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Phenotypic and Genotypic Characterization of *mecA* Gene in Methicillin Resistance *Staphylococcus aureus* Isolated from Smoked Fish

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Abstract

Staphylococcus aureus is a bacterial species responsible for food poisoning and outbreaks of opportunistic, nosocomial and community-acquired diseases. The aim of this study was to characterize *S. aureus* strains resistant to methicillin. Seventy-five (75) samples of smoked fish, including *Scomber scombrus*, *Trachurus trachurus*, *Thunnus* spp., *Cyprinus* spp. and *Sardinella* spp., were studied. The Mueller-Hinton diffusion method was used to determine the phenotypic resistance profile. The coagulase test and thermonuclease detection were used to assess the enzymatic production potential of the strains. The methicillin resistance *mecA* gene was detected by PCR. With a contamination rate of 80%, the prevalence of *S. aureus* varied from 15% to 31.7% in animal products. *S. aureus* strains were DNase (91.7%) and coagulase (50%) producers. The resistance of these strains was 42.7% (cefoxitin), 37.8% (oxacillin) and 26.4% (cefuroxime sodium). They were resistant to tetracycline (62.4%), erythromycin (61.1%), vancomycin (34.6%), levofloxacin (33.3%) and imipenem (12.7%). The prevalence of the *mecA* gene in animal products ranged from 13.9% to 33.4%. The *mecA* gene induction showed different sensitivities with cefoxitin (100%) and oxacillin (56.7%). In addition, all tests showed a specificity of 100%. This work demonstrates the need to strengthen surveillance to prevent the spread of *S. aureus* epidemics in various environments.

Keywords

S. aureus, Methicillin-Resistant, *mecA* Gene, PCR, Smoked Fish

1. Introduction

In many countries around the world, fish is consumed and is considered an important source of dietary protein. In Africa, fish constitutes approximately 17% of animal protein consumed [1]. Fish, and in particular smoked fish, are highly prized by the African population and play a significant role in food and nutritional security. In addition, post-harvest activities in artisanal maritime fisheries generate wealth of 4 million euros and contribute 0.23% of GDP [2]. However, there are problems of post-harvest losses in artisanal maritime fisheries. Indeed, studies reveal that these post-harvest losses are estimated at between 20 and 50% in Africa [2].

In Côte d'Ivoire, smoked fish represents 2/3 of the consumption of fishery products. This form of fish preservation is still obtained using outdated traditional smoking methods that promote the development of micro-organisms in fish. Among these microorganisms, several bacterial genera such as *Escherichia*, *Listeria*, *Pseudomonas*, *Klebsiella*, *Salmonella* and *Staphylococcus* have been isolated from fish [3]. Some bacterial species such as *Staphylococcus aureus* are responsible for food poisoning and epidemics of opportunistic, nosocomial and community diseases throughout the world [3] [4]. Among these bacterial species belonging to the genus *Staphylococcus*, others are resistant to different antibiotics, including methicillin.

Several studies have shown that some strains of *S. aureus* have developed cross-resistance between penicillins M (methicillin, oxacillin) and other β-lactams [5]. This cross-resistance is due to the production of a protein, PBP2a, which is a penicillin-binding protein (PBP2a) and has a low affinity for these compounds [3] [4]. This complex explains the multi-resistance profile of hospital and community methicillin-resistant *S. aureus* (MRSA) [6]. *Staphylococcus aureus* strains possessing the *mecA* gene are therefore resistant to the entire family of β-lactams, in particular to methicillin or oxacillin [3] [6].

Studies have shown that resistance to β-lactams in MRSA strains may be associated with the presence of transferable genomic islands, called SCCmec, in which the mec gene determines resistance to methicillin [3] [6]. Among the different types of SCCmec, there may be *mecA* or *mecC* genes and genes for resistance to other groups of antibiotics such as aminoglycosides, macrolides, lincosamides, streptogramins B, and tetracyclines [4] [6]. The *mecA* gene and its numerous homologues (*mecB*, *mecC*, and *mecD*) share approximately 70% nucleotide sequence similarity. The main function of the *mecA* gene was related to cell wall synthesis, but its evolution into a resistance determinant appears to have occurred through a stepwise process [3] [6].

This *mecA* gene is included in a mobile genetic element: the staphylococcal cassette chromosome mec (SCCmec). The *mecA* gene regulation mechanism is mainly dependent on two genes: *mecI* (*mecA* gene repressor) and *mecR* (antirepressor) genes [3]. This *mecA* gene confers two types of resistance to *S. aureus* strains. The first is expressed by all strains and is said to be homogeneous. The second is said to be heterogeneous and is expressed only by a proportion of daughter colonies from a mother colony expressing resistance.

In strains with heterogeneous resistance to methicillin, the level of resistance is not correlated with the amount of PBP2a, but seems to be dependent on four chromosomal *fem A*, *B*, *C*, *D* genes (factors essential for methicillin resistance) involved in the formation of the pentaglycine interpeptide bridge of the peptidoglycan [5]. These different multi-resistances indicate that the spread of methicillin-resistant *S. aureus* (MRSA) epidemics can be a public health problem. Therefore, laboratory tests and molecular detection of the *mecA* gene are necessary in the surveillance and management of methicillin-resistant *S. aureus* (MRSA) outbreaks.

In Côte d'Ivoire, several studies have been carried out on resistance to antibiotics and in particular to methicillin in *S. aureus* of hospital and community origin [7] [8]. Despite these few studies carried out in Côte d'Ivoire, data are rare, if not non-existent, regarding the characterization of methicillin-resistant *S. aureus* (MRSA) of animal origin. The objective of this study was to characterise methicillin-resistant *S. aureus* (MRSA) strains isolated from local animal products.

2. Material and Methods

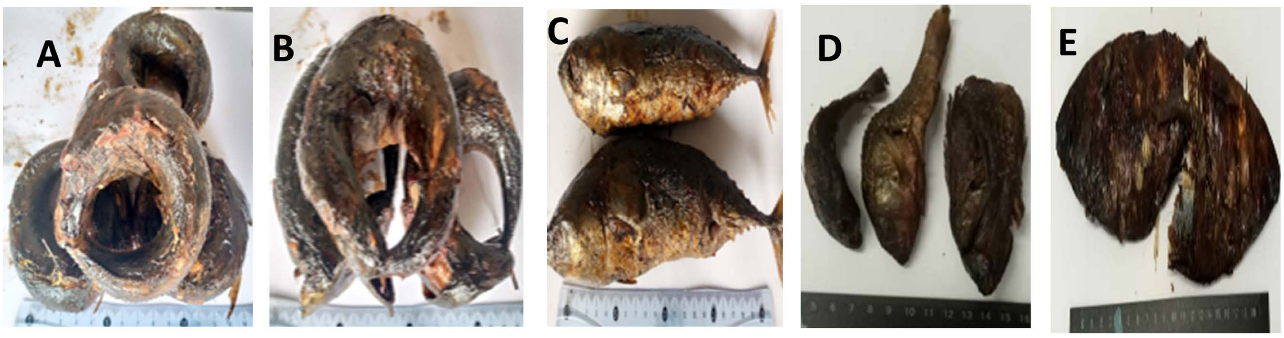
2.1. Sampling Collection

This study is a cross-sectional study in which a total of 75 samples of smoked fish consisting of five different species were randomly collected from female vendors in Abidjan. The five different species of smoked fish consisted of *Scomber scombrus* (mackerel), *Trachurus trachurus* (horse mackerel), *Thunnus spp*. (tuna), *Cyprinus spp*. (carp) and *Sardinella* spp. (sardinella) (**Figure 1**). The purchased smoked fish were immediately placed in Stomacher bags and stored in coolers containing ice packs for analysis the same day in the laboratory.

2.2. Isolation and Identification of *S. aureus*

Different stock suspensions were obtained by grinding 10 g of each smoked fish sample in 90 mL of buffered peptone water (Oxoid, Basingstoke, UK). The stock suspensions and the decimal dilutions obtained were inoculated on the surface of

Baird Parker agar (Biomerieux, France) supplemented with tellurite egg yolk. The plates were incubated at 37˚C for 24 to 48 hours. The characteristic black, shiny colonies surrounded by a clear halo are considered *S. aureus* after the oxidase test and Gram staining.



A: Trachurus trachurus; B: Scomber scombrus; C: Thunnus spp.; D: Cyprinus spp.; E: Sardinella spp.

Figure 1. Species of smoked fish.

2.3. Detection of Coagulase and DNase

2.3.1. Coagulase Test

Well-isolated *S. aureus* colonies on Baird Parker agar were transferred to brain heart broth (BHB) and incubated at 37˚C for 24 hours. After incubation, a volume of 0.2 mL of each culture was transferred to sterile hemolysis tubes containing 0.5 mL of rabbit plasma. The whole was incubated again at 37˚C and examined after 1 and 4 hours. The presence of a firm clot indicates the presence of coagulase-positive *S. aureus* [9].

2.3.2. DNAase Production

DNAase or thermonuclease was sought from suspect colonies isolated on Baird Parker. After incubation at 37˚C for 24 hours on Columbia medium (Bio-Rad), an isolated colony is seeded in 1 cm diameter plaques on DNA agar (OXOIOD). The supernatant is removed 5 minutes after flooding these DNA plates with a 1moL.L-1 hydrochloric acid solution. The presence of a clear zone around the plaque indicates a positive DNase. In the absence of a clear zone, the *S. aureus* strain is said to be DNase negative [10].

2.4. Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing was performed on Mueller Hinton agar (Oxoid, UK) using the diffusion technique as per the guidelines set by CA-SFM, [11]. All antibiotic discs used were from Oxoid, UK. These discs included Tetracycline (TET), Erythromycin (E), Cefepime (FEP), Ceftazidime (CAZ), Imipenem (IPM), Cefoxitin (FOX), Cefuroxime sodium (CXM), Oxacillin (OXA), Levofloxacin (LVX), Vancomycin (VA). The entire surface of the Mueller-Hinton agar was swabbed in a sterile manner from the bacterial suspension in saline solution with a turbidity equivalent to 0.5 McFarland. This inoculum of approximately 10^8

CFU/mL was made from young colonies of 24 hours taken from ordinary agar.

Fifteen minutes after the antibiotic-impregnated discs were placed on the surface of the inoculated agar, the plates were incubated at 37˚C for 24 hours. After 24 hours, the inhibition zones were determined. The interpretation of the results was done according to the rules of the Antibiogram Committee of the French Society of Microbiology [11]. The reference strain *Staphylococcus aureus* ATCC 25923 was used for quality control of the antibiogram.

2.5. Molecular Study

2.5.1. DNA Extraction and Master Mix

DNA from all *Staphylococcus* isolates was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). After DNA extraction, the master mix was prepared as described by the modified method of Kuellmer and Frank [12]. This 25 µL reaction mixture consisted of 13 µL of sterile Milli-Q water (milli-Q™, Millipore Corporation, USA), 5 µL of 5X concentration buffer, 1.5 µL of 2 mM MgCl2 (Promega Corporation, Madison, WI 53711-5399, USA), 1 µL of 10 mM NTPs, 1 µL of each 27F and 1492R primer, 10 mM (TranS, AP111 5U, CHINA), 0.5 µL of Easy Tag® DNA polymerase with a final concentration of 1.5 U (TranS, AP111 5U, CHINA), and 2 µL of the DNA template. Sterile Milli-Q water and reference strain ATCC 25923 were used as negative control and positive control, respectively, for each PCR reaction.

2.5.2. Amplification of *mecA* Genes Encoding MRSA

Amplification of the *mecA* gene encoding methicillin-resistant *Staphylococcus aureus* (MRSA) was performed according to the method described by Momtaz *et al.* [12] using primers F: 5'-AAAATCGATGGTAAAGGTTGGC-3' and R: 5' AGTTCTGCAGTACCGGATTTGC-3' for *mecA* (533bp). The amplification program included an initial denaturation of 5-min at 95˚C followed by 35 cycles including a 30-s denaturation step at 95˚C, a 30-s primer fixation (annealing) step at 60˚C, and a 40-s extension step at 72˚C. The amplification reaction was terminated by a final 5-min elongation at 72˚C. Samples were stored at 4 °C until the thermal cycler was stopped.

2.6. Data Analysis

Statistical analyses were calculated by IBM SPSS version 26 software package (IBM Corporation, Somers, NY) by applying a Pearson's Chi-square test. The statistical significance level was set at $P < 0.05$.

3. Results

3.1. Prevalence of *S. aureus*

The contamination rate of different smoked fish by *S. aureus* varied from 12% (*Scomber scombrus*) to 25.3% (*Thunnus spp*). As for the prevalence of these strains, it was higher in fish of the *Thunnus spp* type (31.6%) (Table 1).

Table 1. Prevalence and contamination rate.

Types of Smoked Fish	Prevalence and Contamination Rate					Total
	Scomber scombrus n = 15	*Trachurus trachurus* n = 15	*Thunnus* spp. n = 15	*Sardinella* spp. n = 15	*Cyprinus* spp. n = 15	Total n = 75
Number of *S. aureus* strains (n)	9	11	19	11	10	60
Contamination Rate (%)	12	14.7	25.3	14.7	13.3	80.0
Prevalence (%)	15	18.3	31.7	18.3	16.7	100

3.2. Enzyme Production Potential in *S. aureus*

The enzymatic production potential showed that *S. aureus* strains were predominantly producers of deoxyribonuclease (DNase) endonuclease with a positivity rate of 91.7%. This positivity rate was 50% for coagulase production (Table 2).

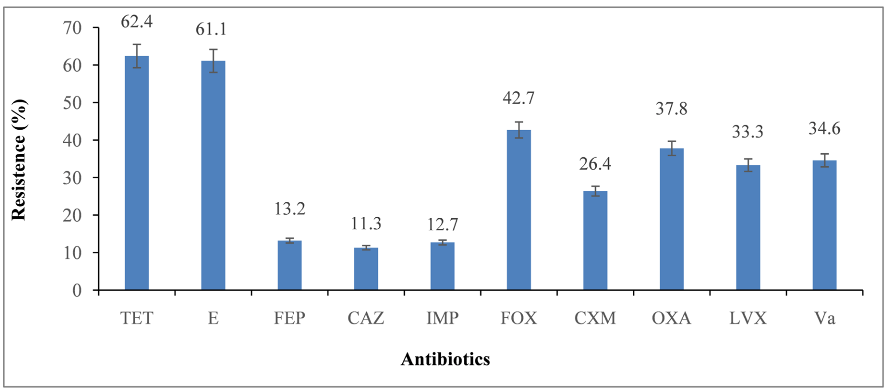
Table 2. Enzyme production potential in *S. aureus*.

Types of Smoked Fish (n = 75)	*S. aureus* (n = 60)	*DNaPSa* (n = 55)	*CoPSa* (n = 30)
Scomber scombrus n = 15	9	8	4
Trachurus trachurus n = 15	11	10	5
Thunnus spp. n = 15	19	17	11
Sardinella spp. n = 15	11	10	5
Cyprinus spp. n = 15	10	10	5
Percentage (%)	**60 (100 %)**	**55 (91.7%)**	**30 (50%)**

CoPSa: Coagulase-Positive *S. aureus*, DNaPSa: DNase-Positive *S. aureus*.

3.3. Antimicrobial Resistance

Staphylococcus aureus strains showed different levels of resistance to antibiotics. This resistance was 42.7% (cefoxitin), 37.8% (oxacillin) and 26.4% (cefuroxime sodium). These strains were also resistant to tetracycline (62.4%), erythromycin (61.1%), vancomycin (34.6%), levofloxacin (33.3%) and imipenem (12.7%) (Figure 2).

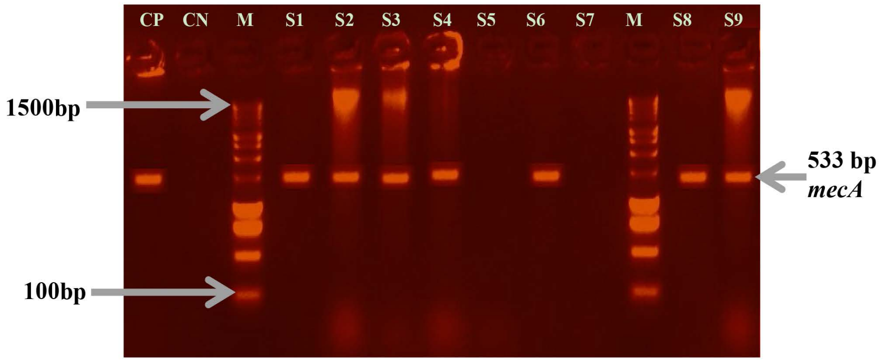


Tétracycline (TET), Erythromycine (E), Cefepime (FEP), Ceftazidime (CAZ), Imipenème (IPM), Céfoxitine (FOX), Céfuroxime sodium (CXM), Oxacilline (OXA), Levofloxacine (LVX), Vancomycine (VA).

Figure 2. Phenotypic resistance to antibiotics.

3.4. Methicillin Resistance Genes (*mecA*) in *S. aureus*

The electrophoretic profile of *mecA* genes showed that some *S. aureus* strains (A1-A4; A6; A8 and A9) harbored methicillin resistance genes (*mecA*) (Figure 3). The prevalence of the *mecA* gene in animal products ranged from 13.9% to 33.4%. The *mecA* gene (33.4%) was predominantly detected in *S. aureus* strains isolated from smoked fish of the Thunnus spp. type (Table 3).



CP: Positive control; CN: Negative control; S1-S4; S6; S8; S9 presence of the *mecA* gene in *S. aureus*, M: Marker Gene Ruler (Bench Top, 1500 bp DNA Ladder, Promega Corporation, USA).

Figure 3. Electrophoretic profile of the *mecA* gene in methicillin-resistant *S. aureus*.

Table 3. Distribution and prevalence of *mecA* gene in *S. aureus*.

Distribution of mecA Genes in Methicillin-Resistant S. aureus						
Types of Smoked Fish	*Scomber scombrus* n = 15	*Trachurus trachurus* n = 15	*Thunnus* spp. n = 15	*Sardinella* spp. n = 15	*Cyprinus* spp. n = 15	Total n = 75
Number of S. aureus (n)	9	11	19	11	10	60
Strains (n) harboring mecA	5	7	12	5	7	36
Prevalence of *mecA* gene (%)	13.9%	19.4%	33.4%	13.9%	19.4%	100%

3.5. Sensitivity and Specificity of Phenotypic and Genotypic Method

Methicillin resistance detected by oxacillin in *S. aureus* strains harboring the *mecA* gene showed only a sensitivity of 56.7% while this sensitivity was 100% with cefoxitin (Table 4). At the level of coagulase production (CoPSa), methicillin resistance tested from cefoxitin DD also indicated the highest sensitivity with 70%. However, oxacillin showed the lowest sensitivity with 56.7% (Table 4). In deoxyribonuclease (DNase)-producing *S. aureus* strains, cefoxitin (87.3%) was the best inducer of the *mecA* gene compared to oxacillin (69.1%). Moreover, all tests showed 100% specificity (Table 4).

Table 4. Sensitivity and specificity of phenotypic and genotypic method.

	S. aureus (n = 60)			
	PCR *mecA*		Sensitivity (%)	Specificity (%)
	Negative (n = 24)	Positive (n = 36)		
Cefoxitin DD Resistance				
Negative	0	0	100.0	0.0
Positive	24	36		
Oxacillin Resistance				
Negative	15	11	56.7	100.0
Positive	0	34		
	CoPSa (n = 30)			
	PCR *mecA*		Sensitivity (%)	Specificity (%)
	Negative (n = 7)	Positive (n = 23)		
Cefoxitin DD Resistance				
Negative	7	2	70.0	100.0
Positive	0	21		
Oxacillin Resistance				
Negative	7	6	56.7	100.0
Positive	0	17		
	DNaPSa (n = 55)			
	PCR *mecA*		Sensitivity (%)	Specificity (%)
	Negative (n = 2)	Positive (n = 53)		
Cefoxitin DD Resistance				
Negative	2	5	87.3	100.0
Positive	0	48		
Oxacillin Resistance				
Negative	2	15	69.1	100.0
Positive	0	38		

CoPSa: Coagulase-Positive S. aureus; DNaPSa: DNase-Positive S. aureus; DD: Disque Diffusion.

4. Discussion

Methicillin-resistant *S. aureus* (MRSA) infections pose real major therapeutic problems given the emergence of hospital and community-acquired strains. This resistance to methicillin is linked to the acquisition by the bacterium of a *mecA* gene encoding an additional penicillin-binding protein (PBP) called PBP2a, which has a reduced affinity for all beta-lactams [13].

The present study characterized strains of methicillin-resistant *S. aureus* isolated from different types of smoked fish consumed locally. The level of contamination of these different smoked fish by *S. aureus* varied from 12% (*Scomber scombrus*) to 25.3% (*Thunnus spp*) with a higher prevalence in fish of the *Thunnus spp* type (31.6%).

These results differ from those of Idrees *et al*. [14] who indicated a prevalence of 10.2% of methicillin-resistant *S. aureus* associated with *mecA* and *mecC* genes.

Indeed, this difference in prevalence could be justified by the number and nature of the specimen studied as a matrix. Thus, several studies have highlighted strains of methicillin-resistant *S. aureus*, with different prevalence in food products [15]-[17].

The enzymatic potential of the strains studied also indicated that they were mainly producers of deoxyribonuclease (DNase) and coagulase. These results, on the one hand, justify the belonging of these strains studied to the species *S. aureus* [18]. On the other hand, these results indicate that these strains could be associated with human foodborne diseases due to the ingestion of staphylococcal enterotoxins (SE) [16] [18].

Indeed, staphylococcal enterotoxins (SE) are proteins produced by coagulase-positive staphylococci, mainly by *S. aureus* [19]. These toxins, if present in sufficient quantity in the food, can trigger the symptoms of poisoning [19] [20].

These strains of *S. aureus* studied also showed different levels of resistance to antibiotics [21]. These strains of *S. aureus* had a high level of resistance to cefoxitin (42.7%), oxacillin (37.8%), cefuroxime sodium 26.4% and imipenem (12.7%). This resistance to beta-lactams of staphylococci could be based on two major types of mechanisms that are identical in *S. aureus* with coagulase [3].

The first could be an extrinsic resistance mechanism by production of enzymes inactivating the antibiotic. The second, an intrinsic resistance mechanism by modification of penicillin-binding proteins (PBPs) [4] [17].

Indeed, beta-lactamases are enzymes produced by bacteria and in particular *S. aureus* that can hydrolyze the beta-lactam cycle of penicillins, making them inactive [3] [17].

The high level of cefoxitin resistance could justify the prevalence of *mecA* gene encoding methicillin resistance detected in this study [17]. The reason for using cefoxitin to detect methicillin-resistant strains in *S. aureus* was mainly related to the fact that methicillin is no longer commercially available. Many researchers have also used oxacillin instead of methicillin, but cefoxitin has been found to be the best inducer of *mecA* gene [5].

Indeed, tests using cefoxitin have given more reproducible and more accurate results than tests with oxacillin [17]. Also, cefoxitin remains the best and most reliable inducer because it can phenotypically reveal MRSA harboring the *mecC* gene having an epidemiological impact on human and animal diseases [5] [14] [15]. In addition, this cefoxitin molecule is currently recommended in clinical studies. The various high levels of resistance encountered can be attributed to the irrational use of antibiotics and the lack of resources for infection control [3].

The results of this study clearly show that these strains of *S. aureus* harboring the *mecA* gene and with positive coagulase isolated from smoked fish could be involved in zoonoses, community and nosocomial infections [5] [17]. In the One Health context, MRSA is a public health problem because it poses a risk of zoonotic transmission not only to those involved in the fish processing industry, but also to the wider community through the food chain [22]-[24].

Among the phenotypic methods used in this work to detect methicillin-resistant *S. aureus*, the cefoxitin disk diffusion test showed the best performance, with a sensitivity of 100% compared to oxacillin (56.7%).

Similar results have been obtained by different authors who have demonstrated a better induction of the *mecA* gene by cefoxitin [3]-[5] [25]. In addition, at the level of strains producing coagulase (CoPSa) and deoxyribonuclease (DNase), cefoxitin remained the best inducer of the *mecA* gene. This performance could be explained by the fact that cefoxitin is a strong promoter of the *mecA* gene which is less affected than oxacillin by the hyperproduction of penicillinase [5]. Similar results have been reported by many researchers who detected that cefoxitin disk was superior to oxacillin [4] [5] [26].

5. Conclusion

The present study showed that smoked fish could be an important vector for the transmission of multi-resistant bacteria. The study confirmed the presence of *S. aureus* harbouring the *mecA* methicillin-resistant gene in smoked fish. It is possible that this pathogen contaminating local products could be transferred from the food chain to humans, with the potential to cause health problems. The molecular characterisation, host specificity, pathogenicity and epidemic potential of methicillin-resistant *S. aureus* remain to be explored.

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Author's Contribution

CKDB, DA, DM and AK conceived and designed the study. CKDB, AK performed the experiments. BCKD, AK, KND, and TN analysed the data. CKDB, TWTA, TA, KTG, and AK contributed reagents/materials/analysis tools. CKDB, TWTA, KND, TA, KTG and AK wrote the paper. All the authors read, reviewed, and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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