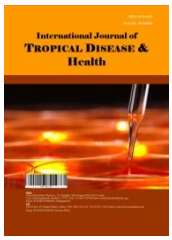

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Prevalence and Characterization of Methicillin-Resistant *Staphylococcus aureus* from Meat Retail Shops and Meat Handlers in the Buea Municipality, Cameroon

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SNE, FAY, WPN and RNN were involved in the conceptualization and design of this study. Authors FAY, WPN, NKK and JAK were involved with collection of samples collection and data on basic hygiene practices. Authors SNE, LMN and RNN supervised the study. Authors SNE, FAY, WPN, NKK and JAK carried out laboratory analysis. Authors SNE, LMN, SIS and RNN were involved in data analysis and result interpretation. Authors SNE, LMN, SIS and RNN provided the resources. Author SNE prepared the original draft manuscript. Authors LMN, SIS and RNN reviewed and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a notorious multidrug-resistant biovar of *S. aureus* associated with infections that result in high morbidity and mortality in humans and animals. Its persistence in both host and non-host environments adds a major ecological dimension to the problem, thereby making it a one health challenge. This study determined the prevalence and

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antibiotic resistance patterns of MRSA from meat retail shops and meat handlers in the Buea municipality in addition to basic hygiene practices implemented in the meat retail shops.
Study Design: In this cross-sectional study, a total of 260 samples (comprising 52 swabs each from weighing balances, butchering slabs, cutting knives, hands of meat handlers and meat samples) were randomly obtained and subjected to microbiological analysis. Data on sociodemographic characteristics of meat handlers and basic hygiene practices were captured in a questionnaire.
Methodology: After enrichment, each sample was inoculated on mannitol salt agar and DNA extracted from presumptive *S*. *aureus* isolates for detection of the *nuc* gene. MRSA was identified by oxacillin disk diffusion and confirmed by amplification of the *mec*A gene. All MRSA isolates were challenged with 10 commonly used antibiotics using the Kirby-Bauer disk diffusion method.
Results: A total of 27 MRSA isolates were recovered from hand swabs (5.8%), meat (15.4%), knives swabs (21.2%) and butchering slabs (9.6%). All (100%) MRSA were resistant to ampicillin and cefepime followed by ofloxacin (92.6%), ciprofloxacin (81.5%) and vancomycin (37%). High susceptibility was observed for gentamicin (100%) and amikacin (100%). All isolates were multidrug-resistant and comprised 11 antibiotypes. Most meat handlers (71.2%) did not have basic meat safety training, while 90.4% of the meat retail shops did not have a nearby water source.
Conclusion: Our results indicate that meat retail shops could be a potential reservoir of MRSA in the environment and meat handlers need more basic meat safety training.

Keywords: Methicillin-resistant Staphylococcus aureus, mecA gene; antibiotics resistance; retail meat shops; Cameroon.

ABBREVIATIONS

MRSA : Methicillin-resistant Staphylococcus aureus
HA-MRSA : Hospital-associated methicillin-resistant Staphylococcus aureus
CA-MRSA : Community-associated methicillin-resistant Staphylococcus aureus
LA-MRSA : Livestock-associated methicillin-resistant Staphylococcus aureus
MSA : Mannitol salt agar
PCR : polymerase chain reaction

1. INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a virulent zoonotic biovar of *S. aureus* that is considered a global public health threat with veterinary importance in both the developed and developing countries [1,2]. It is responsible for a wide range of diseases that are difficult to treat leading to increased mortality, longer treatment, longer stays in hospital, and the use of more expensive and possibly more toxic treatment options [2]. The emergence and spread of MRSA have been exacerbated by faulty human behaviours and the fear of reaching a therapeutic dead end in the treatment of infections caused by this organism may become real due to the “discovery void” in the development of new antibiotics [3].

Initially, MRSA was confined to the hospital environment and was identified as a multi-antibiotic resistant nosocomial pathogen referred to as hospital-associated MRSA (HA-MRSA). It later spread into the community giving rise to a new lineage of MRSA referred to as community-associated MRSA (CA-MRSA) [4]. Livestock-associated MRSA (LA-MRSA) lineage was reported between 2003 and 2005 in Europe in pigs and has progressively been reported to also cause serious infections in other animals and continents with widely varied prevalence [5]. The widespread and improper use of antibiotics in the veterinary sector has resulted in the emergence of MRSA among livestock, suggesting that the infected animal is a permanent reservoir of MRSA, which results in human infections [6]. Reports of CA-MRSA infections continue to increase worldwide, including cases with fatalities. Recent epidemic-molecular investigations have reported that CA-MRSA affects patients within health care settings [7] and there are reports of the isolation of CA-MRSA and HA-MRSA from animals [8]. It has been documented in multiple studies that MRSA strains found in companion animals such as dogs and cats are identical to epidemic strains found in humans and hospital environments [9]. In fact, the epidemiologic changes in MRSA over the

years have shown that the distinction between CA-MRSA and HA-MRSA has become faint [10]. According to literature, the prevalence of these MRSA strains varies by geographical region [1]. So far, MRSA is one of the most successful modern pathogens [11].

In Africa, early reports of MRSA were in 1978 from clinical isolates and four decades after, the coverage and quality of data on MRSA prevalence (except for countries in the Mediterranean basin, South Africa, and Nigeria) is scanty and very inconsistent unlike in developed continents [10]. In Cameroon in particular, although a few sporadic studies have been carried out, the picture of the epidemiology of MRSA is unclear. Kesah et al. [12] reported 21.3% (21/127) MRSA prevalence; while Njoungang et al. [13] documented 13.9% (28/201) from clinical samples in Yaounde. Also, Bissong et al. [14] reported 13.2% from clinical samples in Douala and equally documented that 37 (74%) of the 50 *S. aureus* isolates identified from 289 samples (milk and beef) from the Northwest and Southwest Cameroon were MRSA [15]. All these studies identified MRSA based on phenotypic (oxacillin or cefoxitin screening) methods only. Detection of the *mec*A gene is gold standard for the identification of MRSA [16]. Founou et al. [17] reported a lower prevalence of 7.7% (1/13) in pigs in Cameroon and whole genome analysis revealed that LA-MRSA clonal lineage ST398 is already present in Cameroon.

The development of multidrug resistance is the main obstacle in the treatment of MRSA infections [1]. MRSA always shows a multidrug-resistant pattern, not only for penicillin but also for variable antimicrobial classes including macrolides, fluoroquinolones, aminoglycosides, tetracyclines, and lincosamides [18]. The emergence of multidrug-resistance in MRSA is an important threat to treatment and control. Hence, infection with MRSA is life-threatening for both humans and animals [19].

While CA-MRSA infections continue to spread, the sources of infections in non-epidemic settings remain poorly defined [20]. However, some risk factors for CA-MRSA infection in both natural and built-in environments have been identified [21]. Since there are diverse environmental settings, it is important to understand each environment and its associated risk factors that enable CA-MRSA transmission in order to implement effective primary preventive measures [21]. For example, the exposure of humans to antibiotic resistant bacteria via food and food retail outlets is currently only poorly understood leaving an important gap for intervention design [22]. Moreover, livestock are reservoir for *S. aureus* and MRSA strains and their transmission to humans may occur following exposure to colonized livestock, their food products or even the contaminated environment [23].

This study investigated the meat retail shop environment (the meat and the equipment that are in contact with the meat during the retail activities) for contamination with MRSA using a combination of phenotypic and molecular methods. The antibiotic susceptibility patterns of the MRSA isolates were also determined, in addition to understanding the implementation of basic hygiene practices by the meat handlers.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in the Buea municipality. Buea is the headquarter of the South West Region of Cameroon with coordinates 4°09'9.72" N 9°14'27.60" E of the Greenwich meridian. Buea stands on the eastern slope of mount Cameroon (an active volcano of about 4010 m high), and covers a surface area of 870 km^2 [24]. With its historic nature, the cosmopolitan town had an estimated population of about 300,000 inhabitants in 2013 [25] comprising people of all walks of life. Like the rest of the country, Buea has two seasons (dry and rainy) with an equatorial climate and an annual rainfall of about 3000 mm to 5000 mm [24]. Despite this amount of rainfall, the municipality is faced with huge water crisis most especially in the dry season. The growing water crisis in the Buea municipality has been attributed partly to the rapidly growing population [24]. The drainage system and waste management scheme of the municipality are still very rudimentary [26]. The water scarcity and poor waste management scheme could be risk factors for meat contamination, which may lead to the sale and consumption of unhealthy meat. The Buea municipality has two abattoirs (Buea Town abattoir and Muea abattoir) and many meat retail shops.

2.2 Study Design

This was a cross-sectional study in which random sampling was used to select meat retail shops. The study was carried out from March to August 2020. Meat samples were purchased

from meat that was displayed for sale at each meat retail shop while swab samples were collected from butchery equipment (including knives, butchering slabs, and weighing balances) that were constantly in contact with the meat during retail activities and from the hands of the meat handlers. Only meat handlers who consented to participate in the study were enrolled. A structured questionnaire was used to record observational data that included sociodemographic characteristics of meat handlers and the implementation of basic hygiene practices at the meat retail shops.

2.3 Observational Data Captured in Questionnaire

The structured questionnaire that was administered to each meat handler captured information on sociodemographic characteristics including age, gender, level of education and meat safety training. Knowledge and implementation of basic hygiene practices in the meat retail shops was also assessed. The questionnaire was designed in English and pretested on eight meat handlers in meat retail shops in Mutengene located 12 km from the study area. The finding from pretesting was used to improve on the clarity of one of the questions in the questionnaire. The specific modification was the replacement of the word 'pathogens' with 'germs'.

2.4 Sample Collection

A total of 260 samples including swabs (208) and retail meat samples (52) were collected from 52 meat retail shops and labelled appropriately. Samples were collected from 9 am to 11 am on each sample collection day. The 208 swabs included 52 each from weighing balances, butchering slabs, cutting knives and hands of meat handlers. A total of five samples were collected from each meat retail shop. Sample collection was done aseptically; each meat sample was put in a separate sterile ziploc plastic bag and each swab, collected from a surface of about 20 cm^2, was placed in a sterile test tube. All samples were transported in ice to the Laboratory for Emerging Infectious Diseases, University of Buea, within one hour of collection for microbiological analysis.

2.5 Enrichment of Samples

All samples were enriched in sterile buffered peptone water (BPW) (Oxoid, Hampshire, UK) before bacterial isolation. For each meat sample, 10g were macerated and enriched in 9 mL BPW and incubated at 37 °C for 24 h. Similarly, each swab sample was enriched in 5 mL BPW and also incubated at 37 °C for 24 h.

2.6 Isolation of Presumptive *Staphylococcus aureus*

One hundred microliters of each enriched sample were inoculated on mannitol salt agar (MSA) (Liofilchem, Italy) using the spreading method and plates incubated aerobically at 37 °C for 24 h. After incubation, the plates were examined for evidence of mannitol fermentation, colony characteristics and Gram staining performed for each isolate. From each sample, two colonies that fermented mannitol (evident by the yellow appearance of the medium) and had typical Gram reaction (Gram positive cocci mainly in grape-like clusters) of *S. aureus* were purified on nutrient agar (Liofilchem, Italy). The pure colonies, termed presumptive *S. aureus* isolates, were checked for catalase production, and catalase-positive isolates were subjected to further testing. The pouring of culture media into petri plates, inoculations and other procedures requiring aseptic conditions were carried out in a biological safety cabinet class II (Labconco, USA).

2.7 DNA Extraction from Presumptive *Staphylococcus aureus*

The extraction of genomic DNA was carried out using the Zymo Research genomic DNA isolation kit (Zymo Research, Irvine, USA) and the procedure was performed according to the manufacturer's instructions without any modification. The DNA was extracted from the presumptive *S. aureus* isolates individually and stored at -20 °C until used for polymerase chain reaction (PCR).

2.8 Molecular Identification of *Staphylococcus aureus* by PCR Amplification of the *nuc* Gene

Singlex PCR targeting the *nuc* gene was performed for all presumptive *S. aureus* isolates [27]. The PCR reactions were set up in a PCR Prep Station equipped with timed UV light (Mystaire Inc, USA) to avoid contamination. Barrier tips (Thomas Scientific, New Jersey, USA) and positive displacement micropipettes (Eppendorf, USA) were used. Each PCR reaction had a final volume of 25 µL comprising 5 µL of

DNA template, 12.5 µL of PCR 2x master mix (BioMix Red), 6.5 µL nuclease-free water and 0.5 µL of each primer from a 20 µM working stock. The sense primer (5'-GCGATTGATGGTGATACGGTT-3') and antisense primer (5'-AGCCAAGCCTTGACGAACTAAAGC-3') [27] amplified a fragment of the thermostable nuclease (*nuc*) gene with amplicon size of 280 bp. The PCR was carried out using the following thermal cycling conditions: 1 cycle of initial denaturation at 94^{o}C for 5 min, followed by 40 cycles of denaturation at 94 ^{o}C for 1 min, annealing at 55^{o}C for 1 min and extension at 72^{o}C for 1 min. The final extension was at 72^{o}C for 5 min and the tubes held at 4^{o}C until removed from thermal cycler (MyCycler™ Thermal Cycler BIORAD, USA). Each PCR run had a negative control in which nuclease-free water replaced the DNA template. Electrophoretic separation of PCR products was performed on 1.5 % agarose gel stained with SYBR Safe DNA gel stain (Invitrogen). The PCR products were visualized and photographed with a high performance ultraviolet transilluminator imaging system (25W transilluminator, Upland, CA91786, USA).

2.9 Molecular Identification of Methicillin-Resistant *Staphylococcus aureus* by PCR Amplification of the *mec*A Gene

All confirmed *S. aureus* isolates (*nuc*-positive), were screened for the presence of the *mec*A gene using primer sequences 5'-AAAATCGATAAAGGTTGGC-3' (forward) and 5'-AGTTCGCAGTTACCGGATTTGC-3' (backward) [28] which amplify a 533 bp fragment of the *mec*A gene of *S. aureus*. Each PCR reaction was set up as described above and the thermal profile was the same except for the annealing temperature that was set at 50 °C and the number of cycles reduced to 35. Similarly, electrophoretic separation of PCR products was done in a 1.5 % agarose gel and the PCR products were viewed under ultraviolet light and photographed in a Molecular Imager Gel Doc XR system (BIO-RAD, Hercules, CA, USA).

2.10 Phenotypic Identification of Methicillin-Resistant *Staphylococcus aureus* using Oxacillin Disk Diffusion Method

Methicillin susceptibility was performed using the Kirby-Bauer disk diffusion method following the Clinical Laboratory Standards Institute guidelines [29]. A single oxacillin disk (Hardy Diagnostics, California, USA) of 1 µg potency was used for the detection of phenotypic methicillin resistance in *S. aureus* [30]. The test was performed on Mueller-Hinton agar (Oxoid, Hampshire, England), where a bacterial suspension of each *nuc*-positive *S. aureus* isolate with a turbidity equal to that of 0.5 McFarland standard was inoculated onto the medium by spread plate method, incubated at 35 ^{o}C for 18 to 24 h, and the zone of inhibition was recorded and compared with that on the disk diffusion zone diameter chart for Hardy-Diagnostics (https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/HardyDiskASTProceduresandChart.pdf). All confirmed *S. aureus* isolates (*nuc*-positive), were screened.

2.11 Antibiotic Susceptibility Testing of Methicillin- Resistant *Staphylococcus aureus*

All MRSA isolates where further subjected to antibiotic susceptibility testing using the Kirby-Bauer disk diffusion method to determine their antibiogram and antibiotypes. Antibiotics (Oxoid, Hampshire, England) used in this study included amikacin (AK, 30 µg), ofloxacin (OFX, 5 µg), ciprofloxacin (CIP, 5 µg), vancomycin (VA, 30 µg), cefepime (FEP, 5 µg), erythromycin (E, 5 µg), gentamycin (CN, 30 µg), azithromycin (AZM, 30 µg), doxycycline (DO, 30 µg), and ampicillin (AMP, 30 µg).

Four to five pure colonies of each MRSA isolate from a 24 h culture on nutrient agar were used to prepare the bacterial inoculum in 2.5 mL of sterile normal saline. The turbidity of the inoculum was adjusted to that of 0.5 McFarland standard equivalent to a bacterial density of 1.0 x 10^{8} CFU/mL. The plate was inoculated by spreading 100 µL of the inoculum onto Mueller-Hinton agar and the excess was siphoned by means of a sterile Pasteur pipette. With the aid of forceps, antibiotics disks were dispensed onto the bacterial lawn and gently pressed to ensure contact with the agar surface. Five disks were equidistantly placed at 25 mm from one another and 15 mm from the edge of the plate. Plates were incubated at 35°C for 18 to 24 h. After incubation, plates were examined and diameters of zones of inhibition were measured and interpreted based on the Clinical Laboratory Standards Institute recommendations [29].

2.12 Data Analysis

Data were entered and analyzed using the statistical software SPSS version 23.0.

Descriptive statistics was used to present the frequency of *S. aureus* from different sampling points in the meat retail shops, antimicrobial susceptibility patterns and hygienic conditions. Categorical variable was expressed as percentages. Statistical significance was assessed by using Chi-square. The degree of association was assessed with *p*-values less than 0.05 considered statistically significant.

3. RESULTS

3.1 Sociodemographic Characteristics of the Meat Handlers

A total of 52 meat handlers consented to participate in this study, and all filled the questionnaire. These participants had varied characteristics (Table 1). Majority of the participants were in the age range 30-39, with a frequency of 24 (46.2%) while only six participants (11.5 %) were above 50 years. Male participation was 100 % while female was 0.0 %. Based on the level of education, majority of the participants were secondary school leavers (25, 48.1%), with a very low percentage of illiterates (1, 1.9%). Meat safety skills were acquired by 28.8 % (15/52) of the participants. Eight (15.4 %) of the participants had no health certificate (Table 1).

3.2 Basic Hygiene Knowledge and Implementation of Basic Hygiene Practices by Meat Handlers

Overall, the meat handlers had good basic hygiene knowledge (Table 2). Out of the 52 meat handlers enrolled in this study, 47 (90.4%) knew that 'germs' can be transmitted through their meat retail activities and 48 (92.2%) agreed that improper meat handling is dangerous to health. Similarly, 48 (92.2%) attested that hand washing before handling meat can reduce the risk of contamination. All the meat hamdlers agreed that handling money with bare hands while serving meat can lead to meat contamination. However, up to 18 (34.6%) meat handlers did not know that using different knives and cutting boards for meat retail activities can reduce the risk of meat contamination.

On the implementation of basic hygiene practices in the meat retail shops, 44 (84.6%) meat handlers confirmed to washing their hands before and after handling meat. All meat handlers attested to wearing an apron when handling meat, while 42 (80.8%) washed their aprons after each days' work. Unfortunately, only 5 (9.6%) meat retail shops had a nearby water source.

3.3 Confirmation of *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus*

In this study, a total of 520 (two from each sample) presumptive *S. aureus* isolates were recovered from the 260 samples investigated. These isolates were considered presumptive *S. aureus* based on their growth on mannitol salt agar and their ability to ferment mannitol evident by the production of yellow colonies on the culture medium. They were all Gram positive cocci appearing mostly in grape-like clusters and also catalase positive. Of the 520 *presumptive S. aureus* isolates, 105 (43.6%) were confirmed as *S. aureus* based on the amplification of a 280 bp fragment of the *nuc* gene (Fig. 1). Of the 105 *S. aureus*, only 27 (25.7%) were confirmed to be MRSA based on the amplification of a 533 bp fragment of the *mec*A gene (Fig. 1). The remaining 78 (74.3%) *S. aureus* that lacked the *mec*A gene were methicillin-sensitive *S. aureus*.

3.4 Phenotypic Identification of Methicillin-Resistant *S. aureus* using Oxacillin Disk Diffusion Method

In order to compare detection of the *mec*A gene by PCR with the conventional oxacillin disk diffusion method for the identification of MRSA, all the 105 confirmed *S. aureus* isolates were subjected to oxacillin susceptibility testing. Of the 105 *S. aureus* isolates tested, 35 (33.3%) were MRSA and 70 (66.7%) were methicillin-susceptible *S. aureus*. Since the detection of the *mec*A gene by PCR is considered the 'gold standard' for the identification of MRSA, our PCR results were considered confirmatory.

3.5 Prevalence of *S. aureus* and MRSA in Samples Analysed

A total of 260 samples from the meat retail shops environment were analyzed in this study. Contamination of samples with *S. aureus* was identified in a total of 105 (40.4%) samples. Of these 105 samples, almost half (49, 94.2%) were samples from knives followed by those from meat (24, 46.2%) and butchering slabs (16, 30.8%). Samples from the hands of meat handlers and weighing balances were least

contaminated (Table 3). The difference between *S. aureus* contamination in samples from different sources was statistically significant (χ^2 = 92.34; df = 4; p = 0). A total of 27 (25.7%) of the 105 *S. aureus* positive samples were contaminated with MRSA. Most of the MRSA isolates were from knives swabs (11, 21.2%), followed by meat samples (8, 15.4%). No MRSA isolates were detected in samples from weighing balances. The difference in the prevalence of MRSA among the samples was also statistically significant (χ^2 = 15.13; df = 4; p = 0.004).

Table 1. Sociodemographic characteristics of meat handlers (n = 52)

Variable	Category	Frequency	Percentage (%)
Sex	Male	52	100
	Female	0	0
Age group (years)	20-29	14	26.9
	30-39	24	46.2
	40-49	8	15.4
	≥50	6	11.5
Level of education	No level	1	1.9
	Primary	16	30.8
	Secondary	25	48.1
	Tertiary	10	19.2
Employment status	Voluntary	5	9.6
	Daily basis	21	40.4
	Permanent	26	50.0
Meat safety training	Yes	15	28.8
	No	37	71.2
Health certificate	Yes	44	84.6
	No	8	15.4
Meat retail structure	Open table	15	28.8
	Open table in a building	37	71.2
	Under the tree	0	0

Table 2. Basic hygiene knowledge and implementation of basic hygiene practices by meat handlers

Questions on:	Responses		
	Yes (%)	No (%)	I don't know (%)
Basic hygiene knowledge			
Do you know that pathogens (germs) can be transmitted through your meat retail activities?	47 (90.4)	1 (1.9)	4 (7.7)
Do you know that improper meat handling is dangerous to health?	48 (92.3)	2 (3.8)	1 (1.9)
Do you know that hand washing before handling meat reduces the risk of contamination?	48 (92.3)	1 (1.9)	3 (5.8)
Do you know that using different knives and cutting boards for meat reduces meat contamination?	27 (51.9)	18 (34.6)	7 (13.5)
Do you know that knives, hooks and cutting boards can be a source of meat contamination?	46 (88.5)	1 (1.9)	3 (9.6)
Do you know that handling money with bare hands while serving meat can lead to meat contamination?	52 (100)	0 (0)	0 (0)
Implementation of basic hygiene practices			
Do you wash your hands before / after handling meat?	44 (84.6)	8 (15.4)	0 (0)
Do you wear an apron when handling meat?	52 (100)	0 (0)	0 (0)
Do you wash your apron after each day's work?	42 (80.8)	10 (19.2)	0 (0)
Is there a water source nearby	5 (9.6)	47 (90.4)	0 (0)

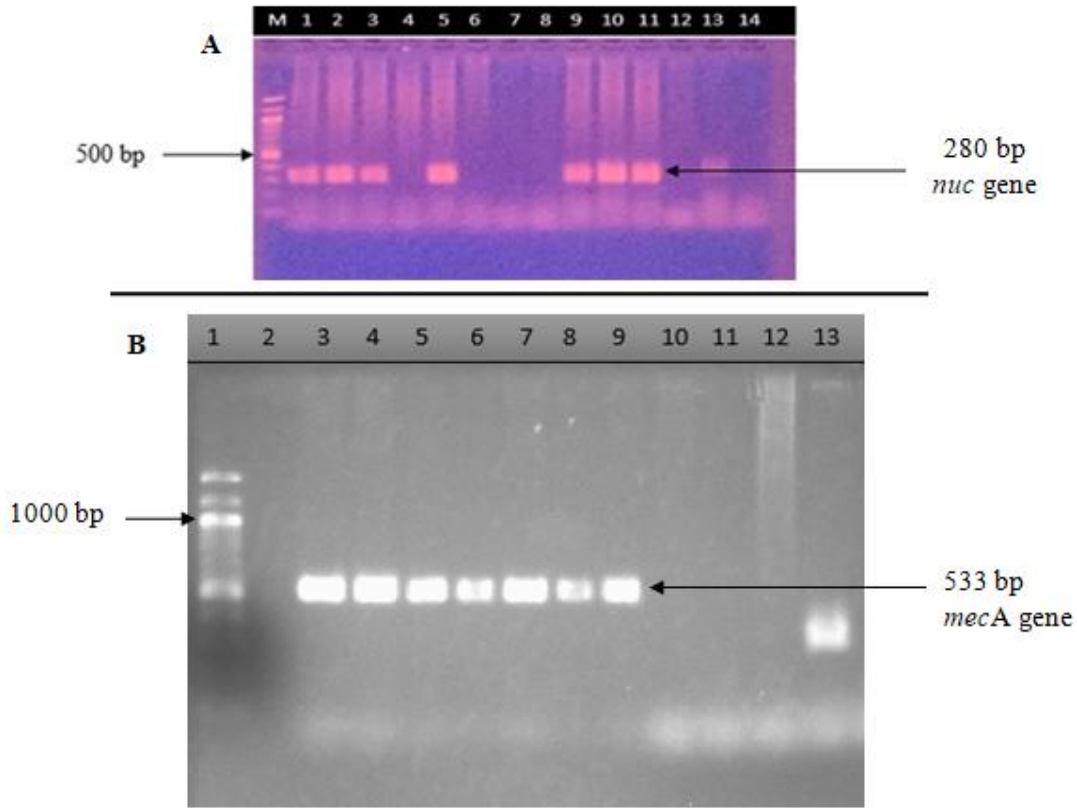

Fig. 1. Visualization of amplified PCR products on 1.5 % agarose gel after electrophoretic separation at 90 V for 1 h. A) *nuc* gene PCR products: 100 bp molecular weight marker (lane M), negative control (lane 7), positive samples (lanes 1-3, 5, 9-11 and 13), and negative samples (lanes 4, 6, 8, 12 and 14). B) *mec*A gene PCR products: lane 1 (100 bp molecular weight marker), lane 2 (negative control), lanes 3-9 (positive samples) and lanes 10-13 (negative samples)

Table 3. Prevalence of *S. aureus* and MRSA in the different sample types

Source of samples	Total samples collected	Number of samples with	
		***S. aureus* (%)**	**MRSA (%)**
Hands	52	8 (15.4)	3 (5.8)
Meat	52	24 (46.2)	8 (15.4)
Knives	52	49 (94.2)	11 (21.2)
Weighing balances	52	8 (15.4)	0 (0)
Butchering slabs	52	16 (30.8)	5 (9.6)
Total	260	105 (40.4)	27 (25.7)

3.6 Antimicrobial Susceptibility Profiles of MRSA Isolates

In this study, antibiotic resistance testing was done against 10 different anti-staphylococcal antibiotics. All the MRSA isolates were resistant to at least three of the antibiotics tested. As expected, the isolates showed very high resistance to ampicillin (100%) and cefepime (100%). High resistance to ofloxacin (92.6%) and ciprofloxacin (81.5%) was also observed. On the contrary, very high susceptibility was observed for gentamicin (100%) and amikacin (100%) followed by doxycycline (81.5%) and vancomycin (63%). A few isolates showed intermediate resistance to erythromycin, ciprofloxacin and ofloxacin (Table 4). Ten (37%) of the MRSA isolates were resistant to vancomycin. Of note is the fact that all the MRSA isolates were multidrug resistant (resistant to at least three or more antibiotic classes) and comprised 11 antibiotypes with the most prevalent being AMP-FEP-OFX-

CIP (40.7%, 11/27) and AMP-FEP-OFX-CIP-E (14.8%, 4/27) (Table 5).

4. DISCUSSION

Susceptible strains of *S. aureus* become resistant to methicillin through the acquisition of the *mec*A gene which encodes the production of a low-affinity penicillin-binding protein called PBP2a. The *mec*A gene can be transferred horizontally thereby conferring resistance to other *S. aureus* strains. Hence, the resistance of *S. aureus* to methicillin, all other β-lactam antibiotics and other classes of antibiotics such as fluoroquinolones, aminoglycosides and tetracyclines continues to increase both in the hospital and community environments [18, 31]. In order to prevent the emergence and further spread of MRSA, continuous tracking, identifying and understanding the risk factors associated with the spread of this pathogen in various environmental settings are necessary [31]. The fight against antimicrobial resistance, especially MRSA, requires surveillance studies that generate real time data to inform policy on resistance [32].

Our study investigated the meat retail shop environment including the persons in contact with the meat for the prevalence, antibiotic-resistant patterns of MRSA and human-associated behaviours that have the potential to exacerbate the spread of this bacterium. The potential route of MRSA spread from the farm to slaughterhouse and possibly to the human population has been reported in several studies [33]. The persistence of MRSA in both host and non-host environments adds a major ecological dimension to the understanding and control of MRSA, thereby making it a one health challenge [34]. Accurate detection of *S. aureus* and MRSA is based on molecular detection methods because phenotypic detection based on conventional biochemical tests may not be reliable, especially in low-income countries like Cameroon where the quality of biochemical reagents purchased from traders may compound the problem [33]. Since the presence of *mec*A gene is highly conserved in methicillin-resistant staphylococci, detection of *mec*A gene remains the 'gold standard' for the identification of MRSA [16]. Although non-*mec*A MRSA strains are also reported, they need to be differentiated from borderline oxacillin-resistant *S. aureus*, another resistant variant in *S. aureus* that has also been reported [35]. Hence, in this study, *S. aureus* and MRSA were identified based on the detection of the *nuc* and *mec*A genes, respectively.

In this study, all (52, 100%) meat handlers were males suggesting that meat retailing in Buea municipality is a male-dominated occupation. Our findings corroborate a very recent study by Al Banna et al. [36] who reported that all 300 Bangladeshi meat handlers included in their study were males. Bafanda et al. [37], in their study carried out in Jammu District of Jammu and Kashmir in India; Thakur et al. [38], in another study in India; Junaidu et al. [39], in Nigeria, all reported 100% male representation of the meat handlers. Of the 52 meat handlers, 24 (46.2%) were within the 30-39 years age group while 14 (26.9%) were in the 20-29 age group, and this is consistent with previous studies that have reported that butchering and meat retailing profession are gender sensitive (a male domain business) and attract more youths because these activities are energy demanding [37]. Majority (37, 71.2%) of the meat handlers in this study did not have meat safety training, a phenomenon that has also been reported in other less developed countries [37, 39]. Majority of the meat handlers in this study had secondary level education contrary to Bafanda et al. [37] who reported that none of the meat handlers had ever received a formal training at any institution.

Table 4. Susceptibility profile of MRSA isolates (n=27) to the various antibiotics

Class of antibiotic	Antibiotic	Susceptible (%)	Intermediate (%)	Resistant (%)
Glycopeptide	Vancomycin	17 (63)	0 (0)	10 (37)
Aminoglycoside	Gentamicin	27 (100)	0 (0)	0 (0)
	Amikacin	27 (100)	0 (0)	0 (0)
Macrolide	Azithromycin	22 (81.5)	0 (0)	5 (18.5)
	Erythromycin	10 (37)	2 (7.4)	15 (55.6)
Tetracycline	Doxycycline	22 (81.5)	0 (0)	5 (18.5)
Quinolone	Ciprofloxacin	0 (0)	5 (18.5)	22 (81.5)
Fluoroquinolone	Ofloxacin	0 (0)	2 (7.4)	25 (92.6)
Penicillin	Ampicillin	0 (0)	0 (0)	27 (100)
Cephalosporin	Cefepime	0 (0)	0 (0)	27 (100)

Table 5. Resistance patterns and antibiotypes of MRSA identified in this study

Antibiotypes	Number of isolates (%)	Resistance category	Sample source
AMP-FEP-OFX	1 (3.7)	Multidrug	Hands
AMP-FEP-OFX-CIP	11 (40.7)	Multidrug	Meat, knives, butchering slabs
AMP-FEP-OFX-VA-E	2 (7.4)	Multidrug	Knives, butchering slab
AMP-FEP-OFX-CIP-E	4 (14.8)	Multidrug	Meat, knives
AMP-FEP-OFX-E-AZM	1 (3.7)	Multidrug	Meat
AMP-FEP-VA-DO-E-AZM	1 (3.7)	Multidrug	Knives
AMP-FEP-OFX-CIP-VA-E	2 (7.4)	Multidrug	Meat, knives
AMP-FEP-OFX-CIP-VA-DO-E	2 (7.4)	Multidrug	Meat, butchering slabs
AMP-FEP-CIP-VA-DO-E-AZM	1 (3.7)	Multidrug	Meat
AMP-FEP-OFX-CIP-VA-E-AZM	1 (3.7)	Multidrug	Butchering slab
AMP-FEP-OFX-CIP-VA-DO-E-AZM	1 (3.7)	Multidrug	Hands
Total	27 (100)		

AMP, ampicillin; FEP, cefepime; OFX, ofloxacin; CIP, ciprofloxacin, VA, vancomycin; E, erythromycin; AZM, azithromycin; DO, doxycyclin

Majority of the meat handlers in this study had good basic hygiene knowledge and acceptable basic hygiene practices. For example, all of them knew that handling money with bare hands while serving meat can lead to meat contamination while 92.3% of them knew that hand washing before handling meat reduces the risk of meat contamination. Many of them (90.4%) knew that 'germs' can be transmitted through their meat retail activities. Similarly, most (≥80.8%) of them implemented good basic hygiene practices such as hand washing, wearing of apron when handling meat and washing of aprons at the end of each day. We found a significant correlation between secondary education and good knowledge, as well as good basic hygiene practices. These findings were in agreement with the finding of Nyamakwere et al. [40] who observed that educational level and professional training of meat handlers were significantly associated with their level of knowledge and food safety practices. Our findings are expected because majority of the meat handlers in this study have at least secondary education.

Of note is the fact that only five (9.6%) of the meat retail shops had a nearby water source. The availability of water in meat retail shops cannot be overemphasized. Water is used to clean hands and surfaces that come into contact with meat. Yenealem et al. [41] identified water scarcity in the meat retail shops as an impediment that prevents the conversion of knowledge to tangible practices that enhance food safety.

In this study, one of the objectives was to determine the prevalence of MRSA in the retail meat shop environment and meat handlers; an overall genotypic prevalence of 25.7 % was recorded, and this was higher than a *mec*A-positive MRSA prevalence of 2.4% (12/500) reported in Italy [42], 4% (4/100) *mec*A-positive MRSA in retail meat in Georgia [43], 1.9% (66/3520) *mec*A-positive MRSA in retail meat in USA [44], 0.7% (29/4264) in retail meat in Korea [45] and 7.3% (9/124) *mec*A-positive MRSA in retail meat in UK [46]. On the contrary, our prevalence was lower than the 45% (45/100) MRSA phenotypic prevalence reported in raw meats sold at various retail outlets in the cape coast metropolis of Ghana [47] and 39.3% (118/300) *mec*A-positive MRSA prevalence observed from slaughter houses and meat retail shops in Pakistan [48]. This difference in prevalence may be accounted for by the difference in sample type, sample size, analytic methods used and even location variability. Generally, while lower levels of MRSA contamination are reported in Europe, USA and Canada, high contamination levels are reported in Asia and Africa [49]. Retail meat may serve as a potential source of exposure to MRSA for humans; therefore, monitoring of meat retail shops and improved hygiene standards should be considered to ensure food safety [42].

Except for the weighing balance, MRSA was detected in all other sample sources with knives recording the highest prevalence (21.2%, 11/52), followed by meat (15.4%, 8/52), butchering slabs (9.6%, 5/52) and hands of meat handlers (5.8%, 3/52). Unlike knives, tables, and the hands of meat handlers that are always in contact with the meat, weighing balances are rarely in contact with the meat in the meat retail shops in Buea.

This could reduce the chances for the weighing balance to be contaminated with MRSA.

Antimicrobial resistance is a global problem and the distribution of antimicrobial resistant staphylococci, especially MRSA present a major challenge to both human and animal health. In this study, the MRSA isolates showed 100 % susceptibility to amikacin and gentamycin followed by 81.5 % for azithromycin and doxycycline and 63% for vancomycin. These results agree with that reported by Aliyu et al. [50], and Fri et al. [51] who also recorded 100 % susceptibility to these antibiotics. However, Savariraj et al. [52] reported 78 % susceptibility to vancomycin, in a similar study.

Reports from previous studies indicate that, there is widespread inappropriate use of antibiotics, for non-therapeutic purposes, such as growth inducers in plant and animal feeds, which have contributed to the emergence and propagation of antimicrobial resistance in MRSA. High levels of resistance were recorded against ampicillin (100 %), cefepime (100 %), ciprofloxacin (81.5 %) and ofloxacin (92.6 %). Similarly, Effah *et al.* [47] reported a 100 % resistance of MRSA to ampicillin in a similar study in the Cape Coast metropolis of Ghana.

Vancomycin resistance (37%), reported in this study is of great public and veterinary health concern since vancomycin is historically regarded as the last resort drug for the treatment of MRSA infections [45]. This result agrees with Al-Amery *et al.* [53] who reported 35 % vancomycin resistance from camel meat and slaughterhouse workers in Egypt. Beyene *et al.* [54] equally reported vancomycin resistance (65.1 %) in a prevalence study that investigated the antimicrobial resistance profile of *Staphylococcus* in dairy farms, abattoir and humans in Addis Ababa, Ethiopia. A higher prevalence of 80% for vancomycin resistance was reported by Bissong et al [15] for *S*. *aureus* from milk and beef from the Northwest and Southwest Regions of Cameroon. In this study, all the MRSA isolates were multidrug resistant a finding which is in line with previous studies that reported multidrug resistance in all MRSA isolates in South Africa [55] and Italy [42].

5. CONCLUSION

MRSA in this study demonstrated high level of resistance against the most commonly used antibiotics in the study area such as ampicillin, cefepime, ofloxacin and ciprofloxacin. This represents both veterinary and public health emergencies, which serves as an indication that these antibiotics are gradually running out of clinical use, particularly in Buea municipality. These findings have profound clinical and veterinary significance and call for urgent intervention to mitigate the situation.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

AVAILABILITY OF DATA AND MATERIALS

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

CONSENT

Informed consent was obtained from all meat handlers who participated in the study. To ensure confidentiality, only unique identification codes were assigned to each questionnaire, and not names. This study was conducted following the relevant guidelines and principles outlined in the Declaration of Helsinki.

ETHICAL APPROVAL

Ethical approval to carry out this study was obtained from the Institutional Review Board of the Faculty of Health Sciences, University of Buea (Ref. No. 2020/1154-03/UB/SG/IRB/FHS of 24 March 2020). Administrative authorizations were obtained from the South West Regional Delegations of Livestock, Fisheries and Animal Industries (Ref No. MINEPIA/RD/SRAG/SW/04/1034 of 31 March 2020) and of Public Health (Ref. No. R11/MINSANTE/SWR/RDPH/PS/620/867 of 27 March 2020). Verbal authorizations were obtained from retail meat shop owners.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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