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Research Article

Ready-to-Eat Foods: A Potential Vehicle for the Spread of Coagulase-Positive Staphylococci and Antimicrobial-Resistant *Staphylococcus aureus* in Buea Municipality, South West Cameroon

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The consumption of ready-to-eat (RTE) foods contaminated with coagulase-positive staphylococci (CoPS) and especially *Staphylococcus aureus* puts consumers at a potential risk of food-borne disease or colonization and subsequent infection. This cross-sectional study determined the levels of CoPS and the presence of *S. aureus* in RTE foods sold in Buea municipality. A total of 420 RTE food samples, comprising 70 each of cake, bread, fruit salad, meat hot-pot, suya, and boiled rice were randomly purchased from February to August 2020. The CoPS counts were determined by culturing on Baird-Parker agar, and *S. aureus* was identified by amplification of the *nuc* gene using the polymerase chain reaction. All *S. aureus* isolates were screened for the presence of classical staphylococcal enterotoxin genes. To determine antimicrobial resistance profiles, each isolate was tested against 11 antimicrobials. Oxacillin-resistant *S. aureus* strains were analyzed for the presence of the *mec*A gene. Overall, 161 (38.3%) samples had detectable levels of CoPS ranging from 2.0 to 5.81 $\log_{10}$ CFU/g. Based on CoPS levels, 37 (8.81%) of the 420 RTE food samples–only fruit salad and meat hot-pot, had unsatisfactory microbiological quality. A total of 72 *S. aureus* isolates, comprising 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. None of the *S. aureus* isolates possessed any of the classical enterotoxin genes. All the isolates were susceptible to vancomycin and ofloxacin, while 68 (94.44%) and 66 (91.67%) were susceptible to oxacillin and ciprofloxacin, respectively. Resistance to penicillin (93.06%) was highest, followed by amoxicillin (91.67%) and erythromycin (79.17%). Four isolates were identified as methicillin-resistant *S. aureus,* all of which carried the *mec*A gene. A total of 24 antibiotypes were identified. Our findings showed that RTE foods sold in the Buea municipality are likely vehicles for the transmission of CoPS and antimicrobial-resistant *S. aureus*.

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 roadside 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 levels, leading to a 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 [9]. 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. 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 food-borne illnesses and outbreaks, mainly due to bacterial contamination [11, 12]. So far, CoPS are considered the main bacterial aetiology of food poisoning [13].

CoPS are opportunistic pathogens that can exist as commensals in humans, companions, and food-producing animals but can cause severe or even lethal diseases [14]. They are facultative anaerobic gram-positive, nonspore-forming spherical-shaped bacteria. At least nine species of CoPS have been identified, and they include *Staphylococcus aureus*, *S. hyicus*, *S. intermedius*, *S. pseudintermedius*, *S. lutrae*, *S. schleiferi* subsp. *coagulans*, *S. delphini*, *S. argenteus*, and *S. schweitzeri* [14, 15]. Although several of these *Staphylococcus* species can produce staphylococcal enterotoxins (SEs), the majority of staphylococcal food poisoning is attributed to SE produced by *S. aureus* [16, 17]. The number of CoPS per gram or per millilitre of RTE food sample can be used to determine the microbiological quality and safety of RTE foods [18].

Staphylococcus aureus is one of the major bacterial agents causing food-borne disease in humans worldwide [19]. Some pathogenic strains of this bacterium elaborate heat-stable SEs, and so far, 23 SEs have been identified, comprising five major classical types (SEs: SEA to SEE) and the nonclassical SE-like toxins (SEl: SEG to SEU) [13, 20]. Approximately 95% of food poisoning outbreaks are caused by classical enterotoxins [21]. Ingestion of food containing SEs can induce severe symptoms, including vomiting, high fever, nausea, and diarrhoea, with rapid onset in typically less than 8 h [22]. Consumption of RTE food contaminated with *S. aureus* has been reported in several developed countries, including the U.S.A., Hong Kong, Germany, Japan, and Italy [1]. In China, several RTE food products have been associated with the introduction of microbiological hazards, including *Listeria monocytogenes*, *Cronobacter*, *Salmonella*, and *S. aureus* [23]. Although evidence on food-borne illnesses in developing countries is still limited [24], cases of staphylococcal food poisoning linked to RTE foods have been reported in some parts of Africa [21] and in two major towns in Cameroon [25]. It is, therefore, important to monitor and control the presence of bacterial contaminants in RTE foods [12, 24].

Over the years, several pathogenic strains of *S. aureus* isolated from RTE food have developed resistance to one or more antimicrobials commonly used in the treatment of infection. Antimicrobial-resistant bacteria (ARB) are a major threat to global public health. In order to effectively counter the threat of infections with antimicrobial-resistant bacteria, it is critical to identify potential ways that humans can be exposed to these bacteria [26]. Although foods have been reported as vehicles for the transmission of antimicrobial-resistant bacteria, the role of RTE foods in the spread of these pathogens is scantily documented [23]. Some RTE foods have been reported to carry antimicrobial-resistant enterococci [5], antimicrobial-resistant *Staphylococcus* species [27], and other bacterial species [28, 29]. Unfortunately, the presence of antimicrobial-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 [21]. Although the circulation of antimicrobial-resistant and multidrug-resistant bacteria among humans, animals, and the environment has been reported as a real and rising public health threat in Cameroon, there is no integrated surveillance of these superbugs in the context of One Health [30]. Although foods, especially of animal origin, have been reported elsewhere as important reservoirs of antimicrobial-resistant bacteria, similar reports are rare in Cameroon. Hence, this study was carried out to investigate the levels of CoPS and determine the prevalence and characteristics of *S. aureus* isolated from commonly consumed RTE food samples collected in Buea, South West Cameroon.

2. Materials and Methods

2.1. Study Area. This study was carried out on RTE foods from vendors in Buea municipality, the administrative headquarter of the southwest 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 1000 m 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 7,000 people relocate to Buea each year, according to the municipal council statistics [33].

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

Six types of RTE foods including fruit salad, suya (roasted beef), cake, boiled rice, bread, and meat hot-pot (cooked meat in tomato sauce) were investigated in this

study. These RTE food types were chosen based on their popularity and round-the-clock availability. They were purchased randomly from street food vendors, and only one sample was collected per vendor.

2.3. Collection of Food Samples. A total of 420 RTE food samples, comprising 70 of each food type, were purchased randomly and twice a week (Mondays and Wednesdays) from street vendors. Upon purchase, a portion of each sample was aseptically put in a sterile ziploc bag, assigned a unique code, and placed in a cool box containing ice packs with temperature maintained at +2 to +6°C. Sample collection was done between 8:00 am and mid-day on each sample collection day. Samples were transported to the Laboratory for Emerging Infectious Diseases, University of Buea, within an hour of collection for processing. The time to transport samples from sampling sites to the lab was at most 30 min.

2.4. Enumeration of CoPS. The surface colony count technique was used for the enumeration of CoPS in each sample, as described previously [18]. Briefly, a 500 μL inoculum from a 10^{-1} dilution (in Peptone saline diluent (Oxoid, Hampshire, UK)) of each RTE food sample homogenate was spread on the surface of Baird-Parker agar medium (Hardy Diagnostics, CA, USA) and the plate was allowed to stand for 15 min to allow the inoculum to be absorbed into the surface of the agar. Each sample was inoculated in duplicate, and the inoculation was done within 45 min of the 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 CoPS (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 CoPS. 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 (Biolab Diagnostics, Budapest, Hungary) 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. A 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 were examined for clotting every 30 min for 4 h by carefully tilting the tubes. If negative after 4 h, the tube was reexamined at 24 h.

The number of CoPS per g of sample was calculated as follows:

$$\text{Count per} g = \frac{\text{No.of colonies confirmed}}{\text{No.of colonies tested}} \times \frac{\text{Presumptive count}}{\text{Volume tested}} \times \text{Dilution factor.} \quad (1)$$

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, 150 μL of phosphate-buffered saline (PBS) were pipetted into a sterile 1.5 mL Eppendorf tube, and a loopful of the bacterial colony was suspended in the PBS. The tubes were vortexed at 14000 rpm for 10 sec. The bacterial solution was heated in a water bath (Thermo Fisher Scientific, MA, USA) at 100°C for 15 min after which it was removed and immediately chilled in ice for another 15 min. The bacterial solution was allowed to thaw at 37°C in the water bath and then chilled on ice for another 15 min. The tubes were centrifuged at full speed (14000 rpm) for 5 min, and the supernatant was pipetted into another labelled tube and used as template DNA in the PCR assay.

Presumptive *S. aureus* isolates were confirmed by amplification of a 280 bp fragment of the *nuc* gene of *S. aureus* using primer sequences previously described (Table 1).

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

The PCR cycling conditions were optimized with an initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 94°C/60 sec, annealing at 54°C/40sec, and extension at 72°C/60 sec. The final extension was at 72°C for 7 min, 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, CA, USA) for 1 h at 90 V 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 [34] 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 60 sec, annealing at

Table 1: Primer sequences of *S. aureus* used for the PCR assay in this study.

Target gene	Oligonucleotide sequence ($5' \longrightarrow 3'$)	Product size (bp)	References
nuc	F: GCGATTGATGGTGATACGGTT R: AGCCAAGCCTTGACGAACTAAAGC	280	[34]
sea	F: TTGGAAACGGTTAAAACGAA R: GAACCTTCCCATCAAAAACA	120	
seb	F: TCGCATCAAACTGACAAACG R: GCAGGTACTCTATAAGTGCC	478	
sec	F: GACATAAAAGCTAGGAATTT R: AAATCGGATTAACATTATCC	257	
sed	F: CTAGTTTGGTAATATCTCCT R: TAATGCTATATCTTATAGGG	317	
see	F: AGGTTTTTTCACAGGTCATCC R: CTTTTTTTTCTTCGGTCAATC	209	[35]
*Mec*A	F: AAAATCGATGGTAAAGGTTGGC R: AGTTCTGCAGTACCGGATTTGC	533	[36]

50°C for 60 sec, and extension at 72°C for 60 sec. A final extension at 72°C for 5 min was done at the end of the cycles. A singleplex PCR run was performed for the 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 *mec*A gene. This gene encodes a modified penicillin-binding protein designated as PBP2a with reduced affinity for β-lactams. The amplification of the 533 bp fragment of the *mec*A gene was optimized under the following conditions: initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, extension at 72°C for 1 min with a final extension at 72°C for 5 min, and cooling to 4°C. Similarly, PCR products were separated electrophoretically, as described above.

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

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

2.9. Data Quality Assurance, Management, and Statistical Analysis. The handling and analysis of samples and bacterial isolates were done following standard operating procedures to ensure the quality and reliability of the study findings. The sterility of culture media was maintained and confirmed by the incubation of uninoculated culture media as a negative control. Based on CoPS count (colony-forming units/gram), the food samples were classified as satisfactory (<20 CFU/g), borderline (20– $\leq 10^4$ CFU/g) or unsatisfactory (potentially injurious to health and/or unfit for human consumption, $>10^4$ CFU/g). Data were entered into Microsoft Excel 2010, exported into SPSS V27.0.1.0, and analyzed using descriptive statistics such as prevalence, percentages, mean, and standard error. The CoPS counts were transformed to $\log_{10}$ CFU/mL prior to statistical analysis. Data on the prevalence of *S. aureus* and multidrug-resistant isolates were analyzed using a chi-squared test to determine whether there were significant differences in their prevalence between sample types. The confidence level was held at 95% and the p value at less than 5% for all analyses.

3. Results

3.1. CoPS Counts. The CoPS counts in the RTE foods ranged from 2.0 to 5.81 $\log_{10}$ CFU/g. Overall, 161 (38.3%) of the RTE food samples had detectable levels of CoPS contamination.

The fruit salad samples were the most contaminated (51, 72.9%), while the suya samples were the least contaminated (12, 17.1%) (Table 2). A total of 652 presumptive staphylococcal isolates were screened in the confirmatory testing for CoPS.

Based on CoPS counts, few (37, 8.81%) of the 420 RTE food samples investigated, comprising only fruit salad and meat hot-pot had unsatisfactory or unacceptable quality. More than half (259, 61.67%) of the RTE food samples had satisfactory quality (Table 3).

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 the required size (280 bp) when separated on a 1.5% (W/V) agarose gel (Figure 1).

Out of the 652 CoPS isolates screened, 72 (17.14%) were confirmed to possess the *nuc* gene and were confirmed to be *S. aureus*. The 72 *S. aureus* isolates comprising 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 samples examined, with some samples having more than one *S. aureus* isolate. Similarly, the fruit salad was the most contaminated sample with *S. aureus,* followed by boiled rice and meat hot-pot (Table 4).

The *mec*A gene (Figure 1) was detected in 5.56% (4/72) of the *S. aureus* isolates from three RTE food types, while none of the *S. aureus* isolates possessed any of the classical enterotoxin genes (Table 4).

3.3. Antimicrobial Susceptibility of Staphylococcus aureus Isolates. The results of antimicrobial susceptibility testing (Figure 2) were interpreted following the guidelines published by the CLSI [37]. All the *S. aureus* isolates were susceptible to vancomycin (100%) and ofloxacin (100%), while 68 (94.44%) and 66 (91.67%) of the bacterial isolates were susceptible to oxacillin and ciprofloxacin, respectively. On the other hand, the isolates showed high resistance rates to penicillin (93.06%), followed by amoxicillin (91.67%) and erythromycin (79.17%) (Table 5). Four isolates were identified as methicillin-resistant *S. aureus* (MRSA) based on their resistance to oxacillin. Each of the MRSA isolates was resistant to at least five antimicrobials. Of the 72 *S. aureus* isolates, 45 (62.5%) were multidrug-resistant. In this study, multidrug resistance was defined as resistance to one antimicrobial in three or more classes of antimicrobials.

3.4. Antibiotypes of S. aureus from the Food Samples. A total of 24 antibiotypes were identified in this study (Table 6). The most prevalent antibiotype was P-AML-E-AZM-C which 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 CoPS, 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 [21]. Staphylococcal food poisoning is usually self-limiting and resolves within 24 to 48 h after onset.

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 food-borne pathogen that quickly acquires resistance to antimicrobials [40, 41]. Therefore, this study, that investigated RTE foods for contamination with CoPS with particular focus on *S. aureus* and its antimicrobial-resistant profiles, has both public health and food safety relevance.

Of the 420 RTE food samples analyzed in this study, 161 (38.3%) had detectable levels of CoPS contamination, with counts ranging from 2.0 to 5.81 $\log_{10}$ CFU/g. Based on the CoPS counts, 37 (8.81%) of the 420 RTE food samples investigated, comprising only fruit salad and meat hot-pot had unsatisfactory or unacceptable quality (Table 3). Our findings corroborate a previous study that analyzed 275 RTE food samples and reported that 25 (9.1%) were inappropriate for consumption according to Turkish microbiological guidelines [42]. Monitoring the CoPS count in RTE foods is very helpful for a risk assessment. The number of CoPS per gram or millilitre of RTE food sample is an indication of the microbiological quality of the food sample [18]. In an earlier study that analyzed 60 RTE foods served in long-term care facilities in Spain, the highest CoPS count in the samples was 1.9 ± 0.3 $\log_{10}$ CFU/g; hence, all samples had acceptable quality based on United Kingdom guidelines [43]. In a very recent study that investigated 83 RTE food samples, 25% (23/83) of those analyzed were classified as unsatisfactory or potentially hazardous [44].

In this study, fruit salad samples showed high levels (51/70, 72.9%) of contamination with CoPS (Table 2). High contamination of RTE fruit salads has been reported in previous studies. Brooks [45] reported a higher rate (90%) of staphylococcal contamination in 20 RTE salad samples analyzed in his study carried out in Calabar, Nigeria. In another recent study that analyzed 36 RTE pineapple slices in Port Harcourt, Nigeria, high levels of bacterial contamination were detected in all the samples, and the contamination levels were even higher for samples collected in the evening than for those collected in the morning from the same vendors [46]. The high levels of bacterial contamination of RTE fruits could be multifactorial and include washing the fruits with a poor-quality source of

Table 2: CoPS 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)			

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

Table 3: Microbiological quality of the RTE food types based on CoPS counts.

RTE food type	Microbiological quality based on CoPS count		
	Satisfactory (<1.3 $\log_{10}$ CFU/g) (%)	Borderline (1.3–≤4 $\log_{10}$ CFU/g) (%)	Unsatisfactory (>4 $\log_{10}$ 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.

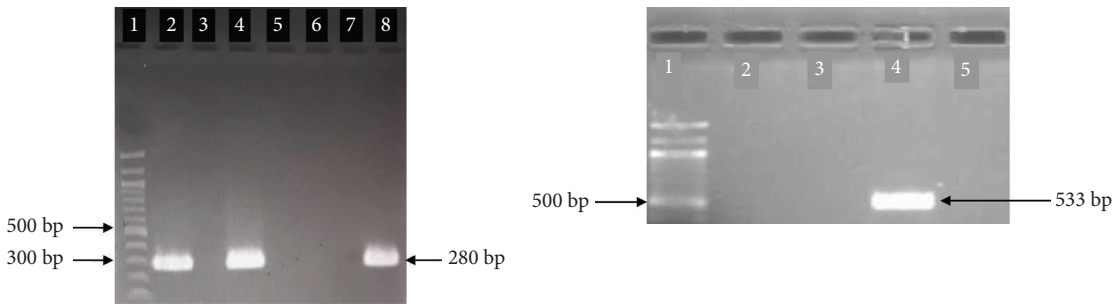

Figure 1: Electrophoretic separation of PCR products on 1.5% agarose gel for detection of *S. aureus* genes: (a) Amplification of *nuc* gene. Lane 1, 100 bp molecular weight marker. Lane 2, positive control. Lanes 3, 5, 6, and 7 are negative samples. Lanes 4 and 8 are positive samples. (b) Amplification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

Table 4: Prevalence of *Staphylococcus aureus* among the ready-to-eat food samples.

RTE food type	*S. aureus* (%)	Number of RTE food with Methicillin-resistant *S. aureus* (%)*	Enterotoxigenic *S. aureus* (%)
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)

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

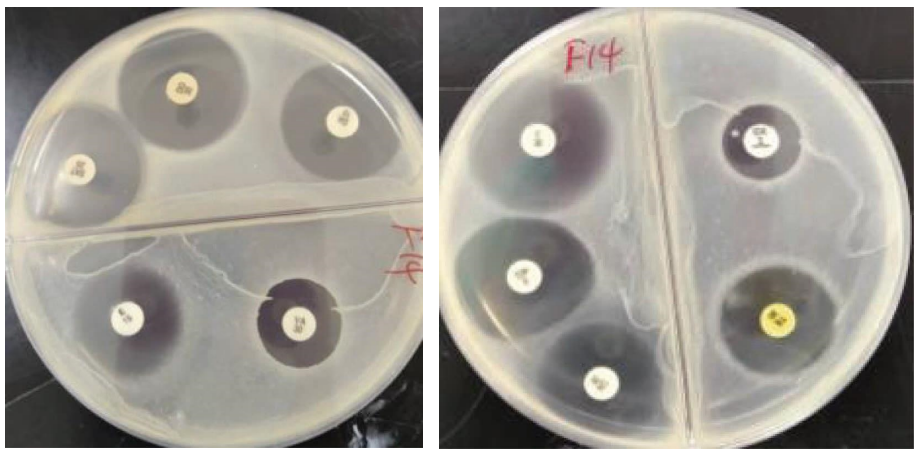

Figure 2: Antimicrobial susceptibility test results of *Staphylococcus aureus* isolate on a Mueller–Hinton agar culture plate using the Kirby–Bauer disk diffusion method. The diameters of the zones of inhibition were measured and values were interpreted as susceptible, intermediate, or resistant according to the guidelines of CLSI [37].

Table 5: Susceptibility of *S. aureus* isolates to the different antimicrobials tested ($n = 72$).

Class of antimicrobial	Antimicrobial	Resistant (%)	Intermediate (%)	Susceptible (%)
Glycopeptide	Vancomycin (VA)	0 (0)	0 (0)	72 (100)
β-lactams	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*.

Table 6: Antimicrobial-resistant patterns of *S. aureus* isolates from RTE food samples ($n = 72$).

Number of antimicrobials 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

Table 6: Continued.

Number of antimicrobials showing resistance	Antibiotype	Number of isolates (%)	Sample origin
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.

water; cross-contamination from other fruits washed in the same water many times [47, 48]; use of dirty trays or dirty processing utensils like knives and slicing tables; not washing the hands thoroughly; and contamination from the air [46].

A total of 12 (17.14%) of the 70 suya samples analyzed in this study had the lowest detectable levels of CoPS, and they ranged from 2 to 2.91 $\log_{10}$ CFU/g (Table 2). While these 12 samples had borderline quality, the remaining 58 (82.86%) samples had satisfactory quality (Table 3). Hence, all the 70 suya samples analyzed in this study were suitable for consumption at the time of sample collection. Falegan et al. [49], in a recent study carried out in Ado-Ekiti Metropolis, Ekiti State, Nigeria, reported comparably higher values of 4.99–5.45 $\log_{10}$ CFU/g for total bacterial counts in the 20 suya samples analyzed and stated that all the samples had unsatisfactory quality. In another earlier study by Egbebi and Seidu [50] carried out in Ado and Akure, South West Nigeria, bacterial counts ranged from 4.48 to 4.60 (in Ado) and 4.48–4.93 (in Akure), and these values placed the suya samples in an acceptable but not satisfactory range. The low values of CoPS in the suya samples reported in our study could be linked to the fact that these samples were purchased shortly after preparation (i.e., freshly prepared), and not much postprocessing contamination had occurred.

In this study, the majority of the samples of boiled rice (75.71%), bread (61.43%), and cake (70.0%) (Table 3) had satisfactory quality, while 24.29% (boiled rice), 38.57% (bread), and 30% (cake) samples had CoPS counts on the high threshold but within microbiologically acceptable limits recommended by regulatory bodies for vended meat and meat products [51, 52]. Our results did not agree with those of Wogu et al. [53], who reported that most of the 48 rice samples from hotels in Benin City, Nigeria, examined in their study had unsatisfactory quality. A very recent study that examined 50 rice samples obtained from cafeterias at Akwa Ibom State University in Nigeria reported that most of the rice samples had unsatisfactory quality [54]. The reasons for the differences in the results reported in these studies could be multifactorial, ranging from rice species differences, methods of preparation, holding temperature and time, and methods of sample analysis.

Staphylococcus aureus was identified in 46 (10.95%) RTE food samples in this study. This overall contamination rate by *S. aureus* reported in our study was higher than the 6.6% (8/120) reported for RTE foods in Egypt [55], 4.31% (6/139) in Libya [56], and 6.4% (48/750) in Istanbul in Turkey [57] but lower than the 29.1% (155/532) in Cambodia [12], 34% (68/200) in Bolgatanga municipality of Ghana [58], 15.42% in Tehran Province in Iran [59], 52.8% (56/106) in Putrajaya, Malaysia, and 12.55% (69/550) in China [23]. The difference in the prevalence rates reported in these studies could be due to the diagnostic method used; while some studies identified *S. aureus* using phenotypic methods, others relied on the more sensitive molecular method.

A total of 72 *S. aureus* isolates, comprising 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 (Table 4) were recovered from the 46 S. aureus-positive samples in this study. Since *S. aureus* is heat-labile and also readily destroyed by most sanitizing agents, its presence in RTE foods may be an indication of poor handling and sanitation [38]. Elsewhere, contamination of RTE foods has been linked to the preparation by small-scale local producers without quality control, the reuse of improperly washed dishes, the open-air distribution environment, and holding temperatures during distribution [60]. We investigated the *S. aureus* isolates for the presence of the classical enterotoxin genes, and none were found to possess any of the genes. In a similar study that investigated 84 *S. aureus* isolates for the presence of the classical enterotoxin genes, only 2 (2.38%) of the isolates possessed the enterotoxin B gene, while the rest of the 82 isolates were negative for other enterotoxins genes [21]. In another study, a high prevalence (58.1%, 18/31) of classical staphylococcal enterotoxin genes was identified in meat samples in Zanjan, Iran [61]. Although a positive PCR confirms the presence of enterotoxins, the negative PCR in our study does not point to the absence of the corresponding operon because there is always a possibility of mutation at the level of the corresponding gene. Furthermore, the differences between our results and those reported elsewhere could also be due to other factors like sample source and geographical origin [21].

The increasing reports that resistant strains of *S. aureus* are present in various RTE foods in different countries [60, 62, 63] and the notorious resistance of this bacterium to several commonly used antimicrobials [62–64] warranted antimicrobial susceptibility testing for all 72 *S. aureus* isolates identified in this study. All the *S. aureus* isolates showed 100% susceptibility to vancomycin and ofloxacin, followed by ciprofloxacin (91.67%) (Table 5). These results agree with those of Sina et al. [65] in a study carried out in Cotonou Benin and those of Wang et al. [66], who reported that all *S. aureus* isolates from food in Shaanxi Province, China, were susceptible to vancomycin. This high susceptibility of the *S. aureus* isolates has great therapeutic relevance because these antimicrobials can be used to treat infections caused by this bacterium in our resource-poor community. High *S. aureus* antimicrobial resistance was observed for penicillin (93.06%), followed by amoxicillin and erythromycin, and our results are comparable to those reported by Tsehayneh et al. [67]. Forty-five (62.5%) *S. aureus* isolates in this study showed multidrug resistance, and this was lower than 98% (54 out of 55 *S. aureus* isolates) recently reported by Tsehayneh et al. [67]. This difference in resistance reported may be due to the extent of usage of antimicrobials in a locality and possibly the type of samples analyzed [60].

In this study, resistance to oxacillin was used to identify methicillin-resistant *S. aureus* (MRSA) strains. A total of four (5.56%) of the 72 *S. aureus* investigated were MRSA, and all possessed the *mec*A gene. Saad et al. [55] reported that two (25%) of the eight *S. aureus* isolates identified in their study were MRSA; Yang et al. [23] reported 10.14% (7/69) prevalence of MRSA, out of which six were *mec*A-positive; Naas et al. [56] reported only one (16.67%) of six *S. aureus* isolates as MRSA but did not screen for the presence of the *mec*A gene. All MRSA strains in this study were susceptible to vancomycin and, as expected, resistant to penicillin and other antimicrobials. MRSA is a pathogen of global concern and is placed on the WHO priority pathogen list [68] because infections caused by it are extremely difficult to treat. Thus, understanding the epidemiology of MRSA and other antimicrobial-resistant strains of *S. aureus*, in the context of the one health approach, is relevant to effective prevention and control [63], especially at this time when the antimicrobial pipeline is nearly empty [68]. 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 CoPS and *S. aureus* cannot be ignored. The contamination of these foods with CoPS, S. *aureus*, and even MRSA poses a potential risk to consumers. To combat the spread of antimicrobial-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.

This study did not take into consideration the duration of each RTE food in exposed air at room temperature before sample collection, as this could favour the growth of microorganisms. Our results emphasize an urgent need for policies to enforce hygienic practices within the RTE food outlets for the improvement of food safety. Food vendors and food handlers should undergo training on basic food hygiene and preservation to enhance the strict implementation of the hygienic practices. To ascertain the enterotoxigenic potential of *S. aureus* isolates from RTE foods, the isolates should be tested for the presence of more staphylococcal enterotoxin genes. More research is also required to understand the epidemiological aspects of MRSA and *S. aureus* isolates in RTE foods.

The first draft of this manuscript was deposited on a preprint server [69].

Data Availability

All relevant data generated during this study are included in this manuscript.

Disclosure

This manuscript has been submitted to BiOrxiv as a preprint: https://www.biorxiv.org/content/10.1101/2021.09.30.462650v1.full.

Conflicts of Interest

The authors declare that they do not have any conflicts of interest.

Authors' Contributions

Seraphine Nkie Esemu and Lucy M. Ndip conceptualized the study. Seraphine Nkie Esemu, Nene Kaah Keneh, and Jerome Achah Kfusi curated the data. Seraphine Nkie Esemu, Nene Kaah Keneh, Sally Tabe Njoh, and Jerome Achah Kfusi performed formal analysis. Seraphine Nkie Esemu, Nene Kaah Keneh, Sally Tabe Njoh, and Jerome Achah Kfusi performed an investigation. Seraphine Nkie Esemu, Lucy M. Ndip, Achiangia Patrick Njukeng proposed a methodology. Seraphine Nkie Esemu, Sally Tabe Njoh, and Lucy M. Ndip were responsible for resources. Seraphine Nkie Esemu and Achiangia Patrick Njukeng supervised the study. Lucy M. Ndip and Achiangia Patrick Njukeng validated the study. Seraphine Nkie Esemu wrote the original draft. Lucy M. Ndip and Achiangia Patrick Njukeng wrote, reviewed, and edited the study.

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