Ready-to-eat foods: A potential vehicle for spread of coagulase-positive staphylococci and antibiotic-resistant *Staphylococcus aureus* in Buea municipality, South West Cameroon

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24 **Abstract**

25 **Background**

26 The consumption of ready-to-eat (RTE) foods contaminated with coagulase-positive staphylococci
27 (CoPS) and especially *Staphylococcus aureus* puts consumers at potential risk of foodborne disease or
28 colonization and subsequent infection. This cross-sectional study determined the levels of CoPS and the
29 presence of *S. aureus* in RTE foods sold in Buea municipality.

30 **Method**

31 A total of 420 RTE food samples comprising 70 each of cake, bread, fruit salad, meat-hot-pot, suya and
32 boiled rice were randomly purchased from February to August 2020. The CoPS counts were determined
33 by culturing on Baird-Parker agar and *S. aureus* identified by amplification of the *nuc* gene using
34 polymerase chain reaction. All *S. aureus* isolates were screened for the presence of classical
35 staphylococcal enterotoxin genes and each isolate challenged with 11 antibiotics to determine their
36 antibiotic susceptibility profiles. Oxacillin-resistant *S. aureus* were analyzed for the presence of *mecA*
37 gene.

38 **Result**

39 Overall, 161 (38.3%) samples had detectable levels of coagulase-positive staphylococci ranging from
40 2.0-5.81 log₁₀CFU/g. Based on CoPS levels, 37 (8.81%) of the 420 RTE food samples, only fruit salad
41 and meat-hot-pot, had unsatisfactory microbiological quality. A total of 72 *S. aureus* isolates,
42 comprising 52.78% from fruit salad, 16.67% from meat-hot-pot, 12.5% from boiled rice, 9.72% from
43 suya, 5.56% from bread and 4.17% from cake were recovered. None of the *S. aureus* isolates possessed
44 any of the classical enterotoxin genes. All the isolates were sensitive to vancomycin and ofloxacin while
45 68 (94.44%) and 66 (91.67%) were sensitive to oxacillin and ciprofloxacin, respectively. Resistance to
46 penicillin (93.06%) was highest followed by amoxicillin (91.67%) and erythromycin (79.17%). Four

47 isolates were identified as methicillin-resistant *S. aureus* all of which carried the *mecA* gene. A total of
48 24 antibiotypes were identified.

49 **Conclusion**

50 Our findings showed that RTE foods sold in the Buea municipality are likely vehicles for transmission
51 of CoPS and antibiotic-resistant *S. aureus*.

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53 **Key words:** Ready-to-eat foods, coagulase-positive staphylococci, *Staphylococcus aureus*, Cameroon

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1. Introduction

Ready-to-eat (RTE) foods are perishable food types that are ready for consumption, without any further heat treatment or washing, most often at the point of sale [1]. Convenience, ease of production, affordability, palatability, availability and unique flavours are some of the appealing factors that make RTE foods very popular and cherished by people of all age groups and particularly workers in urban areas, students and road side dwellers [2-4]. The popularity of RTE foods varies from country to country and depends on the food type and the staple diets of the population [5]. In low- and middle-income countries like Cameroon, RTE vendors have been associated with low literacy level leading to lack of knowledge about good hygiene and food handling practices [3, 6]. Furthermore, some RTE foods are prepared, stored and served under unhygienic conditions and vending is often done in outdoor environments, thereby exposing the foods to aerosols, insects, and rodents, which may serve as sources of food contaminants [7, 8].

Food safety in the community is a major focus area in public health (Gizaw, 2019). Three methods, considered gold standards, have been recommended for the determination of the microbiological quality and safety of RTE foods. These methods are aerobic colony count, identification of hygiene indicator organisms and identification of specific food borne pathogens (Table 1).

Although these methods can be used in combination, each of them is sufficient to provide evidence for the microbiological quality and safety of RTE foods [10]. Despite having many advantages, RTE foods continue to be associated with a growing number of foodborne illnesses and outbreaks mainly due to bacterial contamination [11, 12]. So far, coagulase-positive staphylococci are considered the main bacterial etiology of food poisoning [13].

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94 Table 1: Microbiological guidelines on the interpretation of results for ready-to-eat foods

Test	Result (colony-forming unit (CFU)/gram unless otherwise stated)		
	Satisfactory	Borderline	Unsatisfactory (potentially injurious to health and/or unfit for human consumption)
Aerobic Colony Count*			
Category A	$< 10^4$	$< 10^5$	$\geq 10^5$
Category B	$< 10^5$	$< 10^7$	≥ 10
Category C	N/A	N/A	N/A
Hygiene indicator organisms			
<i>Enterobacteriaceae</i>	$< 10^2$	$10^2 - \leq 10^4$	$> 10^4$
<i>Escherichia coli</i>	< 20	$20 - \leq 10^2$	$> 10^2$
Specific bacterial pathogens			
<i>Salmonella</i> species	n.d. in 25 g	N/A	Detected in 25 g
<i>Campylobacter</i> species (thermotolerant)	n.d. in 25 g	N/A	Detected in 25 g
<i>E. coli</i> 0157: H7 and VTEC	n.d. in 25 g	N/A	Detected in 25 g
<i>Vibrio cholerae</i> (O1 and O139)	n.d. in 25 g	N/A	Detected in 25 g
<i>Shigella</i> species	n.d. in 25 g	N/A	Detected in 25 g
<i>Listeria monocytogenes</i>			
*Food intended for infants	n.d. in 25 g	N/A	Detected in 25 g
*For other RTE foods	< 10	$10 - \leq 100$	> 100
<i>Vibrio parahaemolyticus</i>	< 20	$20 - \leq 10^3$	$> 10^3$
<i>Clostridium perfringens</i>	< 10	$10 - \leq 10^4$	$> 10^4$
Coagulase-positive staphylococci	< 20	$20 - \leq 10^4$	$> 10^4$
<i>Bacillus cereus</i>	$< 10^3$	$10^3 - \leq 10^5$	$> 10^5$

95 Source: Centre for Food Safety [14], Health Canada [15]

96 n.d. = not detected; VTEC = Verotoxigenic *Escherichia coli*; N/A, not applicable because the food, or a
97 component of it, naturally contains high numbers of bacteria (e.g raw fruits or vegetables, fermented or
98 cultured foods).

99 *Category A: applies to ready-to-eat foods in which all components are fully cooked for immediate sale
100 or consumption (eg meat pie)

101 Category B: applies to ready-to-eat foods that are fully cooked with further handling or processing
102 before consumption (eg custard filled pastry)

103 Category C: food that contain uncooked fermented ingredients or fresh fruit and vegetables (eg
104 takeaway fruit and yoghurt mix)

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106 Coagulase-positive staphylococci (CoPS) are opportunistic pathogens that can exist as commensals in
 107 humans, companion and food-producing animals but can cause severe or even lethal diseases [16]. They
 108 are facultative anaerobic Gram-positive, non-spore forming spherical-shaped bacteria. At least nine
 109 species of CoPS have been identified and they include *Staphylococcus aureus*, *S. hyicus*, *S. intermedius*,
 110 *S. pseudintermedius*, *S. lutrae*, *S. schleiferi* subsp. *coagulans*, *S. delphini*, *S. argenteus* and *S.*
 111 *schweitzeri* [16, 17]. Although several of these *Staphylococcus* species can produce staphylococcal
 112 enterotoxins (SEs), the majority of staphylococcal food poisoning is attributed to SE produced by *S.*
 113 *aureus* [18, 19]. The number of coagulase-positive staphylococci per gram or per milliliter of RTE food
 114 sample can be used to determine the microbiological quality and safety of RTE foods [20].

115 *Staphylococcus aureus* is one of the major bacterial agents causing foodborne disease in humans
 116 worldwide [21]. Some pathogenic strains of this bacterium elaborate heat-stable SEs and so far, 23 SEs
 117 have been identified comprising five major classical types (SEs: SEA to SEE) and the non-classical SE-
 118 like toxins (SEI: SEG to SEU) [13, 22]. Approximately 95% of food poisoning outbreaks are caused by
 119 the classical enterotoxins [23]. Ingestion of SEs containing food can induce severe symptoms, including
 120 vomiting and high fever with or without nausea and diarrhea, with rapid onset in typically less than 8 h
 121 (usually between 3 and 4 h) [24]. Consumption of RTE food contaminated with *S. aureus* has been
 122 reported in several developed countries including U.S.A., Hong Kong, Germany, Japan and Italy [1]. In
 123 China, several RTE food products have been associated with the introduction of microbiological
 124 hazards, including *Listeria monocytogenes*, *Cronobacter*, *Salmonella* and *S. aureus* [25]. Contamination
 125 by toxigenic *S. aureus* in RTE food is a major public health challenge in both developing and developed
 126 countries and the prevalence of this bacterium in RTE foods needs to be monitored and controlled [12,
 127 26].

Over the years, several pathogenic strains of *S. aureus* isolated from RTE food have developed resistance to one or more commonly used antibiotics used in treatment of infection. Antibiotic-resistant bacteria (ARB) are a major threat to global public health. In order to effectively counter the threat of infections with antibiotic-resistant bacteria, it is critical to identify potential ways that humans can be exposed to these bacteria [27]. Although foods have been reported as vehicles for the transmission of antibiotic-resistant bacteria, the role of RTE foods in the spread of these pathogens is scantily documented [25]. Some RTE foods have been reported to carry antibiotic-resistant enterococci [5], antibiotic-resistant *Staphylococcus* species [28] and other bacterial species [29, 30]. Unfortunately, the presence of antibiotic-resistant bacteria in RTE food is not routinely investigated in low- and middle-income countries and data are only available from a small number of studies [23]. Hence, this study was carried out to investigate the levels of CoPS, determine the prevalence and characteristics of *S. aureus* isolated from commonly consumed RTE food samples collected in Buea, South West Cameroon.

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141 **2. Materials and methods**

142 **2.1 Study area**

This study was carried out on RTE foods from vendors in Buea municipality; the administrative headquarter of the south west region of Cameroon. Buea is located on the slope of mount Cameroon between latitude 4°14" north of the equator and longitude 9°20" east of the Greenwich Meridian with an altitude of about 1000m above sea level [31]. Buea is a cosmopolitan and multicultural town and had an estimated population of 300,000 inhabitants in 2013 [32]. This town is the seat of many educational institutions at the primary, secondary and tertiary levels and is experiencing a very high rate of population growth and urbanization. At least 7000 people relocate to Buea each year according to the municipal council statistics [33].

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151 **2.2 Study design and RTE food types**

152 This was a cross-sectional study carried out from February to August 2020. Buea was purposively
153 selected because of its large concentration of vendors with intensive food vending activities throughout
154 the week, from Monday through Sunday and from early hours of the day to late evening. These vendors
155 are patronized by people-from-all-walks-of-life particularly shoppers, workers, passers-by and students.

156 Six types of RTE foods (fruit salad, suya (roasted beef), cake, boiled rice, bread and meat-hot-pot
157 (cooked meat in tomato sauce) were included in this study. These RTE food types were chosen based on
158 their popularity and round-the-clock availability. They were purchased randomly from street food
159 vendors and only one sample was collected per vendor.

160 **2.3 Collection of food samples**

161 A total of 420 RTE food samples, comprising 70 of each food type, were purchased randomly and
162 periodically from street vendors. Upon purchase, each sample was aseptically put in a sterile zip lock
163 bag, assigned a unique code, and placed in a cool box containing ice packs with temperature maintained
164 at +2 to +6°C. Samples were transported to the Laboratory for Emerging Infectious Diseases, University
165 of Buea within an hour of collection for processing.

166 **2.4 Enumeration of coagulase-positive staphylococci**

167 The surface colony count technique was used for the enumeration of coagulase-positive staphylococci in
168 each sample as described previously [20]. Briefly, a 500 µL inoculum from a 10⁻¹ dilution (in Peptone
169 saline diluent) of each RTE food sample homogenate was spread on the surface of Baird-Parker agar
170 medium and plate allowed to stand for 15 min to allow the inoculum to be absorbed into the surface of
171 the agar. Each sample was inoculated in duplicate and the inoculation was done within 45 min of
172 preparation of the sample homogenate. The culture plate was incubated at 37 °C for 24-48 h. At the end

of incubation, plates were examined for typical colonies of coagulase-positive staphylococci (grey-black, shiny and convex with a diameter of 1-2.5 mm surrounded by a dull halo). Only plates with colony counts of between 10 and 300 colonies per plate were considered for enumeration.

2.4.1 Confirmation of coagulase-positive staphylococci

Five colonies of each type (or all colonies if less than five) were subcultured for confirmatory testing for CoPS based on DNase production and tube coagulase positivity. Briefly, a DNase agar plate was divided into five segments and each segment was spot inoculated with a different colony. Plates were incubated at 37 °C for 24 h. DNase production was evident by a defined zone of clearing surrounding the inoculated spot after flooding the DNase plate with 1N HCl and decanting excess HCl after about 30 sec. Results were read within 5 min following the application of HCl. Tube coagulase test was performed by aseptically transferring 500 µL of human plasma to a sterile round bottom tube and inoculating the plasma with an equal volume of the suspension of the colony to be tested. Incubation was done at 37 °C and the tubes examined for clotting every 30 min for 4 h by carefully tilting the tubes. If negative after 4 h, the tube was re-examined at 24 h.

The number of coagulase positive staphylococci per g of sample was calculated as follows:

$$\text{Count per g} = \frac{\text{No. of colonies confirmed}}{\text{No. of colonies tested}} \times \frac{\text{Presumptive count}}{\text{Volume tested}} \times \text{Dilution factor}$$

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2.5 Molecular identification of *Staphylococcus aureus*

All colonies that were used for the confirmation of CoPS were further investigated for the identification of *S. aureus* by PCR targeting the species-specific thermonuclease (*nuc*) gene of *S. aureus*. DNA was extracted from each colony using the simple boiling method. Briefly, 150µL of phosphate buffered saline (PBS) were pipetted into a sterile 1.5 mL Eppendorf tube and a loop full of bacterial colony

197 suspended in the PBS. The tubes were vortexed at 14000 rpm for 10 sec. The bacterial solution was
198 heated in a water bath at 100°C for 15min after which it was removed and immediately chilled in ice for
199 another 15min. The bacterial solution was allowed to thaw at 37 °C in the water bath and again chilled in
200 ice for another 15min. The tubes were centrifuged at full speed (14000rpm) for 5 min and the
201 supernatant was pipetted into another labeled tube and used as template DNA in PCR assay.

202 Presumptive *S. aureus* isolates were confirmed by amplification of a 280 bp fragment of the *nuc* gene of
203 *S. aureus* using primer sequences previously described (Table 2).

204 Table 2: Primer sequences of *S. aureus* used for PCR assay in this study

Target gene	Oligonucleotide sequence (5' → 3')	Product size (bp)	Reference
<i>nuc</i>	F: GCGATTGATGGTGATACGGTT R: AGCCAAGCCTTGACGAACATAAGC	280	[34]
<i>sea</i>	F: TTGGAAACGGTTAAAACGAA R: GAACCTTCCCATCAAAAACA	120	
<i>seb</i>	F: TCGCATCAAACCTGACAAACG R: GCAGGTACTCTATAAGTGCC	478	
<i>sec</i>	F: GACATAAAAGCTAGGAATTT R: AAATCGGATTAACATTATCC	257	
<i>sed</i>	F: CTAGTTTGGTAATATCTCCT R: TAATGCTATATCTTATAGGG	317	
<i>see</i>	F: AGGTTTTTTTCACAGGTCATCC R: CTTTTTTTTCTTCGGTCAATC	209	[35]
<i>MecA</i>	F: AAAATCGATGGTAAAGGTTGGC R: AGTTCTGCAGTACCGGATTTGC	533	[36]

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206 Each PCR reaction in this study comprised 2X BioMix Red master-mix (10μL), 1μL of each primer
207 from a 10μM working stock (final concentration, 0.5μM), 1μL DNA template and nuclease free water to
208 make a final volume of 20μL. Each PCR run had a negative control which was nuclease-free water and a
209 positive control which was a previously characterized *S. aureus* isolate stored in the laboratory.

210 The PCR cycling conditions were optimized with initial denaturation at 95°C for 5min followed by 35
211 cycles of denaturation at 94°C / 60sec, annealing at 54°C / 40sec and extension at 72°C / 60sec. Final

extension was at 72°C for 7min and the reaction was stopped by holding tubes at 4°C until removed from the thermal cycler (MyCycler™ Thermal Cycler BIORAD, USA). The PCR products were electrophoresed in a 1.5% agarose gel stained with SYBR Safe DNA Gel stain (Invitrogen) for 1 hour at 90V in 1X Tris borate EDTA, visualized and photographed with a Molecular Imager Gel Doc XR system (BIO-RAD, Hercules, CA, USA).

2.6 Detection of classical enterotoxin genes in *S. aureus*

All confirmed coagulase-positive *S. aureus* isolates were screened for the presence of *S. aureus* enterotoxins types A, B, C and D using multiplex PCR [35] with primers previously described (Table 1). The multiplex PCR run was optimized at initial denaturation for 5 min at 94 °C followed by 30 cycles of denaturation at 94°C for 60sec, annealing at 50°C for 60sec and extension at 72°C for 60sec. A final extension at 72°C for 5min was done at the end of the cycles. A singleplex PCR run was performed for detection of enterotoxin E using the same cycling conditions except for the annealing temperature that was set at 48°C. All PCR products were electrophoretically separated in 1.5% agarose gel as described above.

2.7 Detection of *mecA* gene in methicillin-resistant *S. aureus* isolates

Staphylococcus aureus isolates that were phenotypically confirmed as methicillin-resistant were further screened by PCR for the presence of the *mecA* gene. This gene encodes a modified penicillin-binding protein designated as PBP2a with reduced affinity for β -lactams. The amplification of the 533bp fragment of the *mecA* gene was optimized under the following conditions: initial denaturation at 95°C for 5min, 35 cycles of denaturation at 94°C for 1min, annealing at 50°C for 1min, extension at 72°C for 1min with a final extension at 72°C for 5min, and cooling to 4°C. Similarly, PCR products were separated electrophoretically as described above.

235 **2.8 Antibiotic susceptibility testing of *S. aureus* isolates**

236 Antibiotic susceptibility testing (AST) was performed using the Kirby-Bauer disk diffusion method on
 237 Mueller–Hinton agar (Oxoid, UK) according to the guidelines of the Clinical Laboratory Standards
 238 Institute [37]. Each *S. aureus* isolate was challenged with a panel of 11 antibiotics viz: vancomycin
 239 (VA-30µg), chloramphenicol (C-30µg), gentamicin (CN-30µg), erythromycin (E-15µg), clindamycin
 240 (DA-2µg), ciprofloxacin (CIP-5µg), penicillin (P-10 I.U), ofloxacin (OFX-5µg), amoxicillin (AML-
 241 30µg), azithromycin (AZM-30µg) and oxacillin (OX-1µg). Antibiotics were selected to represent
 242 different antibiotic classes and also comprised commonly used and most available antibiotics for the
 243 treatment of staphylococcal-related infections in human and veterinary medicine.

244 Pure colonies (two to five) of each *S. aureus* isolate from nutrient agar plate were used to prepare the
 245 inoculum for AST. The turbidity of the inoculum was adjusted to that of 0.5 MacFarland standard and
 246 used to inoculate the Mueller-Hinton agar using the spreading method. Antibiotic disks were applied
 247 firmly on the agar surface and plates incubated at 35°C for 24h. The diameter of the zone of inhibition
 248 was measured and interpreted as resistant, susceptible and intermediate according to CLSI [37]
 249 guidelines. *Staphylococcus aureus* isolates that showed resistance to antibiotics in three or more
 250 antibiotic classes were considered multidrug-resistant.

251 **2.9 Data quality assurance, management and statistical analysis**

252 The handling and analysis of samples and bacterial isolates were done following standard operating
 253 procedures to ensure the quality and reliability of study findings. The sterility of culture media was
 254 maintained and confirmed by the incubation of uninoculated culture media as negative control. Data
 255 were entered into Microsoft Excel 2010, exported into SPSS V27.0.1.0 and analyzed using descriptive
 256 statistics such as prevalence, percentages, mean and standard error. Coagulase-positive staphylococci
 257 counts were transformed to log₁₀CFU/mL prior to statistical analysis. Data on the prevalence of *S.*

258 *aureus* and multidrug resistant isolates were analysed using a chi-squared test to determine whether
259 there were significant differences in their prevalence between sample types. The confidence level was
260 held at 95% and *p*-value at less than 5% for all analysis.

261 3. Results

262 3.1 Coagulase-positive staphylococci counts

263 The CoPS counts in the RTE foods ranged from 2.0-5.81 log₁₀CFU/g. Overall, 161 (38.3%) of the RTE
264 food samples had detectable levels of CoPS contamination. The fruit salad samples were the most
265 contaminated (51, 72.9%) while suya samples were the least contaminated (12, 17.1%) (Table 3). A total
266 of 652 bacterial isolates were screened in the confirmatory testing for CoPS.

267 Based on CoPS counts, only 37 (8.81 %) of the 420 RTE food samples investigated and comprising only
268 of fruit salad and meat-hot-pot, had unsatisfactory or unacceptable quality. More than half (259,
269 61.67%) of the RTE food samples had satisfactory quality (Table 4).

270 Table 3: Coagulase-positive staphylococci counts obtained from the ready-to-eat foods analyzed

RTE food type	Number with CoPS (%)	CoPS counts (log ₁₀ CFU/g)		Mean±SE
		Minimum	Maximum	
Suya (n=70)	12 (17.1)	2.0	2.91	2.60±0.07
Fruit salad (n=70)	51 (72.9)	2.38	5.81	4.35±0.10
Boiled rice (n=70)	17 (24.3)	2.3	3.91	3.40±0.13
Bread (n=70)	27 (38.6)	2.0	3.95	2.88±0.11
Meat-hot-pot (n=70)	33 (47.1)	2.48	4.94	3.77±0.11
Cake (n=70)	21 (30.0)	2.23	3.92	2.83±0.09
Total (n=420)	161 (38.3)			

271 RTE, ready-to-eat; CoPS, coagulase-positive staphylococci; CFU, colony forming unit; SE, standard
272 error.

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Table 4: Microbiological quality of the RTE food types based on coagulase-positive staphylococci counts

RTE food type	Microbiological quality based on CoPS count		
	Satisfactory [<1.3 $\log_{10}\text{CFU/g}$] (%)	Borderline [$1.3 \leq 4$ $\log_{10}\text{CFU/g}$] (%)	Unsatisfactory [>4 $\log_{10}\text{CFU/g}$] (%)
Suya (n=70)	58 (82.86)	12 (17.14)	0 (0.0)
Fruit salad (n=70)	19 (27.14)	23 (32.86)	28 (40.0)
Boiled rice (n=70)	53 (75.71)	17 (24.29)	0 (0.0)
Bread (n=70)	43 (61.43)	27 (38.57)	0 (0.0)
Meat-hot-pot (n=70)	37 (52.86)	24 (34.29)	9 (12.86)
Cake (n=70)	49 (70.0)	21 (30.0)	0 (0.0)
Total (n=420)	259 (61.67)	124 (29.52)	37 (8.81)

RTE, ready-to-eat; CoPS, coagulase-positive staphylococci; CFU, colony forming unit

3.2 Prevalence of *Staphylococcus aureus* in food samples

Molecular confirmation of *S. aureus* relied on the amplification of the *nuc* gene by singleplex PCR. The amplified PCR products showed the desired band of required size (280 bp) when separated on a 1.5 % (W/V) agarose gel (Fig1).

Fig 1: Electrophoretic separation of PCR products on 1.5% agarose gel for detection of *S. aureus* genes: (A) Amplification of *nuc* gene. Lane 1, 100 bp molecular weight marker. Lane 2, positive control. Lanes 3, 5, 6 and 7 are negative samples. Lanes 4 and 8 are positive samples. (B) Amplification of *mecA* gene. Lane 1, 100bp molecular weight marker. Lanes 2, 3 and 5 are negative samples. Lane 4 shows positive sample.

Out of the 652 CoPS isolates screened, 72 (17.14 %) were confirmed to possess the *nuc* gene and confirmed to be *S. aureus*. The 72 *S. aureus* isolates comprised 52.78% from fruit salad, 16.67% from meat-hot-pot, 12.5% from boiled rice, 9.72% from suya, 5.56% from bread and 4.17% from cake were recovered from 46 RTE food samples. Overall, *S. aureus* was detected in 10.95 % of all RTE food

294 samples examined with some samples having more than one *S. aureus* isolates. Similarly, fruit salad was
295 the most contaminated sample with *S. aureus* followed by boiled rice and meat-hot-pot (Table 5).

296 Table 5: Prevalence of *Staphylococcus aureus* among the ready-to-eat food samples

RTE food type	Number of RTE food with		
	<i>S. aureus</i> (%)	Methicillin-resistant <i>S. aureus</i> (%)*	Enterotoxigenic <i>S. aureus</i> (%)
Suya (n=70)	5 (7.14)	0 (0.0)	0 (0.0)
Fruit salad (n=70)	21 (30.0)	1 (3.33)	0 (0.0)
Boiled rice (n=70)	8 (11.43)	0 (0.0)	0 (0.0)
Bread (n=70)	3 (4.29)	1 (33.33)	0 (0.0)
Meat-hot-pot (n=70)	7 (10.0)	2 (28.57)	0 (0.0)
Cake (n=70)	2 (2.86)	0 (0.0)	0 (0.0)
Total (n=420)	46 (10.95)	4 (8.69)	0 (0.0)

297 *Percentage calculated on the number of RTE food with *S. aureus*

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299 The *mecA* gene (Fig 1) was detected in 5.56 % (4/72) of the *S. aureus* isolates from three RTE food
300 types while none of the *S. aureus* isolates possessed any of the classical enterotoxin genes (Table 5).

301 3.3 Antibiotic susceptibility of *Staphylococcus aureus* isolates

302 All the *S. aureus* isolates were sensitive to vancomycin (100 %) and ofloxacin (100 %) while 68 (94.44
303 %) and 66 (91.67 %) of the bacterial isolates were sensitive to oxacillin and ciprofloxacin, respectively.
304 On the other hand, the isolates showed high resistance to penicillin (93.06 %) followed by amoxicillin
305 (91.67 %) and erythromycin (79.17 %) (Table 6). Four isolates were identified as methicillin-resistant *S.*
306 *aureus* (MRSA) based on their resistance to oxacillin. Each of the MRSA isolates was resistant to at
307 least five antibiotics. Of the 72 *S. aureus* isolates, 45 (62.5 %) were multidrug resistant. In this study,
308 multidrug resistance was defined as resistance to one antibiotic in three or more classes of antibiotics.

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Table 6: Susceptibility of *S. aureus* isolates to the different antibiotics tested (n=72).

Class of antibiotic	Antibiotic	Resistant (%)	Intermediate (%)	Sensitive (%)
Glycopeptide β-lactams	Vancomycin (VA)	0 (0)	0 (0)	72 (100)
	Penicillin (P)	67 (93.06)	0 (0)	5 (6.94)
	Amoxicillin (AML)	66 (91.67)	1 (1.39)	5 (6.94)
	Oxacillin (OX)	4 (5.56)*	0 (0)	68 (94.44)
Quinolone	Ciprofloxacin (CIP)	6 (8.33)	0 (0)	66 (91.67)
Macrolides	Erythromycin (E)	57 (79.17)	7 (9.72)	8 (11.11)
	Azithromycin (AZM)	43 (59.72)	3 (4.17)	26 (36.11)
Lincosamide	Clindamycin (DA)	31 (43.06)	10 (13.89)	31 (43.06)
Aminoglycoside	Gentamicin (CN)	22 (30.56)	6 (8.33)	44 (61.11)
Fluoroquinolone	Ofloxacin (OFX)	0 (0)	0 (0)	72 (100)
Chloramphenicol	Chloramphenicol (C)	15 (20.83)	0 (0)	57 (79.17)

*Methicillin-resistant *S. aureus*

3.4 Antibiotypes of *S. aureus* from the food samples

A total of 24 antibiotypes were identified in this study (Table 7). The most prevalent antibiotype was P-AML-E-AZM-C that was detected in 9 (12.5%) of the 72 *S. aureus* isolates. More antibiotypes were identified in fruit salad followed by meat-hot-pot.

4. Discussion

The high rate of consumption of RTE foods in Cameroon and especially in the Buea municipality coupled with the scarcity of data on the microbiological quality of these foods is a call for concern. Elsewhere, there are several reports that highlight the role of RTE foods in the emergence or reemergence of microbial threats to health, by providing information on the contamination of RTE foods by bacteria or resistant bacterial species to guide public health and food safety interventions [38]. Staphylococcal food poisoning, caused by the consumption of one or more of the 23 heat-stable enterotoxins and many variants elaborated by coagulase-positive staphylococci and particularly *Staphylococcus aureus*, is a global public health concern [13, 39]. Most food types, including RTE foods, support the growth of staphylococci and are ideal for enterotoxin production [23]. Although staphylococcal food poisoning is usually self-limiting and resolves within 24 to 48 h after onset,

Table 7: Antibiotic-resistant patterns of *S. aureus* isolates from RTE food samples (n=72)

Number of antibiotics showing resistance	Antibiotype	Number of isolates (%)	Sample origin
2	P-DA	2 (2.78)	Fruit salad, suya
	E-CN	1 (1.39)	Meat-hot-pot
	P-AML	1 (1.39)	Cake
	AML-E	2 (2.78)	Fruit salad
3	E-AZM-C	1 (1.39)	Meat-hot-pot
	P-AML-E	1 (1.39)	Suya
	P-AML-DA	3 (4.17)	Rice, Bread
	AML-E-CN	2 (2.78)	Fruit salad
	P-AML-AZM	6 (8.33)	Cake, rice, bread
4	P-E-AZM-C	1 (1.39)	Meat-hot-pot
	P-AML-E-DA	6 (8.33)	Meat-hot-pot, rice
	P-E-AZM-CN	1 (1.39)	Fruit salad
	P-AML-E-CN	2 (2.78)	Fruit salad
	P-AML-E-AZM	8 (11.11)	Suya, bread, cake
	P-AML-DA-CN	1 (1.39)	Meat-hot-pot
5	P-AML-E-AZM-C	9 (12.5)	All the RTE foods
	P-AML-E-DA-CN	5 (6.94)	Fruit salad, rice
	P-AML-CIP-E-CN	2 (2.78)	Meat-hot-pot
	P-AML-E-AZM-DA	7 (9.72)	Rice, bread, cake
	P-AML-E-AZM-CN	1 (1.39)	Fruit salad
6	P-AML-OX-CIP-E-C	1 (1.39)	Fruit salad
	P-AML-E-AZM-DA-CN	6 (8.33)	Rice, suya
7	P-AML-OX-CIP-E-AZM-C	2 (2.78)	Meat-hot-pot
9	P-AML-OX-CIP-E-AZM-DA-CN-C	1 (1.39)	Bread
Total		72 (100)	

P, penicillin; AML, amoxicillin; OX, oxacillin; CIP, ciprofloxacin; E, erythromycin; AZM, azithromycin; DA, clindamycin; CN, gentamicin; C, chloramphenicol; RTE, ready-to-eat

331

occasionally it can be severe and require hospitalization [1]. *Staphylococcus aureus* is ubiquitous in the environment, a commensal in the human body and a notorious zoonotic and major foodborne pathogen that quickly acquires resistance to antibiotics (Kakoullis et al., 2021; Guo et al., 2020). Therefore this study that investigated RTE foods for contamination with coagulase-positive staphylococci with particular focus on *S. aureus* and its antibiotic-resistant profiles, has both public health and food safety relevance.

338 Of the 420 RTE food samples analyzed in this study, 161 (38.3 %) had detectable levels of CoPS
 339 contamination with counts ranging from 2.0-5.81 log₁₀CFU/g. Based on the CoPS counts, 37 (8.81%) of
 340 the 420 RTE food samples investigated and comprising only of fruit salad and meat-hot-pot, had
 341 unsatisfactory or unacceptable quality (Table 4). Our findings corroborate a previous study that analyzed
 342 275 RTE food samples and reported that 25 (9.1%) were inappropriate for consumption according to
 343 Turkish microbiological guidelines [40]. Monitoring CoPS count in RTE foods is very helpful for a risk
 344 assessment. The number of CoPS per gram or milliliter of RTE food sample is an indication of the
 345 microbiological quality of the food sample [20]. In an earlier study that analyzed 60 RTE foods served
 346 in long-term care facilities in Spain, the highest CoPS count in the samples was $1.9 \pm 0.3 \log_{10}\text{CFU/g}$,
 347 hence all samples had acceptable quality based on United Kingdom guidelines [41]. In a very recent
 348 study that investigated 83 RTE food samples, 25% (23/83) of those analyzed were classified as
 349 unsatisfactory or potentially hazardous [42].

350 In this study, fruit salad samples showed high levels (51/70, 72.9 %) of contamination with CoPS (Table
 351 3). High contamination of RTE fruit salads has been reported in previous studies. Brooks [43] reported a
 352 higher rate (90 %) of staphylococcal contamination of 20 RTE salad samples analyzed in his study
 353 carried out in Calabar, Nigeria. In another recent study that analyzed 36 RTE pineapple slices in Port
 354 Harcourt, Nigeria, high levels of bacterial contamination were detected in all the samples and the
 355 contamination levels were even higher for samples collected in the evening than for those collected in
 356 the morning from the same vendors [44]. The high levels of bacterial contamination of RTE fruits could
 357 be multifactorial and include: washing the fruits with poor quality source of water, cross contamination
 358 from other fruits washed in the same water many times [45, 46], use of dirty trays or dirty processing
 359 utensils like knives and slicing tables, not washing the hands thoroughly and contamination from the air
 360 [44].

361

362 A total of 12 (17.14%) of the 70 suya samples analyzed in this study had the lowest detectable levels of

363 CoPS and it ranged from 2 - 2.91 log₁₀CFU/g (Table 3). While these 12 samples had borderline quality,

364 the remaining 58 (82.86%) samples had satisfactory quality (Table 4). Hence, all the 70 suya samples

365 analyzed in this study were suitable for consumption at the time of sample collection. Falegan et al. [47]

366 in a recent study carried out in Ado-Ekiti Metropolis, Ekiti State, Nigeria, reported comparably higher

367 values of 4.99 - 5.45 log₁₀CFU/g for total bacterial counts in the 20 suya samples analyzed and stated

368 that all the samples had unsatisfactory quality. In another earlier study by Egbebi and Seidu [48] carried

369 out in Ado and Akure, South West Nigeria, bacterial counts ranged from 4.48 - 4.60 (in Ado) and 4.48 –

370 4.93 (in Akure), and these values placed the suya samples in acceptable but not satisfactory range. The

371 low values of CoPS in the suya samples reported in our study could be linked to the fact that these

372 samples were purchased shortly after preparation (i.e freshly prepared) and not much post-processing

373 contamination had occurred.

374

375 In this study, majority of the samples of boiled rice (75.71%), bread (61.43%) and cake (70.0%) (Table

376 4) had satisfactory quality while 24.29% (boiled rice), 38.57% (bread) and 30% (cake) samples had

377 CoPS counts on the high threshold but within microbiologically acceptable limits recommended by

378 regulatory bodies for vended meat and meat products [14, 15]. Our results did not agree with that of

379 Wogu et al. [49] who reported that most of the 48 rice samples from hotels in Benin City, Nigeria

380 examined in their study had unsatisfactory quality. A very recent study that examined 50 rice samples

381 obtained from cafeterias in Akwa Ibom State University in Nigeria reported that most of the rice

382 samples had unsatisfactory quality [50]. The reasons for the differences in the results reported in these

383 studies could be multifactorial ranging from rice species differences, methods of preparation, holding

384 temperature and time and methods of sample analysis.

385 *Staphylococcus aureus* was identified in 46 (10.95 %) RTE food samples in this study. This overall
386 contamination rate by *S. aureus* reported in our study was higher than the 6.6% (8/120) reported for
387 RTE foods in Egypt [51], 4.31% (6/139) in Libya [52] and 6.4% (48/750) in Istanbul in Turkey [53] but
388 lower than the 29.1% (155/532) in Cambodia [12], 34% (68/200) in Bolgatanga municipality of Ghana
389 [54], 52.8% (56/106) in Putrajaya, Malaysia and 12.55% (69/550) in China [25]. The difference in the
390 prevalence rates reported in these studies could be due to diagnostic method used; while some studies
391 identified *S. aureus* using phenotypic methods, others relied on the more sensitive molecular method.

392 A total of 72 *S. aureus* isolates comprising 52.78% from fruit salad, 16.67% from meat-hot-pot, 12.5%
393 from boiled rice, 9.72% from suya, 5.56% from bread and 4.17% from cake (Table 5) were recovered
394 from the 46 *S. aureus*-positive samples in this study. Since *S. aureus* is heat-labile and also readily
395 destroyed by most sanitizing agents, its presence in RTE foods may be an indication of poor handling
396 and sanitation [38]. Elsewhere, contamination of RTE foods has been linked to the preparation by small-
397 scale local producers without quality control, re-use of improperly washed dishes, open-air distribution
398 environment and holding temperatures during distribution [55]. We investigated the *S. aureus* isolates
399 for the presence of the classical enterotoxin genes and none was found to possess any of the genes. In a
400 similar study that investigated 84 *S. aureus* isolates for the presence of the classical enterotoxin genes,
401 only 2 (2.38%) of the isolates possessed the enterotoxin B gene while the rest of 82 isolates were
402 negative for other enterotoxins genes [23]. In another study, a high prevalence (58.1%, 18/31) of
403 classical staphylococcal enterotoxin genes was identified in meat samples in Zanjan, Iran [56]. Although
404 a positive PCR confirms the presence of enterotoxins, the negative PCR in our study does not point to
405 the absence of the corresponding operon because there is always a possibility of mutation at the level of
406 the corresponding gene. Furthermore, the differences between our results and those reported elsewhere
407 could also be due to other factors like sample source and geographical origin [23].

408 The increasing reports that resistant strains of *S. aureus* are present in various RTE foods in different
 409 countries [55] and the notorious resistance of this bacterium to several commonly used antibiotics [57]
 410 warranted antibiotic susceptibility testing for all 72 *S. aureus* isolates identified in this study. All the *S.*
 411 *aureus* isolates showed 100% sensitivity to vancomycin and ofloxacin followed by ciprofloxacin
 412 (91.67%) (Table 6). These results agree with that of Sina et al. [58] in a study carried out in Cotonou
 413 Benin and that of Wang et al. [59], who reported that all *S. aureus* isolates from food in Shaanxi
 414 Province, China were sensitive to vancomycin. This high sensitivity of the *S. aureus* isolates has great
 415 therapeutic relevance because these antibiotics can be used to treat infections caused by this bacterium
 416 in our resource poor community. High *S. aureus* antibiotic resistance was observed for penicillin
 417 (93.06%) followed by amoxicillin and erythromycin and our results are comparable to that reported by
 418 Tsehayneh et al. [60]. Forty-five (62.5%) *S. aureus* isolates in this study showed multidrug resistance
 419 and this was lower than 98% (54 out of 55 *S. aureus* isolates) recently reported by Tsehayneh et al. [60].
 420 This difference in resistance reported may be due to the extent of usage of antibiotics in a locality and
 421 possibly the type of samples analyzed [55].

422
 423 In this study, resistance to oxacillin was used to identify methicillin-resistant *S. aureus* (MRSA) strains.
 424 A total of four (5.56%) of the 72 *S. aureus* investigated were MRSA and all possessed the *mecA* gene.
 425 Saad et al. [51] reported that two (25%) of the eight *S. aureus* isolates identified in their study were
 426 MRSA, Yang et al. [25] reported 10.14% (7/69) prevalence of MRSA out of which six were *mecA*-
 427 positive, Naas et al. [52] reported only one (16.67%) of six *S. aureus* isolates as MRSA but did not
 428 screen for the presence of the *mecA* gene. All MRSA strains in this study were sensitive to vancomycin
 429 and as expected, but resistant to penicillin and other antibiotics. MRSA is a pathogen of global concern
 430 and is placed in the WHO priority pathogen list [61] because infections caused by it are extremely
 431 difficult to treat. Thus, understanding the epidemiology of this pathogen and its sources in the

environment are relevant towards effective prevention and control, especially at this time when the antibiotic pipeline is nearly empty [61]. A total of 24 antibiotypes were identified among the 72 *S. aureus* isolates in this study and they did not show any clustering with the origin of the *S. aureus* isolates.

5. Conclusion

This study presents very important findings in terms of public health and food safety because these RTE foods are consumed without further heat treatment. The potential role of these RTE foods in the spread of CoNS and *S. aureus* cannot be ignored. The contamination of these foods with CoNS, *S. aureus* and even MRSA poses a potential risk to consumers. To combat the spread of antibiotic-resistant *S. aureus* and particularly MRSA, it is necessary and urgent to identify all possible sources and potential pathways by which these pathogens spread. Moreover, our study also provides insight into the antibiotypes of *S. aureus* circulating in the study area.

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Fig 1