Identification of *Escherichia* spp. strains in street-vended beverages and associated preparation surfaces using 16S rRNA analysis

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Abstract

Street-vended beverages are commonly prepared by mechanical extraction of the pulp, usually unpasteurised and requires multiple handling steps. Foodborne pathogens transmitted via skin of street vendors or via faecal-oral route may contaminate the preparation surfaces and beverages. The aim of this study is to identify *Escherichia* spp. strains of street-vended beverages and their associated preparation surfaces using 16s rRNA analysis. The hygienic practice of vendors was represented by *Staphylococcus* spp. analysis and *Staphylococcus aureus* is not detected in beverages and associated preparation surfaces. A total of 80 samples (18 beverages, 15 swab samples and 47 direct film samples) were collected followed by enumeration of microbial load. Polymerase Chain Reaction (PCR) amplification and 16S ribosomal ribonucleic acid (rRNA) sequencing were carried out. Results of 16S rRNA sequence analysis indicated that three gram-negative isolates were identified as *Escherichia coli* RM9387 (Accession no. CP009104.1), *Escherichia coli* c164 (Accession no. JQ781646.1) and *Escherichia fergusonii* E10 (Accession no. KJ626264.1) with similarity value of 99% respectively.

Keywords: coconut water, *Escherichia fergusonii*, hygiene, *Staphylococcus aureus*, sugarcane juice

Introduction

In developing countries, beverages sold by street-vendors are widely consumed by the society (Al Mamun *et al.*, 2013). In urban as well as rural area, street-vended consumables are becoming popular as they are inexpensive, convenient and attractive (World Health Organisation, 1996). Besides, such beverages are readily available and affordable to the population (Mahale *et al.,* 2008). The World Health Organization (WHO) defined street-vended foods as "foods and beverages prepared and/or sold by vendors in streets and other public places for immediate consumption or consumption at a later time without further processing or preparation" (WHO, 1996). Unpasteurised clouded, untreated juices are widely consumed because of the satisfactory flavour attributes. For instance, coconut water and sugarcane juice can be easily prepared by mechanical extraction of the pulp and liquid of mature fruits (Nonga *et al.,* 2014).

In Malaysia, the preponderance of foodborne disease as an important public health problem is alarmingly high as it causes significant impact on economic and trade of the country (Sharifa *et al.,* 2013). Based on the current society, there are a few phenomenon that are detected and encountered. The consumers emphasize on sensory taste instead of the hygienic level of beverages prepared by vendors. Furthermore, the contamination of beverages and unhygienic preparation surfaces that leads to foodborne outbreaks and will harm the safety of consumers. Sources of contamination may vary (Tambekar *et al.,* 2009) and pathogenic organisms are able to enter and contaminate foods and beverages through damaged or injured plant and fruit surfaces that occur during growing, harvesting as well as improper

washing during preparation. Such beverages are identified as potential sources of bacterial pathogens notably coliform, *Escherichia coli*, and *Staphylococcus aureus* (Mukhopadhyay *et al.,* 2011). Tan *et al.* (2013) reported that in Malaysia, transmission of bacteria such as *S. aureus* into food is mainly due to the unhygienic practice of food handlers.

Various types of pathogenic microbes in water source including bacteria, viruses, and protozoa have been linked to human disease caused by the usage of contaminated food and water (Kinge *et al.,* 2008). These water-related diseases include diarrhoea, typhoid, hepatitis, cholera, and dysentery (Kinge *et al.,* 2008). In developing countries, limited access to safe water and substandard sanitation continues to jeopardise human health. According to WHO (2003), since then, pathogenic bacteria have been identified as the utmost etiological agent in the vast majority of the waterborne outburst reported worldwide. Hence a study of the street-vended beverages was undertaken to assess the safety for human consumption and as possible sources of bacterial pathogens.

Materials and Methods

Sampling

Fresh, unpasteurised sugarcane juice (n=9) and coconut water samples (n=9) were collected from street vendors in Jeli District, Malaysia. All samples were stored below 4˚C and transported to the laboratory for microbiological analyses. The pH values of all sampled beverages were measured.

Swabbing of preparation surfaces

Samples were collected from vendors' left and right palm (72 cm^2 on each palm) using sterile 3M swabs pre-moistened with 0.1% sterile peptone water (Oxoid, England). The surface points (upper pressing unit, lower pressing unit) (100 cm^2) of sugarcane extractor, coconut opener (54 cm^2) and apron (100 cm^2) were swabbed. Swabbing was performed horizontally and vertically (Harrigan, 1998). In addition, Sanita-kun (JNC Corporation, Japan) direct stamping was also carried out on all preparation surfaces. Then, the swabs and Sanita-kun film samples were stored and transported to laboratory under cold-storage conditions (Tan *et al.,* 2014).

Bacteriological Examination

Total Plate Counts (TPCs) were determined using plate count agar (Oxoid, England) pour plate method and incubated at 37˚C for 24 hours. Visible colonies were counted using Bacterial Colony Counter (Funke Gerber, Germany) (Harrigan, 1998). The existence of coliform was determined using multiple-tube fermentation technique and the three-tube most-probable-number (MPN) method. Appropriate dilutions of samples were inoculated into triplicates of Lauryl Sulfate Tryptose broth (Merck, Germany) (Bagci and Temiz, 2011). Then, one loopful of culture from presumptive positive Lauryl Sulfate Tryptose tubes were confirmed by sub-culturing into Brilliant Green Bile broth (R and MJL Chemicals, U.K.) and further incubated at 37˚C for 24 hours (Bagci and Temiz, 2011).

Determination of Escherichia coli

One loopful of positive Lauryl Sulfate Tryptose broth were transferred into EC broth (Oxoid, England) and incubated. Presumptive positive tubes were further isolated on Eosin Methylene Blue agar (Merck, Germany) (Mallmann and Darby, 1941) and subjected to IMViC test. For indole production, 0.3 ml of Kovác's reagent (Merck, Germany) were added into incubated tryptone broth (Merck, Germany) containing presumptive *E. coli* colony (Tan *et al.,* 2014). Indole positive colony from nutrient agar was inoculated on Simmons Citrate agar (Oxoid, England) slant for citrate test.

Determination of Staphylococcus aureus

For each dilution to be plated, 1 ml of inoculum was aseptically distributed equitably to 3 plates of Baird-Parker agar (Merck, Germany) with Egg Yolk Tellurite Emulsion (Oxoid, England). Presumptive *Staphylococcus aureus* colonies from Baird-Parker agar were transferred into 0.2 ml Brain Heart Infusion broth (Merck, Germany) and emulsified thoroughly for coagulase test. Then, 0.3 ml of rabbit coagulase plasma with EDTA (Remel, United Kingdom) were added to Brain Heart Infusion culture and examined periodically for clot formation (Harrigan, 1998; Kateete *et al.,* 2010).

DNA extraction and agarose gel electrophoresis

Presumptive positive *E. coli* samples were preserved using glycerol stock. Bacteria cells were then inoculated into 5 ml Luria Bertani broth (Merck, Germany) and incubated overnight until the optical density (OD) value of culture falls within the range of 0.8-1.0 at 600 nm (Kinge *et al.,* 2008; Magray *et al.,* 2011). Genomic DNA were then extracted (Fattahi *et al.,* 2013) using G-spin Total Genomic DNA Kit (Intron, Korea). Horizontal agarose 1% w/v slab gel was prepared using agarose (Vivantis, USA), 1X Tris-Acetate-EDTA (40 mM tris-acetate; 20 mM glacial acetic acid and 1.0 mM EDTA, pH 8.0) and 10 µl of 1 mg/ml ethidium bromide (Kinge *et al.,* 2008). DNA ladder of 1 kb and 100 bp and each DNA template with loading dye (Vivantis, USA) were loaded into the well prior to electrophoresis. The agarose gel was electrophoresed for 45 minutes at 80 V and EtBr-stained agarose gel was visualised under UV transilluminator (Kazemi and Hajizadeh, 2012).

Polymerase Chain Reaction (PCR)

Extracted DNA from isolated bacteria were then used for PCR and 16S rRNA analysis using 16S rRNA universal primers (Forward primer 5'-AGAGTTTGATCCTGGCTCAG-3' and reverse primer 5'-CGGCTACCTTGTTACTT-3') (Magray *et al.,* 2011). PCR reaction was carried out in 50 µl PCR reaction mixture (2.5 U Taq DNA polymerase, 10 p moles each of the forward and reverse primers, 1X Taq buffer, 0.2 mM dNTP mix, 1.5 mM $MgCl_2$, DNA template and sterile distilled water) (1st Base, Singapore). PCR amplification was performed in a Thermal Cycler (Eppendorf AG 22331 Hamburg, Germany) under conditions such as heat

denaturation at 94°C for 4 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute; annealing at 59°C for 1 minute and extension at 72°C for 1 minute. This was followed by a final extension step at 72°C for 7 minutes and 4°C hold (Kinge *et al.,* 2008; Pathak *et al.,* 2013). Also, the purity of DNA were determined using Nanodrop Spectrophotometer (DeNovix, United State) at $A_{260/280}$ and $A_{260/230}$ (Pathak *et al.,* 2013). Vivantis Gel DNA Purification Kit was used to perform gel extraction purification.

16S rRNA Sequencing

Purified PCR products were subjected for 16S rRNA fragment sequencing. The relative molecular sizes of the PCR products were compared against the sequences from the Gen Bank database, National Centre for Biotechnology Information (NCBI) using Basic Local Alignment Search Tool (BLAST) analysis program (Fattahi *et al.,* 2013).

Results and Discussion

The microbiological standard of beverages and preparation surfaces concerned were compared against Ministry of Health (MOH) Malaysia drinking water quality as well as World Health Organisation (WHO), U.S. Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) drinking water regulations. Prior to any microbial test, the pH of sugarcane juice were taken as it may contribute to microbial growth. The pH of sugarcane juice were recorded from stall A (pH 4.38), stall B (pH 5.35) and

179 stall C (pH 4.67). These values are in the range of Oliveira *et al*. (2006) and
180 Mahale *et al*. (2008) studies (pH 4.89). Although low pH value may have
181 inhibitory effects to microbial contamination (Nonga *et al.,* 2014) and the
182 survival of pathogenic bacteria in beverages, large groups of bacteria are
183 able to grow at pH greater than 4 (Oliveira *et al*., 2006).

184 Based on the distribution of bacteria from total plate counts of sugarcane
185 juice, stall B showed the highest bacteria load at 2.63±0.01 log CFU/ml
186 (P<0.05) compared to Stall A and C's sugarcane juice (Table 1). The
187 detection of coliform contamination in juice samples clearly indicated that it
188 does not fulfil WHO and EPA's standard where no coliform should be
189 detected. All sugarcane juice samples recorded 240 MPN/ml for presumptive
190 *Escherichia coli* counts (ECCs). Finding presumptive pathogenic *E. coli*
191 species in beverages ready for consumption at all three stalls is generally
192 viewed as an indication of faecal contamination (Lambrechts *et al.,* 2014).

193 *Staphylococcus aureus* had been reported in street-vended juice
194 samples (Suneetha *et al.,* 2011). In this study, stall A sugarcane juice
195 reported the highest staphylococci load (2.62±0.02 log CFU/ml) (Table 1). In
196 contrast, staphylococci were not detected in *S. aureus* selective agar in stall
197 C's sugarcane juice sample. This indicated better hygiene management of
198 beverages preparation since human skin and mucous membrane are the
199 natural carrier of *S. aureus* (Lambrechts *et al.,* 2014). Based on the
200 coagulase test, none of the samples from stall A and stall B sugarcane juice
201 showed coagulation. The possible species on Baird-Parker agar but
202 responded as negative-coagulase is *Staphylococcus epidermidis*.

For coconut water, Yong *et al.* (2009) claimed that the pH of fresh coconut water for consumption should fall within the range of 4.6–5.6. The present study showed the average pH of coconut water from stalls A, B and C were 4.80, 5.27 and 4.78 respectively which is compatible with the study. Coliform counts in coconut water ranged between 43 MPN/ml to >1000 MPN/ml (Table 1) which is also incompatible with WHO and EPA standard as no coliform should be present. The presumptive positive *E. coli* species detected in coconut water was non-pathogenic Enterobacteriaceae bacteria. For identification of *Staphylococcus*, stall A recorded the highest staphylococci bacteria load followed by stall B while stall C's samples did not show characteristic feature of staphylococci colony.

Pearson correlation showed that the average TPCs with presumptive *Staphylococcus aureus* counts (SACs) of sugarcane juice and coconut water, from all three selected vendors were highly correlated, r=0.861. It delineated that causal relationship exist between TPC and ECC of beverages as well as among TCC and SAC of beverages collected from all three stalls. *Staphylococcus* counts were detected in 60% (9/15) of the associated preparation surfaces and 26.67% (4/15) of *E. coli* counts were detected on the examined preparation surfaces using Sanita-kun direct stamping method. The linear correlation coefficient between the Baird-Parker agar *Staphylococcus aureus* counts (SACs) and Sanita-kun *Staphylococcus aureus* counts (SSCs) method was r=0.956 which showed high correlation. Morita *et al.* (2003) and Park and Kim (2013) reported that the plate count

and Sanita-kun film count correlation coefficient were r=0.99 and therefore Sanita-kun is an acceptable alternative to various agar medium.

Table 2 showed that there were no significant difference (P>0.05) in TPCs between both left and right hands of the street vendors. The TPCs of stall B handler's left and right hands recorded the highest bacteria load at 2.96±0.02 and 2.90±0.02 log CFU/ml respectively. Stall B's handler also recorded the highest *Staphylococcus* sp. count for sugarcane juice (2.63±0.01 log CFU/ml) and coconut water (2.56±0.01 log CFU/ml). The TPCs on both hands were compatible with Christison *et al.* (2008) who stated that the TPCs of handlers' hands were comparably higher than beverages. Likewise, similar trends were observed for stall A and stall C vendor's hands. It can be deduced that cross-contamination occurs where partial microbial load was transmitted from the preparation surface during handling process (Mahale *et al.,* 2008) and from handler's hands to the sugarcane juice and coconut water (Tan *et al.,* 2014).

Among all five types of preparation surfaces examined, sugarcane extractors revealed the highest TPCs (4.13±0.01 and 3.91±0.01 log CFU/ml) for stall B and stall C respectively (Table 2). Gilbert *et al.* (2000) suggested that several factors attributed to the prevalence of coliform on preparation surfaces i.e. the unhygienic practices of vendors, equipment design (e.g. the uneven and ragged surface of the upper and lower pressing unit of sugarcane extractor that build-up food debris and microorganisms) and cleaning and sanitation procedures. Results from the present study showed

249 that sugarcane juice extractor from stall C exhibited positive citrate test with
250 the highest presumptive *E. coli* counts (240 MPN/ml, 2.79±0.01 log CFU/cm^2).
251 The microbial status of the coconut opener which is a knife used by
252 street vendors is also shown in Table 2. The total coliform counts and
253 presumptive *Escherichia coli* MPN test results were negative for all coconut
254 opener samples analysed. The microbiological quality of street vendors'
255 aprons with TPCs ranged from < 2.40 to 3.77±0.02 log CFU/ml. There is a
256 vast difference between the total microbial load of stall B vendor's apron
257 (3.77±0.02 log CFU/ml) compared with stall A and stall C (Table 2).
258 This study showed high bacterial load in beverages ranging from
259 2.12±0.01 to 2.63±0.01 log CFU/ml. The TPCs for preparation surfaces
260 fluctuates and covers a wide range, i.e. from an acceptable range of <2.40
261 to 4.13±0.01 log CFU/ml. This reflects the inadequate sanitary of the
262 sugarcane juice, coconut water and utensils used (Oliveira *et al.* 2006).
263 Results showed that different street vendors will significantly affect (P<0.05)
264 the presumptive *Staphylococcus aureus* load for each preparation surfaces.
265 The staphylococci microbial load for left and right hands, sugarcane extractor
266 and aprons using both BPA and Sanita-kun *S. aureus* direct stamping
267 method were in the order of stall B > stall A > stall C. However, all preparation
268 surfaces were tested as coagulase negative and eliminated the possibility of
269 the presence of pathogenic *Staphylococcus aureus* species.
270 High surrounding temperature of street-vended beverages favours
271 bacteria growth (Nonga et al. 2014). Furthermore, heavy vehicular traffic also
272 contributes to high TPCs of street-vended stalls (Mugampoza *et al.,* 2013)

and are susceptible to contamination by dust-laden microorganisms. With regards to waste disposal, dumping of wastes on-site with piles of uncovered waste is another unhygienic source that causes further cross-contamination to the consumable goods (Nonga *et al.,* 2014).

In some circumstances, both swabbing and dry culture medium methods have pros and cons. The direct stamping method using sheet medium can effectively pick up bacteria present at the surface examined. While for sterile swabs, the collection surface is notably smaller and if excess moisture is applied on the sampling surface, it can cause residual to remain on the tested surface. However, swab samples are best suited for use on irregular surfaces such as sugarcane extractor in this study while flat sheet medium is less preferred. The Sanita-kun *E. coli* and coliform chromogenic medium were metabolised by the growing microorganism. The coloured colonies were easily distinguished between *E. coli* and other coliform colonies because the dry medium was hydrolysed by galactosidase from coliforms and will produce X-Gal visible blue dye upon coliform growth. Meanwhile, salmon-glucuronic acid composed in the medium was hydrolysed by glucuronidase from *E. coli* to produce a red-purple dye (Ushiyama and Iwasaki, 2010). The Sanita-kun *Staphylococcus aureus* dry culture medium contains chromogenic agent and a specific fluorescence enzyme substrate (Waku *et al.* 2013). A buffering agent is also present to adjust and maintain the pH at approximately 7.0 to favour the growth of bacteria. As bacteria grow, the blue and black colour colonies will develop. This eases the direct enumeration of *S. aureus* compared with enumeration on Baird-Parker agar where *S. aureus* was

297 distinguished among black colonies by identifying opaque ring clear zones
298 (Waku *et al.,* 2013).

299 DNA extractions were conducted on *E. coli* strains isolated from
300 sugarcane juice of all stalls and sugarcane extractor from stall C as only
301 replicates from these four isolates were identified as pathogenic *E. coli*
302 through IMViC test. Replicates of extracted DNA samples were subjected to
303 gel electrophoresis. All DNA samples migrated from the cathode towards the
304 anode, showing bands of DNA fragments at respective wells while elution
305 without DNA samples was set as the negative control. The rate of travel of
306 DNA towards the positive pole of agarose gel varies inversely with its
307 molecular weight. This is explained by the large size of DNA fragments
308 entangled in the gel matrix and which migrated slowly (Magray *et al.,* 2011).
309 Since the initial DNA extracts often contains RNA and protein, it caused
310 smearing of bands and may interfere with the extracted DNA and need to be
311 purified to retain only targeted DNA fragment. DNA products of all samples
312 were subjected to PCR to multiply the copies of DNA from a single strand of
313 DNA extracted. Through PCR, the targeted DNA was amplified to enable the
314 selection of *E. coli*. Universal primers were used for the amplification of 16S
315 rRNA gene fragment (Magray *et al.,* 2011).

316 The negative control in the above agarose gel for gel electrophoresis of
317 PCR product received the PCR mixture containing no DNA template (Fattahi
318 *et al.,* 2013). High intensity of PCR products in Figure 1 indicates that
319 selective amplification of *E. coli* was successfully attained using universal
320 primers during annealing at 72˚C with high concentration of DNA fragments

obtained (Sabat *et al.,* 2000). From the gel electrophoresis of PCR products, only PCR products with high intensity and clear band under UV illumination (S3, S4, S6 and S9) from each type of sample were selected for PCR purification.

Purification step was performed with the aid of Vivantis Gel DNA Recovery Kit for rapid purification of residual reagents during PCR reaction and elimination of the unspecific DNAs that caused smearing of bands observed in S3, S4, S6 and S9. The clear and single sharp purified bands of 1500 bp (Figure 2) represents good quality DNA and these enable the 16S rRNA fragments (Fattahi *et al.,* 2013) of samples from sugarcane juices and sugarcane juice extractor to be subjected to DNA sequencing to identify strains of *Escherichia coli*.

The sequencing results were obtained from MyTACG Bioscience Enterprise. All four isolates were aligned using nucleotide BLAST to ascertain the sequence similarity against the NCBI database. Based on the results obtained, the genera with highest occurring frequency are *Escherichia* spp. in all four samples (Table 3).

Escherichia coli strain RM9387 was previously linked to contaminated milk and is commonly isolated from cattle faeces (Yan *et al.* 2015). *E. coli* RM9387 were also associated with recreational and municipal drinking water outbreaks (Kaper *et al.* 2004). *Escherichia fergusonii,* isolated from stall C's sugarcane juice extractor is the closest related species to *Escherichia coli* and both has been reported as pathogenic species in *Enterobacteriaceae*. *Escherichia fergusonii* and *Escherichia coli* can be isolated from faeces and

345 water samples (Maheux *et al.* 2014). *Escherichia fergusonii* had recently
346 been shown to cause disease in animals and humans (Forgetta *et al.* 2012)
347 as it can influence the intestinal function of both poultry and mammals
348 (Hariharan *et al.* 2007). The presence of *E. coli* on sugarcane plant could be
349 due to contamination from animals' or pests' faeces (Duncan and Colmer
350 1964; Heaton and Jones 2008) instead of cross-contamination from handlers
351 as *E. coli* were not detected on handlers' hands or aprons. Furthermore,
352 when transporting the sugarcane plant, the exposed ends of sugarcane plant
353 can be a favourable environment for the multiplication of *E. coli*.
354
355 **Conclusion**
356
357 *Escherichia coli* and *Staphylococcus* spp. were present in juice samples
358 and some preparation surfaces. The highest total plate count was recorded
359 in both sugarcane juice and coconut water from stall B. The detection of *E.*
360 *coli* strain RM9387 in stalls A and B sugarcane juice, *E. coli* strain c164 in
361 stall C's sugarcane juice sample and *E. fergusonii* strain E10 in stall C
362 sugarcane juice extractor showed that contamination of street-vended
363 beverages or preparation surfaces may present health risk and leads to
364 foodborne illnesses.
365 Street-vended beverages are prepared for immediate consumption and
366 are usually untreated. The results of this study demonstrated that beverages
367 extracted from fresh fruits and associated preparation surfaces are identified
368 as potential sources of bacterial pathogens. The isolated pathogenic *E. coli*

strains may present health risk and leads to foodborne illnesses. Accordingly, adoption of strict hygienic measures should be followed during the preparation of street-vended beverages. Regular monitoring of the quality of street-vended beverages is vital to avoid foodborne illnesses. The public need to have greater awareness with respect to food safety while the authority should enforce regulations by making basic Food Handling Course compulsory to food handlers and further training as required by the relevant authority. Associated preparation surfaces such as sugarcane extractor and coconut opener are potential sources of contamination and should be cleaned and sanitised regularly.

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545 Table 1. Microbiological quality of sugarcane and coconut juice samples

Stall	Total Plate Count Mean (log CFU/ml)		Total Coliform Count Mean (MPN/ml)		*Escherichia coli* Mean (MPN/ml)		*Staphylococcus* spp. Mean (log CFU/ml)	
	Sugarcane	Coconut	Sugarcane	Coconut	Sugarcane	Coconut	Sugarcane	Coconut
A	2.49 ± 0.02[b]	2.50 ± 0.05[a]	93	150	240	93	2.62 ± 0.02[a]	2.76 ± 0.02[a]
B	2.63 ± 0.01[a]	2.56 ± 0.01[a]	>1100	>1100	240	460	2.28 ± 0.01[b]	2.36 ± 0.01[b]
C	2.32 ± 0.01[c]	2.12 ± 0.01[b]	>1100	43	240	<3.0	ND	ND

546 ND: Not Detected
547 Data are expressed as means ± standard deviation (n=3).
548 [abc] values with different superscripts in the same column indicate significant difference (P<0.05).
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553 Table 2. Bacterial load of associated food contact surfaces

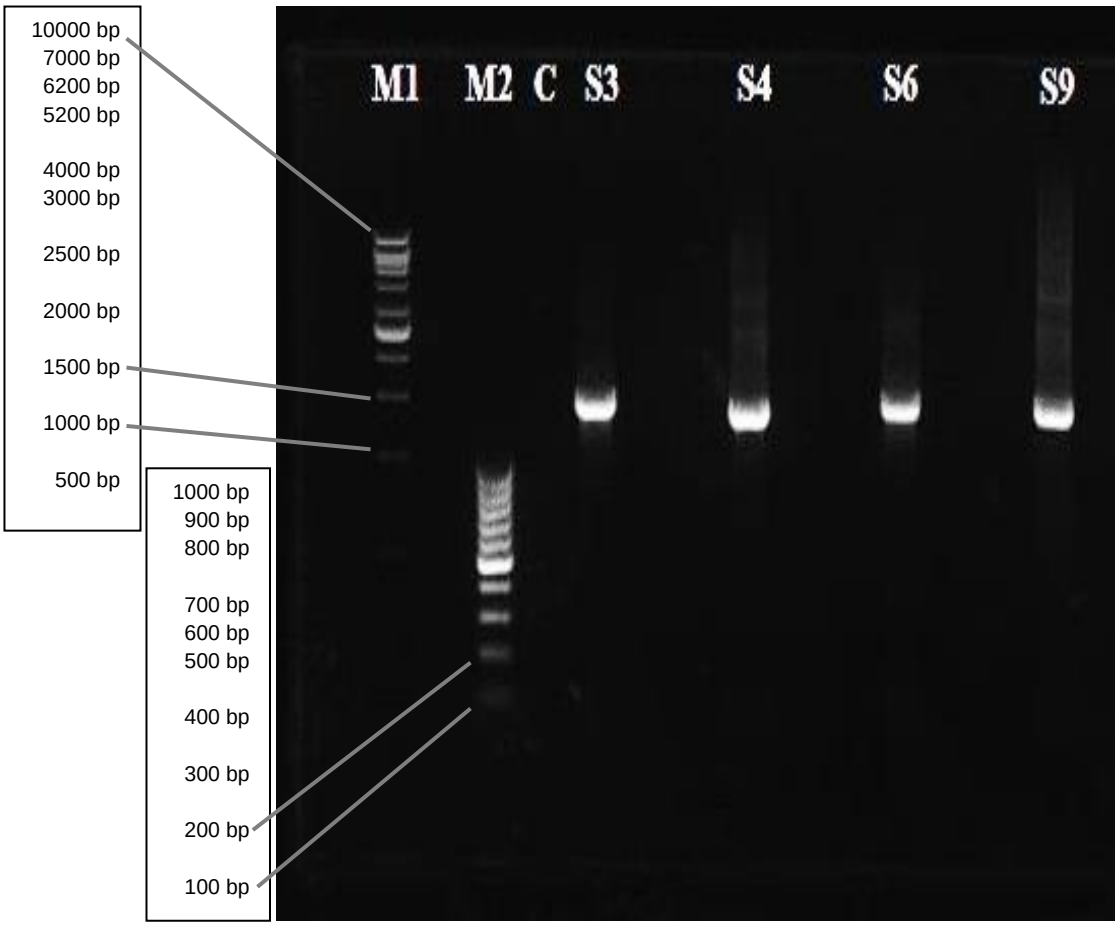
Sample type / Stall	Total Plate Count (log CFU/ml)	Total Coliform Count (MPN/ml)	*Escherichia coli*		*Staphylococcus* spp.	
			MPN/ml	log CFU/cm^2	log CFU/ml	log CFU/cm^2
Left hand						
A	2.64 ± 0.04^b	240	43	ND	<2.40	<2.40
B	2.96 ± 0.02^a	460	240	ND	2.53 ± 0.01	2.63 ± 0.01
C	<2.40	23	23	ND	ND	ND
Right hand						
A	2.69 ± 0.06^b	240	43	ND	<2.40	<2.40
B	2.90 ± 0.02^a	460	240	<2.40	2.61 ± 0.04	2.69 ± 0.04
C	<2.40	23	23	ND	ND	ND
Aprons						
A	<2.40	<3.0	<3.0	ND	<2.40	<2.40
B	3.77 ± 0.02^a	43	23	ND	3.08 ± 0.01	3.11 ± 0.01
C	1.87 ± 0.02^b	93	43	<2.40	ND	ND
Sugarcane juice extractors						
A	<2.40	<3.0	<3.0	ND	ND	ND
B	4.13 ± 0.01^a	460	93	<2.40	2.46 ± 0.05	2.44 ± 0.01
C	3.91 ± 0.01^b	240	240	2.79 ± 0.01	ND	ND
Coconut openers						
A	<2.40	<3.0	<3.0	ND	2.62 ± 0.02^a	<2.40
B	2.91 ± 0.01^a	<3.0	<3.0	ND	2.28 ± 0.01^b	2.50 ± 0.01
C	2.86 ± 0.02^b	<3.0	<3.0	ND	<2.40	ND

554 ND: Not Detected
555 Data are expressed as means ± standard deviation (n=3)
556 [ab] values with different superscripts in the same column indicate significant difference (P<0.05) for each type of food contact
557 surfaces

Table 3. Similarity of 16S rRNA gene sequences of samples based on the NCBI database using BLAST analysis

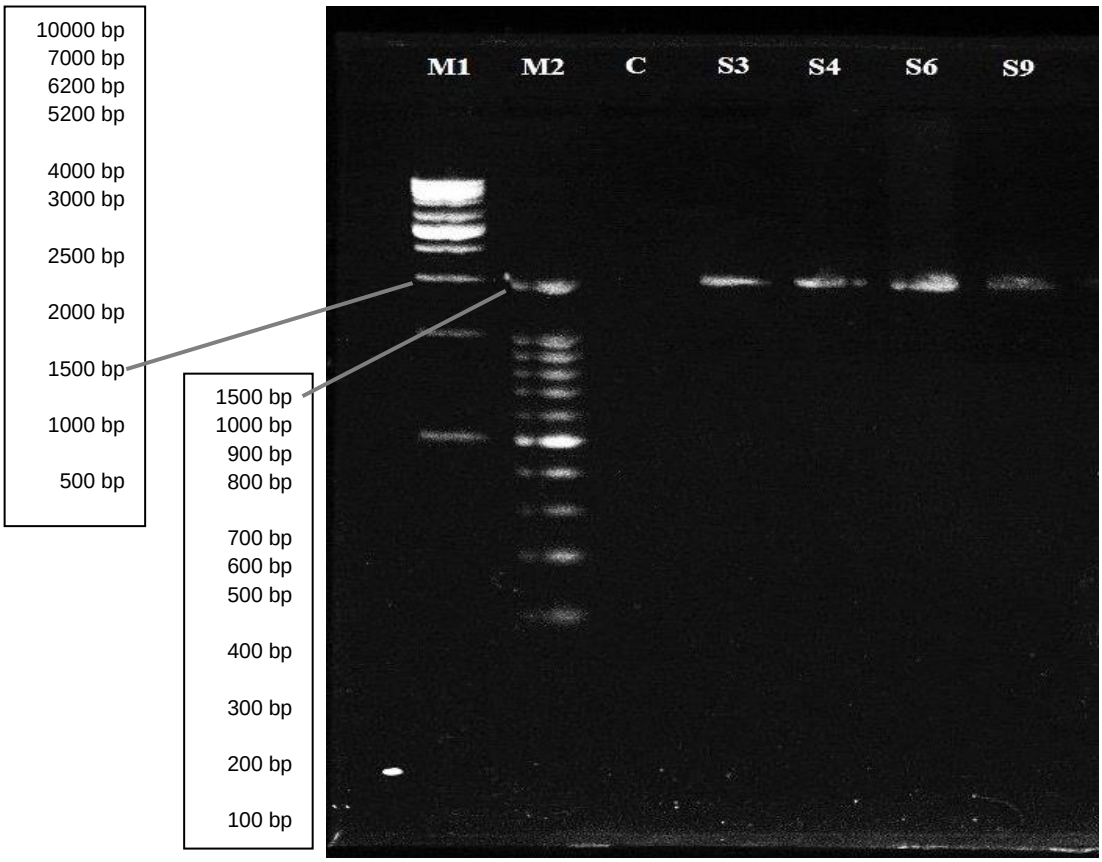
Isolates	**Similarity from BLAST**	**Identity**	***Accession***
S3	*Escherichia coli* strain RM9387	99%	CP009104.1
S4	*Escherichia coli* strain RM9387	99%	CP009104.1
S6	*Escherichia coli* strain c164	99%	JQ781646.1
S9	*Escherichia fergusonii* strain E10	99%	KJ626264.1

Note: Isolate S3: Stall A sugarcane juice
Isolate S4: Stall B sugarcane juice
Isolate S6: Stall C sugarcane juice
Isolate S9: Stall C sugarcane juice extractor



M1: 1 kb ladder; M2: 100 bp ladder; C: Negative control; S3: PCR product from stall A sugarcane juice; S4: PCR product from stall B sugarcane juice; S6: PCR product from stall C sugarcane juice extractor; S9: Stall C sugarcane juice

Figure 1. PCR products visualised under UV illumination on 1.0% agarose gel



M1: 1 kb ladder; M2: 100 bp ladder; C: Negative control; S3: PCR product from stall A sugarcane juice; S4: PCR product from stall B sugarcane juice; S6: PCR product from stall C sugarcane juice extractor; S9: Stall C sugarcane juice

Figure 2. Purified PCR products visualised under UV illumination on 1.0% agarose gel