

## Article

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

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

3

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12

13 **Abstract**

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

31

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

34

35 **Introduction**

36 In developing countries, beverages sold by street-vendors are widely  
37 consumed by the society (Al Mamun *et al.*, 2013). In urban as well as rural  
38 area, street-vended consumables are becoming popular as they are  
39 inexpensive, convenient and attractive (World Health Organisation, 1996).  
40 Besides, such beverages are readily available and affordable to the  
41 population (Mahale *et al.*, 2008). The World Health Organization (WHO)  
42 defined street-vended foods as “foods and beverages prepared and/or sold  
43 by vendors in streets and other public places for immediate consumption or  
44 consumption at a later time without further processing or preparation” (WHO,  
45 1996). Unpasteurised clouded, untreated juices are widely consumed  
46 because of the satisfactory flavour attributes. For instance, coconut water  
47 and sugarcane juice can be easily prepared by mechanical extraction of the  
48 pulp and liquid of mature fruits (Nonga *et al.*, 2014).

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

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

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

76

## 77 **Materials and Methods**

78

### 79 *Sampling*

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

84

85 *Swabbing of preparation surfaces*

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

95

96 *Bacteriological Examination*

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

108

109 *Determination of Escherichia coli*

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

118

119 *Determination of Staphylococcus aureus*

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

129

130 *DNA extraction and agarose gel electrophoresis*

131 Presumptive positive *E. coli* samples were preserved using glycerol  
132 stock. Bacteria cells were then inoculated into 5 ml Luria Bertani broth (Merck,  
133 Germany) and incubated overnight until the optical density (OD) value of  
134 culture falls within the range of 0.8-1.0 at 600 nm (Kinge *et al.*, 2008; Magray  
135 *et al.*, 2011). Genomic DNA were then extracted (Fattahi *et al.*, 2013) using  
136 G-spin Total Genomic DNA Kit (Intron, Korea). Horizontal agarose 1% w/v  
137 slab gel was prepared using agarose (Vivantis, USA), 1X Tris-Acetate-EDTA  
138 (40 mM tris-acetate; 20 mM glacial acetic acid and 1.0 mM EDTA, pH 8.0)  
139 and 10 µl of 1 mg/ml ethidium bromide (Kinge *et al.*, 2008). DNA ladder of 1  
140 kb and 100 bp and each DNA template with loading dye (Vivantis, USA) were  
141 loaded into the well prior to electrophoresis. The agarose gel was  
142 electrophoresed for 45 minutes at 80 V and EtBr-stained agarose gel was  
143 visualised under UV transilluminator (Kazemi and Hajizadeh, 2012).

144

#### 145 *Polymerase Chain Reaction (PCR)*

146 Extracted DNA from isolated bacteria were then used for PCR and 16S  
147 rRNA analysis using 16S rRNA universal primers (Forward primer 5'-  
148 AGAGTTTGATCCTGGCTCAG-3' and reverse primer 5'-  
149 CGGCTACCTTGTTACTT-3') (Magray *et al.*, 2011). PCR reaction was  
150 carried out in 50 µl PCR reaction mixture (2.5 U Taq DNA polymerase, 10 p  
151 moles each of the forward and reverse primers, 1X Taq buffer, 0.2 mM dNTP  
152 mix, 1.5 mM MgCl<sub>2</sub>, DNA template and sterile distilled water) (1st Base,  
153 Singapore). PCR amplification was performed in a Thermal Cycler  
154 (Eppendorf AG 22331 Hamburg, Germany) under conditions such as heat

155 denaturation at 94°C for 4 minutes, followed by 30 cycles of denaturation at  
156 94°C for 1 minute; annealing at 59°C for 1 minute and extension at 72°C for  
157 1 minute. This was followed by a final extension step at 72°C for 7 minutes  
158 and 4°C hold (Kinge *et al.*, 2008; Pathak *et al.*, 2013). Also, the purity of DNA  
159 were determined using Nanodrop Spectrophotometer (DeNovix, United  
160 State) at  $A_{260/280}$  and  $A_{260/230}$  (Pathak *et al.*, 2013). **Vivantis Gel DNA**  
**161 Purification Kit** was used to perform gel extraction purification.

162

### 163 *16S rRNA Sequencing*

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

169

## 170 **Results and Discussion**

171

172 The microbiological standard of beverages and preparation surfaces  
173 concerned were compared against Ministry of Health (MOH) Malaysia  
174 drinking water quality as well as World Health Organisation (WHO), U.S.  
175 Environmental Protection Agency (EPA) and Food and Drug Administration  
176 (FDA) drinking water regulations. Prior to any microbial test, the pH of  
177 sugarcane juice were taken as it may contribute to microbial growth. The pH  
178 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  $2.63 \pm 0.01$  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 ( $2.62 \pm 0.02$  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*.

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

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

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

228 Table 2 showed that there were no significant difference ( $P>0.05$ ) in  
229 TPCs between both left and right hands of the street vendors. The TPCs of  
230 stall B handler's left and right hands recorded the highest bacteria load at  
231  $2.96\pm 0.02$  and  $2.90\pm 0.02$  log CFU/ml respectively. Stall B's handler also  
232 recorded the highest *Staphylococcus* sp. count for sugarcane juice  
233 ( $2.63\pm 0.01$  log CFU/ml) and coconut water ( $2.56\pm 0.01$  log CFU/ml). The  
234 TPCs on both hands were compatible with Christison *et al.* (2008) who stated  
235 that the TPCs of handlers' hands were comparably higher than beverages.  
236 Likewise, similar trends were observed for stall A and stall C vendor's hands.  
237 It can be deduced that cross-contamination occurs where partial microbial  
238 load was transmitted from the preparation surface during handling process  
239 (Mahale *et al.*, 2008) and from handler's hands to the sugarcane juice and  
240 coconut water (Tan *et al.*, 2014).

241 Among all five types of preparation surfaces examined, sugarcane  
242 extractors revealed the highest TPCs ( $4.13\pm 0.01$  and  $3.91\pm 0.01$  log CFU/ml)  
243 for stall B and stall C respectively (Table 2). Gilbert *et al.* (2000) suggested  
244 that several factors attributed to the prevalence of coliform on preparation  
245 surfaces i.e. the unhygienic practices of vendors, equipment design (e.g. the  
246 uneven and ragged surface of the upper and lower pressing unit of  
247 sugarcane extractor that build-up food debris and microorganisms) and  
248 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,  $2.79 \pm 0.01$  log CFU/cm<sup>2</sup>).

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  $3.77 \pm 0.02$  log CFU/ml. There is a  
256 vast difference between the total microbial load of stall B vendor's apron  
257 ( $3.77 \pm 0.02$  log CFU/ml) compared with stall A and stall C (Table 2).

258 This study showed high bacterial load in beverages ranging from  
259  $2.12 \pm 0.01$  to  $2.63 \pm 0.01$  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  $4.13 \pm 0.01$  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)

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

277 In some circumstances, both swabbing and dry culture medium methods  
278 have pros and cons. The direct stamping method using sheet medium can  
279 effectively pick up bacteria present at the surface examined. While for sterile  
280 swabs, the collection surface is notably smaller and if excess moisture is  
281 applied on the sampling surface, it can cause residual to remain on the tested  
282 surface. However, swab samples are best suited for use on irregular surfaces  
283 such as sugarcane extractor in this study while flat sheet medium is less  
284 preferred. The Sanita-kun *E. coli* and coliform chromogenic medium were  
285 metabolised by the growing microorganism. The coloured colonies were  
286 easily distinguished between *E. coli* and other coliform colonies because the  
287 dry medium was hydrolysed by galactosidase from coliforms and will produce  
288 X-Gal visible blue dye upon coliform growth. Meanwhile, salmon-glucuronic  
289 acid composed in the medium was hydrolysed by glucuronidase from *E.*  
290 *coli* to produce a red-purple dye (Ushiyama and Iwasaki, 2010). The Sanita-  
291 kun *Staphylococcus aureus* dry culture medium contains chromogenic agent  
292 and a specific fluorescence enzyme substrate (Waku *et al.* 2013). A buffering  
293 agent is also present to adjust and maintain the pH at approximately 7.0 to  
294 favour the growth of bacteria. As bacteria grow, the blue and black colour  
295 colonies will develop. This eases the direct enumeration of *S. aureus*  
296 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

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

325 Purification step was performed with the aid of Vivantis Gel DNA  
326 Recovery Kit for rapid purification of residual reagents during PCR reaction  
327 and elimination of the unspecific DNAs that caused smearing of bands  
328 observed in S3, S4, S6 and S9. The clear and single sharp purified bands of  
329 1500 bp (Figure 2) represents good quality DNA and these enable the 16S  
330 rRNA fragments (Fattahi *et al.*, 2013) of samples from sugarcane juices and  
331 sugarcane juice extractor to be subjected to DNA sequencing to identify  
332 strains of *Escherichia coli*.

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

338 *Escherichia coli* strain RM9387 was previously linked to contaminated  
339 milk and is commonly isolated from cattle faeces (Yan *et al.* 2015). *E. coli*  
340 RM9387 were also associated with recreational and municipal drinking water  
341 outbreaks (Kaper *et al.* 2004). *Escherichia fergusonii*, isolated from stall C's  
342 sugarcane juice extractor is the closest related species to *Escherichia coli*  
343 and both has been reported as pathogenic species in *Enterobacteriaceae*.  
344 *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*

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

379

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544

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

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

546 ND: Not Detected

547 Data are expressed as means ± standard deviation (n=3).

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

554 ND: Not Detected

555 Data are expressed as means  $\pm$  standard deviation (n=3)

556 <sup>ab</sup> values with different superscripts in the same column indicate significant difference ( $P<0.05$ ) for each type of food contact  
557 surfaces

558

559 Table 3. Similarity of 16S rRNA gene sequences of samples based on the

560 NCBI database using BLAST analysis

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

561 Note: Isolate S3: Stall A sugarcane juice

562 Isolate S4: Stall B sugarcane juice

563 Isolate S6: Stall C sugarcane juice

564 Isolate S9: Stall C sugarcane juice extractor

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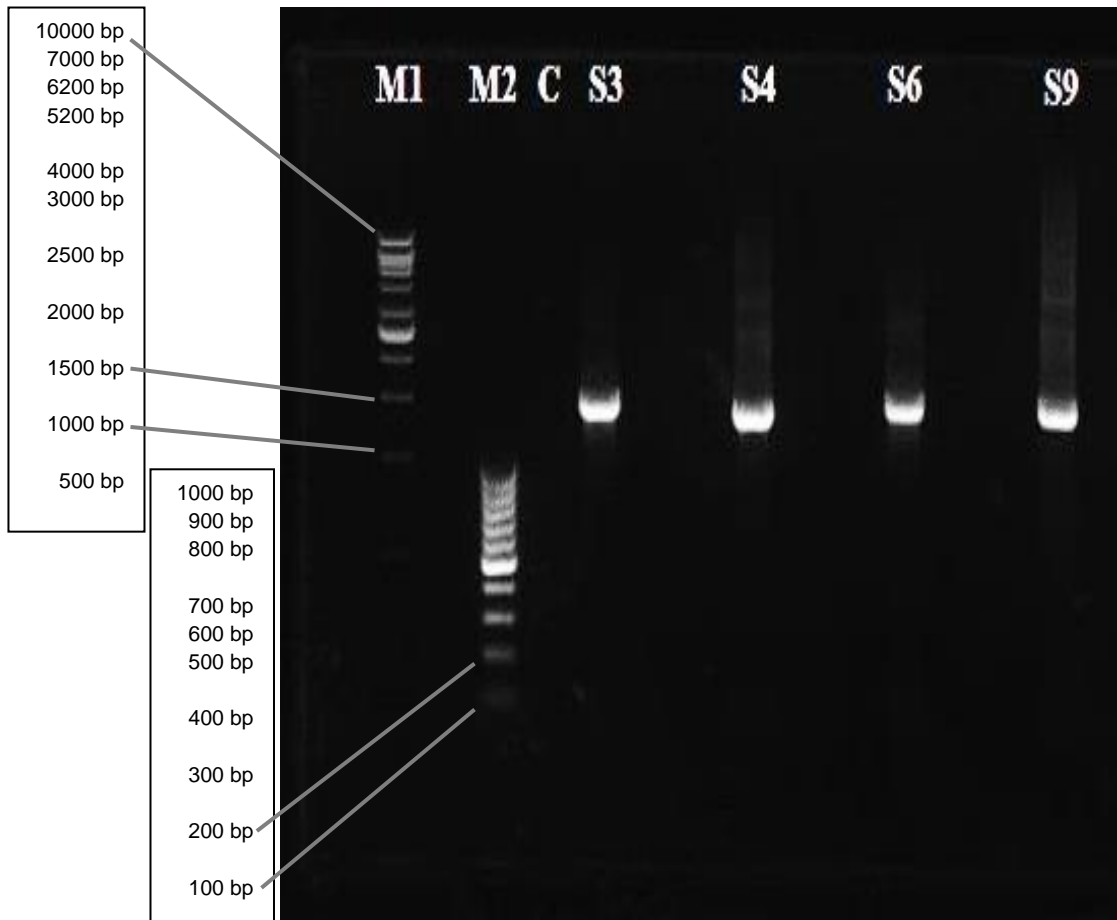
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579 M1: 1 kb ladder; M2: 100 bp ladder; C: Negative control; S3: PCR product  
 580 from stall A sugarcane juice; S4: PCR product from stall B sugarcane juice;  
 581 S6: PCR product from stall C sugarcane juice extractor; S9: Stall C  
 582 sugarcane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

583

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

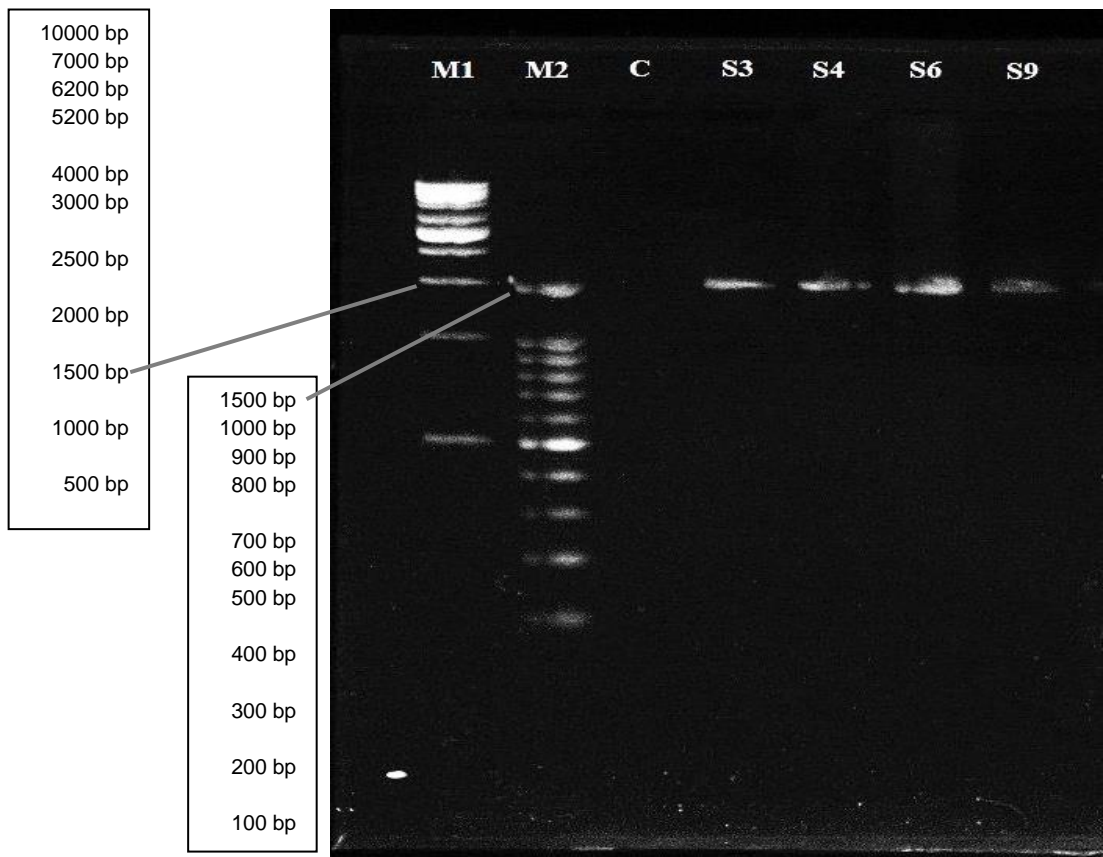
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592 M1: 1 kb ladder; M2: 100 bp ladder; C: Negative control; S3: PCR product  
 593 from stall A sugarcane juice; S4: PCR product from stall B sugarcane juice;  
 594 S6: PCR product from stall C sugarcane juice extractor; S9: Stall C  
 595 sugarcane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

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

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