile distilled water to remove excessive crystal violet stain and air-dried at 37°C. Next, 160 µL of 95% ethanol was added to test wells

to resolubilise the dye bound to the adhered cells. Biofilm formation level was quantified by measuring absorbance value using a 96-well microplate reader (Thermofisher, USA) at 650 nm. Experiments were repeated three times independently. The average OD reading for each sample and biofilm formation level for each bacterial isolate was classified according to Stepanović et al. (2004).

Statistical Analysis

The bacterial concentration results were presented as mean ± standard deviation (SD). One-way ANOVA followed by Games-Howell post hoc test were conducted to compare the bacterial contamination levels between meat contact surfaces. The statistical tests were performed using SPSS version 23.0. Results are deemed significant at $p<0.05$ unless otherwise stated.

RESULTS

The microbial loads of total aerobic plate count (APC), Enterobacteriaceae and *E. coli* found on meat contact surfaces from the selected abattoirs in Selangor were summarized in Table 1. Overall results showed that the average APC for all contact surfaces was 4.77±1.14 log CFU/cm^2 (mean±SD). Moreover, 75% of the samples were found to be positive with Enterobacteriaceae at 3.31±1.14 log CFU/cm^2 while only 11.36% of the contact surfaces were contaminated with *E. coli* (2.91±1.00 log CFU/cm^2).

Based on Table 1, knives sampled prior to slaughter had the lowest average count at 3.61±0.81 log CFU/cm^2. However, this is not significantly different ($p>0.05$) as compared to APC level for air curtain (4.28±0.65 log CFU/cm^2). Splitting tools sampled after slaughter were identified as

Table 1
Microbial load of Total Aerobic Plate Count, Enterobacteriaceae and E. coli *on meat contact surfaces from local abattoirs in Selangor*

Meat contact surface (n=20)[1]	Microorganisms detected on meat contact surfaces					
	Total Aerobic Plate Count		Enterobacteriaceae		*E. coli*	
	No. (%)[2]	Mean[3]±SD	No. (%)	Mean ± SD	No. (%)	Mean ± SD[4]
Air curtain	8 (100)	4.28±0.65^{cd*}	4 (50)	2.07±0.19^{b}	0	0
Knife (Before)	20 (100)	3.61±0.81^{d}	7 (35)	2.55±1.04^{ab}	1 (5)	2.20
Knife (After)	20 (100)	4.73±0.87^{bc}	19 (95)	3.54±1.08^{a}	7 (35)	2.78 ± 0.85
Splitting tool (Before)	20 (100)	5.27±1.06^{ab}	17 (85)	3.23±1.00^{a}	1 (5)	1.61
Splitting tool (After)	20 (100)	5.66±0.83^{a}	19 (95)	3.65±1.24^{a}	1 (5)	1.61
Total (N=88)	40 (100)	4.77±1.14	66 (75)	3.31±1.14	10 (11)	2.91±1.00

[1]No. of samples for each meat contact surfaces except for air curtains (n=8)
[2]No. of positive samples
[3]Mean bacterial counts expressed in Log CFU/cm^2
[4]Standard deviation data was only expressed for *E. coli* readings with more than one positive samples
*Different letters indicate significant difference between meat contact surfaces within the same column ($p>0.05$)

the most contaminated surface (5.66±0.83 log CFU/cm^2; p<0.05) as compared to other meat contact surfaces, save for splitting tools sampled prior to slaughter (p>0.05) with APC count of 5.27±1.06 log CFU/cm^2. Table 2 showed the bacterial isolates obtained from the APC counts, identified using the 16S rDNA method. Out of all isolates obtained, 63.6% are Gram-positive bacteria with the remaining 36.3% are Gram-negative (Table 2). From the APC counts of the isolates, 10 bacterial families were identified, with Staphylococcaceae has the highest frequency (27.3%), followed by Bacillaceae (22.7%), Enterobacteriaceae (13.63%) and Flavobacteriaceae (9.1%). Other families include Enterococcaceae, Moraxellaceae, Planobacteriaceae, Sphingobacteriaceae, Microbacteriaceae and Micrococcaceae (4.5% each), as depicted in Figure 1.

Save for one sample, both knives and splitting tools (95%) after slaughter were found to be positive with Enterobacteriaceae, recorded at 3.54±1.08 log CFU/cm^2 and 3.65±1.24 log CFU/cm^2 respectively (Table 1). The Enterobacteriaceae level on the meat contact surfaces showed an increase in the average bacterial count as compared to samples collected prior to slaughtering. The increase was also observed in APC

Table 2
Bacterial identification of isolates from Aerobic Plate Count (APC) plates via 16S rDNA PCR method

ID	Gram staining	Bacterial species
A3	Positive	*Staphylococcus fleuretti*
A4	Positive	*Bacillus cereus*
A7	Positive	*Staphylococcus saprophyticus*
A9	Positive	*Enterococcus hirae*
A10	Positive	*Bacillus thuringiensis*
A11	Negative	*Acinetobacter schindleri*
A12	Negative	*Wautersiella falsenii genomovar 2*
A14	Positive	*Kurthia populi*
A16	Negative	*Sphingobacterium daejeonense*
A31	Negative	*Macrococcus bovicus*
B1	Negative	*Enterobacter xianfangenesis*
B2	Negative	*Empedobacter falsenii*
B3	Negative	*Proteus mirabilis*
B4	Positive	*Microbacterium esteraromaticum*
G2	Positive	*Glutamicibacter creatinolyticus*
G14	Positive	*Bacillus cereus*
G18	Positive	*Staphylococcus sciuri subsp. sciuri*
G21	Positive	*Macrococcus caseolyticus*
G24	Positive	*Bacillus pumilus*
G29	Positive	*Staphylococcus sciuri*
G30	Positive	*Bacillus flexus*
G34	Negative	*Cronobacter malonaticus*

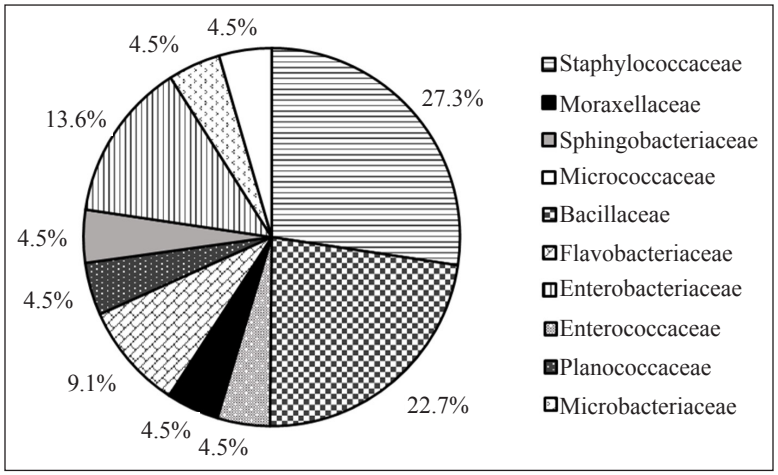

Figure 1. Diversity of bacterial isolates obtained from Aerobic Plate Count (APC) samples from meat contact surfaces at selected beef processing environment in Selangor

readings. However, there is no significant difference (p>0.05) in these readings save for the APC counts for knives. Air curtains had the lowest Enterobacteriaceae count at 2.07±0.19 log CFU/cm^2.

For *E. coli* detection, knives sampled after slaughter had the highest positive *E. coli* (58%) at 2.78 ± 0.85 log CFU/cm^2. No *E. coli* was found on the air curtains. Only one sample was found to be positive with *E. coli* for other contact surfaces. No statistical analysis was conducted to compare the average bacterial count between the surfaces due to insufficient samples that were tested positive. Analysis on the presence of *Salmonella* spp. showed that 25% of total samples were found to be positive. Out of them, splitting tools sampled after slaughter had the highest *Salmonella* spp. (41%) whereas knives sampled before slaughter had the lowest (Figure 2).

To compare the biofilm formation ability between the isolates, biofilm formation level was classified into four categories according to Stepanovic et al. (2004); non-adherent, weakly adherent, moderately adherent and strongly adherent. Table 3 compares the difference in biofilm formation

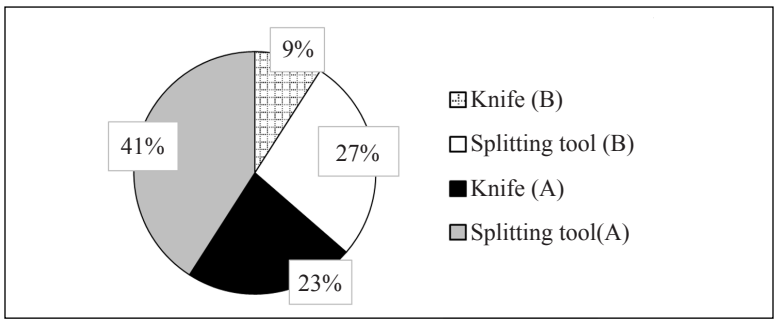

Figure 2. Distribution of *Salmonella* spp. positive samples on different types of meat contact surfaces (n=22)

Table 3
Classification of biofilm forming ability of isolates obtained from meat contact surfaces according to Stepanovic et al. (2004)[1]

ID	Temperature		
	4˚C	25˚C	37˚C
Isolates from APC plates			
A3	Non-adherent	Weak adherent	Weak adherent
A4	Non-adherent	Weak adherent	Weak adherent
A7	Moderate adherent	Moderate adherent	Weak adherent
A9	Non-adherent	Non-adherent	Weak adherent
A10	Weak adherent	Non-adherent	Weak adherent
A11	Non-adherent	Weak adherent	Weak adherent
A12	Non-adherent	Non-adherent	Weak adherent
A14	Non-adherent	Non-adherent	Non-adherent
A16	Weak adherent	Weak adherent	Weak adherent
A31	Weak adherent	Non-adherent	Moderate adherent
B1	Weak adherent	Weak adherent	Non-adherent
B2	Non-adherent	Weak adherent	Non-adherent
B3	Moderate adherent	Moderate adherent	Weak adherent
B4	Weak adherent	Weak adherent	Moderate adherent
G2	Non-adherent	Weak adherent	Weak adherent
G14	Non-adherent	Non-adherent	Weak adherent
G18	Weak adherent	Weak adherent	Weak adherent
G21	Weak adherent	Weak adherent	Weak adherent
G24	Non-adherent	Non-adherent	Non-adherent
G29	Weak adherent	Weak adherent	Weak adherent
G30	Weak adherent	Weak adherent	Moderate adherent
G34	Weak adherent	Weak adherent	Weak adherent
E.coli isolates			
A1	Moderate adherent	Strong adherent	Moderate adherent
EC3	Weak adherent	Weak adherent	Weak adherent
EC4	Weak adherent	Weak adherent	Weak adherent
EC7	Non-adherent	Weak adherent	Weak adherent
EC36	Weak adherent	Weak adherent	Weak adherent
EC37	Non-adherent	Weak adherent	Weak adherent
Salmonella isolates			
SL2	Non-adherent	Weak adherent	Weak adherent
SL4	Weak adherent	Weak adherent	Weak adherent
SL7	Weak adherent	Weak adherent	Weak adherent
SL8	Weak adherent	Weak adherent	Weak adherent
SL20	Weak adherent	Weak adherent	Weak adherent
SL21	Weak adherent	Weak adherent	Moderate adherent
SL25	Weak adherent	Weak adherent	Weak adherent
SL26	Non-adherent	Weak adherent	Weak adherent
SL27	Weak adherent	Weak adherent	Weak adherent

[1][$OD \leq OD_C$ = non-adherent, $OD_C < OD \leq 2 \times ODC$ = weak adherent; $2 \times OD_C < OD \leq 4 \times OD_C$ = moderate adherent and $4 \times OD_C < OD$ = strong adherent. OD= Optical density value for inoculated wells; OD_C = Optical density value for negative control wells]

ability between the isolates. Overall, results showed that there were two bacterial strains (A14 and G24) isolated from the APC plates with no ability to form biofilm at any temperatures whereas five isolates (A16, G18, G21, G29 and G34) showed weak biofilm adherence at all temperatures. Three isolates (A9, A12 and G14) only produced biofilm at 37°C while B2 produced biofilm at 25°C, although the biofilm adherence was weak. Other isolates that showed moderate biofilm adherence were A7 (25°C and 37°C), B3 (4°C and 25°C), A31, B4 and G30 (all at 37°C). Only one *E. coli* strain (A1) showed strong biofilm adherence but solely at 25°C. This particular isolate only formed moderate biofilm adherence at other temperatures. Other *E. coli* isolates formed weak biofilm adherence at all temperatures except for EC7 and EC37 that did not produce any biofilm at 4°C. Similar observation was seen with *Salmonella* isolates, where most strains showed weak biofilm adherence at all temperatures but both SL2 and SL26 did not form any biofilm at 4°C. However, one *Salmonella* isolate (SL21) had moderate biofilm adherence at 37°C. Based on Table 4, more than 50% of the isolates formed weak biofilm adherence at all temperatures. Meanwhile, the isolates had lower biofilm formation ability at 4°C in comparison to other temperatures.

DISCUSSION

Meat contact surfaces are identified as one of the contributing factors to microbial contamination of meat products in meat processing chain (Gounadaki et al., 2008). Regular inspection in terms of hygiene is essential to assure the quality and safety

Table 4
Distribution of biofilm production level of isolates obtained from meat contact surfaces across temperatures tested (4, 25 and 37°C)

Temperature	Biofilm Production Level	Total isolates	Percentage (%)
4˚C	Strong	0	0
	Moderate	3	8.1
	Weak	20	54.1
	Non-adherent	14	37.8
	Total	37	100
25˚C	Strong	1	2.7
	Moderate	2	5.4
	Weak	27	73.0
	Non-adherent	7	18.9
	Total	37	100
37˚C	Strong	0	0
	Moderate	5	13.5
	Weak	28	75.7
	Non-adherent	4	10.8
	Total	37	100

of the final meat products before being distributed to the public. The hygiene of the meat contact surfaces can be evaluated via indicator bacteria such as total aerobic mesophilic bacteria and Enterobacteriaceae counts (Tomasevic et al., 2016). In this study, the bacterial strains isolated are the bacteria commonly associated with microbial beef contamination (Doulgeraki et al., 2012). The average contaminations of the indicator microorganisms on the meat contact surfaces ranged from 3.61 to 5.66 log CFU/cm^2 and from 2.07 to 3.65 log CFU/cm^2 respectively. Currently, there is no specific regulation on the permissible range of microbial loads on meat contact surfaces in Malaysia. However, this study adopted the guidelines used by Australia and European countries, which suggest 10 CFU/cm^2 for total aerobic plate count (APC) and 1 CFU/cm^2 for Enterobacteriaceae (Gómez Ariño et al., 2012; New South Wales Government Food Authority [NSWFA], 2013). Based on the guidelines, the sanitation level of the meat contact surfaces in the selected abattoirs is deemed as poor.

It is a common practice for operators in the abattoirs worldwide to clean the hand tools such as knives by first rinsing them to remove any soil and have them sanitized in hot water at 82°C (Desmarchelier et al., 2007; Eustace et al., 2007; "EU Regulation 853/2004", 2004). This procedure is conducted for each carcass to prevent cross-contamination. However, based on the observation in the selected abattoirs involved in this study, this practice is not done rigorously, which may explain the high amount of APC and Enterobacteriaceae found on the meat contact surfaces. Another cause can be contamination due to the operators' hands. A study conducted in local abattoirs in Peninsular Malaysia (Shamsul et al., 2016) found that there was high prevalence of microbial contamination on the operators' hands during the meat processing procedures, which were due to inadequate practice of hand washing among the operators. However, this aspect is not covered in this study, whether the operators are aware and fully trained, or there is laxity among the operators in carrying out the procedures. Nevertheless, different meat processing environments apply different hygiene practices and the operators' knowledge, attitude and compliance with the abattoir laws vary between one another (Abdullahi et al., 2016; Ansari-Lari et al., 2010).

Although the number of samples positive with *E. coli* and *Salmonella* was low in this study, their presence on the meat contact surfaces poses a high risk of contamination, that will lead to foodborne illness if the meat products are consumed by the consumers. In fact, *E. coli* is one of the common microorganisms associated with contamination of beef products (Sofos 2008). Although *Salmonella* is more common in poultry products, there is a prevalence of this microorganism in Malaysian retail beef products (Tan et al., 2019). Hence there is a need to monitor the prevalence of the microorganisms in the abattoir environment to ensure the biosafety of beef products. The *E. coli* concentration found on meat contact surfaces indicates the level of pathogenic *E. coli* present in the abattoir environment

(Brown et al., 2000). The highest *E. coli* contamination level in this study was more than 2.5 log CFU/cm^2. This is dangerous as it has been established that the infectious dose for pathogenic *E. coli* such as O157:H7 is as low as 10-100 cells (Desmarchelier & Fegan, 2003). Furthermore, *Salmonella* spp. has the ability to colonise and persist on food contact surfaces and keep on being a contaminant to the final meat products (Joseph et al., 2001; Sallam et al., 2014).

It is surprising to find that the microbial contamination levels on knives and splitting tools prior to slaughter was already high. This raises the question on the effectiveness of cleaning and disinfection procedures in the abattoirs. However, detections of microorganisms on cleaned surfaces, which is up to 10^5 CFU/cm^2 were previously reported (Marouani-Gadri et al., 2009; Schlegelova et al., 2010). If the carcass gets contaminated with microorganisms due to the knives used during dehiding process, the contamination level will increase as the carcass moves along the production line and it can transfer the microorganisms to other meat contact surfaces. Although there is no significant difference, microbial loads on the meat contact surfaces showed an increase of microorganisms sampled after slaughtering procedures. A sufficient cleaning and disinfection regime must be at place to reduce microbial contamination in meat processing environment (Tomasevic et al., 2016).

Deficiency in hygiene practice during the cleaning and disinfection procedures will result in buildup of meat and fat residues on meat contact surfaces (Gill & McGinnis, 2004). This will cause the microbial concentration to accumulate on automated tools such as the splitting tools used for evisceration, which are much harder to clean. The complex structure of the tools causes meat residues to easily accumulate but hard to be accessed during cleaning. This in turn will support bacterial growth on the surfaces (Giaouris et al., 2014; Rivera-Betancourt et al., 2004). This is one of the factors that splitting tools were found to be the most contaminated contact surface in this study. The high microbial contamination subsequently increases the risk of contamination of the final meat products. In our previous study at the same meat processing environment (Chong et al., 2017a), beef carcasses were found to be contaminated with average APC of 4.00 log CFU/cm^2 with the presence of *Salmonella* spp. in some of the samples, that might be due to cross-contamination from the meat contact surfaces. Continuous accumulation and persistence of microorganisms on the meat contact surfaces can lead to biofilm formation on these surfaces (Sofos & Geornaras, 2010).

The high APC and Enterobacteriaceae levels found on the surfaces are sufficient to initiate biofilm formation. Biofilm formation involves many factors that include surface characteristics, properties of bacterial strains as well as environmental factors such as nutrient level, presence of antimicrobial agents, pH and temperature (Chmielewski & Frank, 2003; Giaouris et al., 2014). Although there is a limitation to reproduce the field conditions of the meat

contact surfaces in this study, microplate-based biofilm assays are extensively used to screen the biofilm formation capacity of bacterial isolates (Azredo et al., 2017). Although the surface materials used in the study are different than the actual meat contact surfaces available in the abattoir, the results can still be an indicator of the bacterial isolates' biofilm formation ability.

In this study, although more than 50% of the isolates obtained from the meat contact surfaces are regarded as weak biofilm producers at all temperatures, it still poses a threat to the meat processing environment, because there are reports that the standard sanitation procedures are not sufficient in removing biofilms (Giaouris & Simões, 2018; Joseph et al., 2001; Srey et al., 2013; Vogeleer et al., 2014). This can interfere with the cleaning procedures thus increase the risk of contamination of the meat products (Ayalew et al., 2015; Srey et al., 2013). Most *E. coli* and *Salmonella* isolates obtained in this study demonstrated weak biofilm production, which is in contrast with the previous studies (Speranza et al., 2011; Vogeleer et al., 2016; Wang et al., 2016). However, there are reports on variations between strains, even from the same species (Lianou & Koutsoumanis, 2012; Nesse et al., 2014; Pui et al., 2011; Wang et al., 2013, 2016). In this study, the variations of the isolates' biofilm formation ability at three temperatures are consistent with other reports (Di Bonaventura et al., 2008; Dourou et al., 2011; Rode et al., 2007).

Contamination of meat contact surfaces that leads to carcass contamination in the abattoir is inevitable during the slaughter and after slaughter (Niyonzima et al., 2015). The contamination resulting from the meat contact surfaces to the uncontaminated meat products must be strictly controlled. Even it is difficult to fully eliminate microbial contaminants from the meat processing environment, every meat producer is responsible to minimise the contamination level (Lowe et al., 2001). Good hygienic practice is essential to maintain the abattoir sanitation level, thus ensuring safe and top quality meat products. This can be achieved by the implementation of sanitation standards (HACCP, Good Hygiene Practices, Standard Sanitation Operating Procedures, etc.) and regular microbial quality assessment of the meat products and meat contact surfaces in meat processing establishments (Gómez et al. 2012; Tomasevic et al., 2016). The operators should be provided with training and regular supervision to ensure they understand the importance and roles of the programmes to make it a success (Kusumaningrum et al., 2003).

CONCLUSION

Meat contact surfaces were found to be contaminated even before slaughtering. The microbial contamination level of the meat contact surfaces was much higher than the permissible levels suggested by the international guidelines. Most of the isolates obtained from the surfaces were found to produce biofilms albeit their strength was low. This study has emphasized the need for better monitoring of local abattoir sanitation level by the relevant authorities.

Implementation of sanitation standards is suggested to facilitate better intervention strategies and policy in maintaining high hygiene level in the abattoirs, thus ensuring the safety and quality of the local beef supply.

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